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PROGRAMME

PROGRESS REPORT

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EURATOM

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Programa

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

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

V O L U M E I

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** Tätigkeitsbericht
Progress report
Rapport annuel

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Contract prolonged in 1990-1991
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V O L U M E I I

V O L U M E I I I

III E

GENETISCHE WIRKUNGEN IONISIERENDER STRAHLEN

GENETIC EFFECTS OF IONIZING RADIATION

EFFETS GENETIQUES DES RAYONNEMENTS IONISANTS

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-E-148-NL

Organisation for Health Research
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Title of the research contract:

The genetic and biochemical basis of radiation sensitivity in cultured human and other mammalian cells.

List of projects:

1. Biochemical analysis of DNA repair functions in mammalian cells.

Title of the project no.: 1

Biochemical analysis of DNA repair functions in mammalian cells

Head(s) of project: Dr.G.P. van der Schans and Dr. F. Berends.

Scientific staff: Dr. G.P. van der Schans and Drs. L. Roza

I. Objectives of the project:

This project aims at the identification of various DNA lesions in irradiated mammalian cells and the elucidation of their repair. Special attention will be given to the development of methods for the detection of a variety of lesions in DNA of irradiated mammalian cells, and on the study of their repair. The agents which will be used in these studies for the induction of lesions are ionizing radiation and ultraviolet light of different wavelengths.

II. Objectives for the reporting period:

Our attention was focussed on 2 approaches for the detection of various radiation-induced lesions:

(i) *immunochemical methods*: Production of antibodies against radiation-induced lesions (thymine glycols, local single-strandedness) and development of immunochemical assays based on the antisera obtained;

(ii) *biochemical methods*: Development of an alkaline elution method for the senstive detection of single-strand breaks, with fluorometric quantitation of DNA, meant to be applicable after *in vitro* as well as *in vivo* irradiation.

Next, the methods developed were applied in studies on the induction and repair of DNA lesions in cells exposed to ionizing radiation or UV, either *in vitro* or *in vivo*.

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. Two such "DNA-damages" were synthesized, *i.e.* thymine glycol, and thymine-dimer. The latter lesion, the main damage induced by ultraviolet light (UV), is also important as a product of exposure to ionizing radiation. It was obtained by dimerization of the two thymines present in the tetranucleotide GpTpTpG. For the former damage we used thymidylic acid glycol or poly(dT) in which glycols were induced by treatment with OsO_4. For another lesion induced by ionizing radiation, hydroxymethyldeoxyuridine (HMdU, an oxydation product of thymine), commercially available hydroxymethyluridine (HMU) was chosen as the antigen. All products used were coupled to a carrier protein, and the complexes were used for immunization.

Polyclonal antisera

To obtain polyclonal antisera, rabbits were immunized with

(i) thymidylic acid glycol, coupled to chicken-γ-globulin,
(ii) poly(dT), treated with OsO_4,
(iii) UV-irradiated DNA and
(iv) double-stranded BrdU-containing DNA; the last three electrostatically coupled to methylated bovine serum albumine.

Monoclonal antibodies

These antibodies were obtained after immunizations of mice with

(i) OsO_4-treated poly(dT), coupled to methylated bovine serum albumin and
(ii) HMU, and
(iii) GpTpTpG containing the thymine dimer; the last two coupled to chicken-γ-globulin.

From spleen cells of the mice, after fusion with mouse myeloma cells, a large number of hybridomas were obtained which were selected for antigen-specificity.

As a by-product from fusions of mouse myeloma cells with spleen cells isolated from a mouse immunized with DNA treated with benz(a)pyrene-diolepoxide, a monoclonal antibody (D1B) directed against single-stranded DNA was obtained.

Immunochemical detection methods

Immunochemical detection was carried out in 3 different ways:

(i) Direct ELISA, in which the lesion-containing DNA was coated to the wall of the wells in a 96-well plate and the amount of antibody-binding was detected by binding of a second antibody, directed against the first and conjugated to an enzyme which converts a substrate into a light-absorbing or fluorescing product.
(ii) "Competitive" ELISA: various amounts of lesion-containing DNA were 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"). In this method, white blood cells (WBC) from ^{60}Co-γ-irradiated blood are lysed on a membrane filter. When the detection of endonuclease-susceptible damages is intended, the DNA on the filter then is treated with a *Micrococcus luteus*-extract which recognizes several types of base damages induced by ionizing radiation, making an incision in the sugar-phosphate backbone, close to the damage. Subsequently, the DNA on the filter is washed

and treated with alkali, resulting in unwinding and simultaneously slow elution of single-strand pieces of DNA through the filter. The eluted DNA is quantified by fluorescence measurement after addition of Hoechst 33251. The fraction of DNA remaining on the filter is plotted semi-logaritmically *vs* elution time. The slope of such curves is a measure for the number of breaks (directly induced + *M-luteus*-induced breaks; without *M-luteus*-extract, only directly induced breaks are detected). In an alternative procedure, SSB and endonuclease-sensitive sites were also assayed via alkaline sucrosegradient centrifugation.

Immunochemical detection of radiation-induced single-strandedness

This method is based on the binding of anti-single-stranded-DNA monoclonal antibody to single-stranded DNA. Local single-strandedness is induced by ionizing radiation at each SSB induced, and at other damages leading to distortion of the double-helix. This limited single-strandedness can be amplified enormously by a controlled time-dependent partial unwinding of the cellular DNA under strictly defined, mildly alkaline conditions. After neutralization, immediately followed by sonication (to prevent restoration of the double-helix), the percentage of single-strandedness can be detected by the binding of the antibody, in a competitive ELISA (100% single-strandedness is obtained by performing sonication before neutralization, yielding the 100% value). 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. Recent developments permit the detection of the percentage of single-strandedness also in a direct ELISA. This makes the assay much simpler, only half the amount of cells is required and the same sensitivity is obtained.

UV-endo assay

The detection of pyrimidine dimers, after selective conversion into SSB by UV-endonuclease, via alkaline sucrose gradient sedimentation, was carried out after irradiation of cells both *in vitro* and *in vivo*. This technique requires very low concentrations of DNA. With cultured cells, radioactive labelling of DNA was applied; where radioactive labelling was not possible, we used a specially developed, very sensitive immunochemical quantitation of DNA in the gradient fractions.

Results and discussion

Ionizing radiation damage

In the development of sensitive methods for the detection of various radiation-induced lesions and their repair, three approaches were followed.

(i) immunochemical detection of radiation damage;
(ii) sensitive detection of DNA breaks and
(iii) measurement of repair synthesis of DNA.

The studies aim at methods applicable to WBC and suitable for the monitoring of human populations.

(i) *Immunochemical detection of radiation damage*

Immunization of rabbits with thymidylic acid glycol did not yield the desired antibodies specificity for thymine glycols in DNA. Immunization of rabbits with OsO_4-treated poly(dT) yielded IgG-antibodies which react specifically with DNA treated with a low concentration of OsO_4. After chromatographic fractionation of the polyclonal antiserum, some fractions could be isolated with which it was possible to detect thymine glycols in mammalian cells exposed to 20 Gy of ^{60}Co-γ-rays.

Immunization of mice with poly(dT), treated with OsO_4, yielded antisera recognizing glycols in DNA. Hybridomas obtained after fusion showed some specificity for thymine glycols in DNA. However, all clones characterized so far, produce antibodies of the less specific IgM-type, instead of the IgG-type wanted. The attempts to prepare more specific monoclonal antibodies against thymine glycol, which would lower the detection level to 1-10 Gy, failed so far.

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 have been raised, but their affinity and selectivity for HMdU in irradiated DNA have not been established yet.

As pointed out before, beside thymine glycols 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 at the single-cell level, after doses of ^{60}Co-γ-rays as low as 10 Gy. This technique, which still needs improvement, will be developed further.

The immunochemical assay of radiation-induced single-strandedness, amplified by mild alkaline treatment, allows detection of damage induced with 1 Gy of radiation, both in exposed human blood and in mice (in the WBC as well as the bone marrow cells). Not only the rapidly repairable SSB can be detected by this method but also some other, more persistent damage, both in human WBC and murine WBC and bone marrow cells. This technique now has been simplified in such a way that much more samples can be analysed within a shorter time. Furthermore, we were able to isolate monoclonal antibodies against single-stranded DNA of the IgG type to replace the IgM-type antibodies presently applied. Since the accessibility of lesions is expected to be higher for IgG than for IgM, these antibodies would be particularly useful for the detection of damage at the single-cell level.

Germ cells

The method was applied in a collaborative study with Dr. A. Grootegoed (Biochemistry II, Erasmus University, Rotterdam) on the induction and repair of damage in germ cells in different stages of spermatogenesis of the Syrian goldhamster. In all stages an initially rapid removal of damage was detected followed by a slow repair. However, in the stage just before the conversion into spermatozoes, the so-called elongated spermatids, no significant repair was observed during the first 60 min, both after *in vivo* and *in vitro* exposure.

Leukemia patient

In another application we were able to detect SSB in WBC from a leukemia patient undergoing treatment with a cytostatic drug (endoxan) followed by two consecutive 4.5-Gy total-body irradiations before receiving an allogenic bone marrow transplantation. From this experiment it was concluded that

- before treatment with endoxan the amount of damage in the DNA of the patient was low and not different from that of a healthy donor.
- treatment with endoxan led to a substantial increase of persistent DNA damage (still present at 2 days after the last administration of endoxan).
- immediately after irradiation a significant additional increase of single-strandedness occurred which disappeared only partly during the first 30 min after the irradiation.

(ii) *sensitive detection of DNA breaks*

With the alkaline elution method, DNA breaks could be measured after irradiation of both human blood and mice with ionizing radiation down to 1 Gy. In irradiated mice, the induction and repair of SSB could be studied not only in the DNA of the white blood cells, but also in bone marrow cells (in the whole population of cells from one femur). Experiments are in progress for studying the induction and repair of SSB in gut cells after irradiation of mice.

Also base damage could be detected via "alkaline elution". Base-damage induction in DNA of WBC in human blood, exposed to ionizing radiation, increased linearly with dose. The removal of base damage is slower than that of radiation-induced SSB. Even at 90 min after irradiation, base damage is still detectable after a dose of 3 Gy. Base damage was also detected after a 8.6-Gy total body irradiation of a leukemia patient, followed by a 90 min repair period. After this time the directly induced SSB had disappeared.

Furthermore, with alkaline elution it was established that a radiation-sensitive mutant derived from V79 Chinese hamster cells (XR-V15B) has normal removal of SSB, whereas with neutral elution it could be shown that after 4 h of repair more than 50% of the double-strand breaks remained, in comparison to 3% in the original V79 cells.

Ultraviolet light (UV) induced damage

Irradiation of cultured human cells with UV leads to DNA damage, which may result in cell death or mutations. In our investigations both UV-induced pyrimidne dimers and non-dimer UV-lesions were studied. The experiments were performed with human and rodent cells which were irradiated, either *in vivo* or *in vitro* 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 dimers, detected as UV-endonuclease susceptible sites, was much less in cultured rat cells and Chinese hamster cells than in human cells, whereas unscheduled DNA synthesis after UV-C exposure was only a factor of 2 less. With the BrdU-photolysis method the patch-sizes in both cell types were shown not to be different; the number of repaired sites differed only by a factor of 2. These results indicate that the extent to which different repair mechanisms are responsible for the removal of damage differs for cells from different species.

In a Chinese hamster cell mutant, which almost completely lacks repair of pyrimidine dimers, the removal of these lesions appeared to be restored to the level of the wild type cells after the introduction of the human ERCC1 gene. In another mutant of V79 Chinese hamster cells (V-B11), belonging to a new (seventh) complementation group, accumulation of incision breaks after UV-C irradiation during incubation in the presence of hydroxyurea and β- arabinofuranosylcytosine, at 2 h after UV exposure, was about 30% of that found in wild type cells. Furthermore, phenotypic heterogeneity within the first complementation group of UV-sensitive mutants of Chinese hamster cell lines could be demonstrated by using the same technique.

(ii) *Photoreactivation of UV damage*

This process has been described to occur in 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 human cells *in vivo*. 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 of the serum; 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 (clone-forming ability assay). 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 recognize thymine dimers in DNA. Immunochemical detection with the use of this antibody appeared to be sufficiently sensitive for our study on the induction and repair of UV-damage in human skin cells, both *in vitro* and *in vivo*, and on the occurrence of photoreactivation. Experiments with cultured human cells injected with yeast photoreactivating enzyme indicated that photoreactivation of thymine dimers, if occurring, should be demonstrable with this antibody assay. However, no photoreactivation could be observed with non-injected cells, which confirmed our earlier conclusion about the absence of this process in cultured human cells. Further results with microinjected cells showed that - surprisingly - illumination immediately after UV-irradiation does not reduce UDS, whereas photoreactivation at later moments (1 h) results in a substantial decrease of UDS during the subsequent period. Since the antibody assay demonstrated very fast removal of the thymine dimers upon illumination, also immediately after UV, these data suggest that the

initially detected UDS is mainly due to types of damage other than thymine dimers, possibly (6-4)photoproducts, of which the removal by the incision-excision repair mechanism is known to be much faster than that of pyrimidine dimers.

With the same antibodies, thymine dimers can be detected *in situ*, with immunofluorescence microscopy, in cryostat sections of UV-B-irradiated human skin (200 mJ/cm^2). Quantitation of the fluorescent signal is difficult since not all cells are in focus and because the fluorescence varies with the distance to the horny layer. Nevertheless, it appeared to be possible to establish a dose-effect relation by averaging the fluorescence over all cells in the epidermal layer that are in focus. The results obtained compared well with the data resulting from measurements on cell suspensions prepared from irradiated skin. None of the persons locally exposed to UV-B followed by a partial exposure to photoreactivating light, showed an enhanced removal of dimers in comparison to parts not exposed to this light. For persons, exposed to a fractionated dose of UV-B with intervals of 2.5 h and subsequent illumination after each UV-B irradiation, an enhanced removal of pyrimidine dimers was observed. However, if the interval between the fractionated UV-B doses was of the order of one day and exposure occurred over a period of one week, this phenomenon was no longer observed at the end of the exposure period. Evidently, photoreactivation can be observed in humans, but only under rather specific conditions.

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 TNO Medical Biological Laboratory, Rijswijk (Dr. R.A. Baan)

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-E-330-NL

Laboratory for Radiobiology
University of Amsterdam
AMC, FO-K212
Meibergdreef 9
NL-1105 AZ Amsterdam

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

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Title of the research contract:

Automated detection of radiation induced chromosome aberrations by slit-scan flow karyotyping

List of projects:

Automated detection of radiation induced chromosome aberrations by slit-scan flow karyotyping

Title of the project no.: 1

AUTOMATED DETECTION OF RADIATION INDUCED CHROMOSOME ABERRATIONS BY SLIT SCAN FLOW KARYOTYPING

Head(s) of project:

Dr.J.A.Aten, Prof Dr.G.W.Barendsen

Scientific staff:

Dr.W.Rens, Dr.G.Boschman, Dr.G.W.Barendsen, Dr.J.A.Aten.

I. Objectives of the project:

The investigations are directed at developing a method for the rapid analysis of radiation-induced chromosome aberrations in human cells to assess doses received in accidental exposures.

To achieve this aim, karyotype abnormalities will be detected by slit-scanning of fluorescent chromosomes in suspensions prepared from irradiated cells. Flow cytometry techniques will be adapted for this purpose.

Chromosomes with profiles showing dicentrics will be detected and counted in real-time, i.e. during the passage through the laser beam of the flow cytometer. This detection will allow the activation of the cell sorter module and abnormal chromosomes can thus be collected separately for visual analysis on slides and verification of the counted aberrations.

II. Objectives for the reporting period:

This project started 1-1-1989.

Chromosome aberrations in cultured human cells are detected on the basis of the DNA content, the number of centromeres per chromosome and changes in the centromeric index as derived from slit-scanning profiles. To determine frequencies of aberrations at low radiation doses it is necessary to reduce the background of chromosomal debris and aggregates. The slit-scanning analysis will be carried out with a commercially available flow cytometer to which the hard-ware required for evaluation of the chromosome profile can be connected. By combining high speed slit-scanning with sorting of selected chromosome profiles, a rapid and dependable method for biological radiation dosimetry will be developed.

III. Progress achieved:

The method of slit-scanning involves the illumination by a ribbon-shaped laser beam of elongated chromosomes stained with DNA specific dyes. The chromosomes are aligned in the direction of the flow canal perpendicular to the laser beam. The morphology of the chromosomees is analysed through the digital time dependent registration of the fluorescence signal as the chromosome passes through the narrow laser beam.

In this completed one year project the optical system was improved using a new detector lens system. This allowed an improvement of the resolution from 2.7 um to 1.0 um.

For the analysis of chromosome profiles computer programmes were developed to recognise dips in the profiles which indicate the place of the centromere. A pulse shape analyser was build which detects dips with an amplitude of 8% of the plateau value and a width of 1 um. A shape parameter named Pulse Dip Index (PDI) was defined as the ratio of the integrated signal from the beginning of the pulse until the first dip, relative to the integrated signal of the complete profile. This PDI is similar to the Centromeric Index of chromosomes.

The composition of aggregates in mixtures of fluorescent particles of different sizes was evaluated by PDI analysis. In our experiments the PDI was determined within 30 us from the onset of the pulse-profile and particles with a specified morphology of interest were selected for on-line registration of their profiles as digitized pulse-shapes. In a cell sorter system, the PDI can be used as a parameter for sorting.

In general, particles with dips in their fluorescence profiles have elongated shapes inducing them to align in the direction of flow. This process facilitates the slit-scanning analysis of particles such as sperm, cell aggregates and chromosomes. Using pulse-shape analysis, objects with equal fluorescence intensities but of different shape can be distinguished by the PDI criterium. In this way peaks with overlapping fluorescence intensity distributions can be separated. Our results show that the PDI-analysis can be used as an alternative method to increase the resolution of a measure-

ment when dual-beam equipment for 2 parameter fluorescence analysis is not available.

A detailed discription of the procedure for the preparation of chromosomes and the analysis of their centromeric index will be presented elsewhere, but it should be mentioned that the main requirement for a succesful application to small chromosomes is that they are isolated according to procedures developed to produce elongated chromosomes.

The length of the chromosomes was increased by incubating the unfixed chromosome suspension in a 37^{o}C waterbath under constant movement. This procedure resulted in an appropriate length of the chromosomes without noticeable deterioration of chromosome quality. This was assessed by microscopic and slit-scanning analysis of chromosome morphology, and by flow cytometric analysis of the chromosome fluorescence. The length of the larger chromosomes from human cells could in this way be increased up to 20 um. The chromosomes were kept on melting ice until flow cytometric sorting. About 10 minutes before sorting paraformaldehyde was added to a final concentration of 0.025% (w/v). This was found to be necessary for preservation of the morphology during the process of sorting and slide preparation.

Propidium iodide was chosen for isolation and staining because its intercalating action results in long chromosomes and it shows no base specificity.

It is generally accepted that only a fraction of the primary changes caused by a dose of ionizing radiation results in effects observable as asymmetric chromosomal exchanges. In flow karyotyping analysis, on the other hand, it is to be expected that both asymmetrical and symmetrical translocations are detected. It has been suggested that damage in chromosomes, normally not expressed as breaks, might be exposed by the isolation of the metaphase chromosomes from the protective environment of the cell. Changes in the degree of condensation of the chromatin induced by radiation treatment, moreover, might affect the interaction of the incalating fluorochrome propidium iodide with the DNA. This could result in a change in fluorescence intensity. These different factors

could offer an explanation for the relatively large number of radiation-induced events deduced from changes in the flow karyotypes.

The developments described make it now possible to apply the automated detection of aberrations to derive dose effect relationships for radiation exposure of human cells and finally to assess exposure of persons who received radiation e.g. as a consequence of working conditions or treatments.

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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Contract no.: BI6-E-204-I

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

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Title of the research contract:

Development of biochemical and immunological assays for DNA recombination and repair

List of projects:

1. Purification and characterization of the eukaryotic analogs of recA protein.
2. Development of immunological reagents and analysis of the induction of the recA analogs following DNA damaging treatments.
3. Cloning of the genes encoding the analogs of recA protein in yeast and mammalian cells.
4. Development of a general method for the identification of proteins involved in DNA recombination and repair.

Title of the project no.:

1. Purification and characterization of the eukaryotic analogs of recA protein.
2. Development of immunological reagents and the analysis of the induction of the recA analogs following DNA damaging treatments.
3. Cloning of the genes encoding the analogs of recA protein in yeast and mammalian cells.

Head(s) of project:

Dr. Marco Bianchi
Dr. Giampiero Sironi

Scientific staff:

Dr. Marco Bianchi
Dr. Giovanna Lucchini
Dr. Lucia Panzeri

I. Objectives of the project:

The identification of DNA recombination proteins in yeast and mammalian cells. The development of suitable assays to determine their expression levels under various physiological conditions, and following irradiation or exposure to DNA-damaging agents.

II. Objectives for the reporting period:

The characterization of a yeast protein that cross-reacts with affinity-purified antibodies against recA protein of E. coli.

III. Progress achieved:

METHODOLOGY

Damage to DNA can result in cell death, mutagenesis and cancerogenesis, or complete recovery of cell functions following repair of the lesions. A large fraction of DNA damages gets handled and corrected through recombination: in this process, the information on one parental molecule is used to restore the information lost on the damaged homologous double helix. In addition, several of the enzymes and DNA structures involved in other types of DNA repair are also common to recombination pathways. Although the biochemical mechanisms of recombination are fairly well known in *Escherichia coli*, comparable detailed information is lacking for eukaryotic cells.

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 moderate length, leaving limited scope for deviation from an optimized structure. This argues in favour of an evolutionary conservation across phylogenetic barriers. We have examined the possibility of detecting analogs of the prokaryotic recA protein in eukaryotes using polyclonal antibodies as probes.

A single protein with strong immunological cross-reactivity with recA protein was detected by Western blotting in extracts of the yeast *Saccharomyces cerevisiae*. This protein was shown by immunological methods to be induced by irradiation with UV light and X-rays, to be induced during meiosis in concomitance with DNA synthesis, and to be localized in the cell nucleus. The protein was partially purified and was biochemically tested for recombination activities. Finally, a DNA clone encoding the protein was selected by immunoscreening and sequenced.

RESULTS

Antibodies were raised in rabbits against homogeneous recA protein, and purified by affinity chromatography on immobilized recA. The affinity purified antibodies detected a single band of 39 kD in Western blots of recA$^+$ *E.coli* strains; no band was detected in extracts of a strain with a deletion encompassing the recA gene. In Western blots of total cell extracts of the yeast *S. cerevisiae,* the antibodies reacted strongly with a single protein of about 43 kDal, which we code-named p43. We determined that p43 is present in all strains of *S. cerevisiae*, whether haploid or diploid. The intracellular concentration of p43 increases transiently following UV or X-ray irradiation, as is the case for recA protein. Maximal induction (about fivefold above basal level) is reached 3 hours after irradiation of diploid cells with a UV dose of 50 Jm^{-2}. By immunochemical methods, we also determined that the expression of p43 increases during meiosis, attaining a peak of about threefold over basal level slightly after premeiotic DNA synthesis. Both in irradiated cells and in cells undergoing meiosis, the protein is localized in cell nucleus.

Since the expression pattern and cellular localization of p43 matched with those expected for a recombinase, we undertook its biochemical characterization. We partially 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. A highly enriched preparation of p43 was obtained by conventional chromatographic methods, and was tested for DNA-dependent ATPase activity and strand transfer capacity. Neither of these activities were associated with the protein. Recombination-promoting activities of the protein were tested in an assay where the production of recombinant bla$^+$ plasmids is brought about by a yeast whole-cell extract acting on two different bla$^-$ plasmids; the two substrate plasmids carry a mutation in different positions of the *bla* gene, conferring resistance to ampicillin. Depletion or enrichment of p43 in the yeast extract had no effect on its ability to carry out recombination.

Concurrently, we had tried to clone the gene coding for p43. We screened with the anti-recA antibodies a library of yeast genomic DNA in the expression vector lambda gt11. 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). Comparison of the sequence data from the different labs indicated that also our clone for p43 contained the same gene, *rnr2*. 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 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. Also in collaboration with Dr. Johnston, we were able to show that the transcription of other yeast genes involved in the biosynthesis of DNA is responsive to DNA damage caused by UV light. The concentration of the 5.2 kb transcript for DNA polymerase I 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 other DNA polymerases may also play an essential role. In addition, it is likely that the elements controlling transcription in response to DNA damage may be common to a number of genes involved in DNA metabolism.

DISCUSSION

Using affinity-purified anti-recA antibodies, we identified a yeast protein which displayed several of the properties expected for a recombinase: (i) induction after UV irradiation (ii) increased expression in concomitance with recombination at meiosis (iii) nuclear localization. The protein was partially purified, but no activities that could be associated with a function in recombination were detected. The analysis of the sequence of the corresponding gene indicated that the protein showing immunological crossreactivity to recA is the small subunit of ribonucleotide reductase. The epitope recognized by the anti-recA antibodies was localized to the four C-terminal amino acids of recA and ribonucleotide reductase. This epitope (-Asn-Glu-Asp-Phe-COOH), thanks to its extremely limited size, C-terminal localization and strong immunoreactivity, could find an application as a convenient tag to be appended to various proteins.

We also showed that ribonucleotide reductase is not alone in responding to UV irradiation: to the contratry, this characteristic may be shared by most proteins involved in DNA biosynthesis. In general, both the immunological crossreactivity and the pattern of expression after UV irradiation were shown to be poor correlates with the biochemical activities of enzymes directly involved

in DNA recombination and repair. Consequently, we concentrated increased attention to the development of more reliable general methods for the identification of recombination proteins (see project number 4).

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

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V. Publications:

1. Publications in scientific journals

Johnston L.H., White J.H.M., Johnson A.L., Lucchini G. and Plevani P.
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 (1987).

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 (1987)
Dr. Monica Beltrame (1988-1989)

I. Objectives of the project:

Development of methods based on DNA-protein interactions to identify proteins involved in DNA recombination and repair.
The identification of rat liver proteins that recognize cruciform DNA (Holliday junctions).

II. Objectives for the reporting period:

III Progress achieved:

METHODOLOGY

Damage to DNA can result in cell death, mutagenesis and cancerogenesis, or complete recovery of cell functions following repair of the lesions. A large fraction of DNA damages gets handled and corrected through recombination: in this process, the information on one parental molecule is used to restore the information lost on the damaged homologous double helix. Although the biochemical mechanisms of recombination are not well known in eukaryotic cells, it is to be expected that the highly peculiar DNA structures that have been shown to be involved in recombination processes in *Escherichia coli* will also be recombination intermediates in mammalian cells. Some of these structures, for example Z-DNA, cruciform DNA and triple helices, can be produced in large quantities by utilising chemically synthesized oligonucleotides, or plasmids carrying appropriate sequences. These DNA molecules can then be used as probes to identify proteins that interact specifically with them, and therefore are likely to be involved in recombination processes.

We have investigated two model systems (i: interaction of recA protein with Z-DNA and with triple helices), and have undertook a far reaching investigation on mammalian proteins capable of inteacting with cruciform DNA, the structure adopted by Holliday junction in recombination (ii).

RESULTS

(i) interaction of recA protein with Z-DNA and with triple helices

Holloman and co-workers have shown that the protein rec1, a recA analog from the fungus *Ustilago maydis*, can bind with high affinity to Z-DNA, presumably because Z-DNA is an intermediate formed in the enzymatic reaction catalysed by rec1 (Kmiec et al., Cell 40, 139-145). In collaboration with Dr. Alfred Nordheim (ZMBH, Heidelberg), we have investigated whether binding to Z-DNA is a general property of recombination proteins, and therefore can be used as an assay for their purification. We studied as test case the properties of recA, the prototypical recombination protein.

An alternating GC sequence, capable of forming Z-DNA under the effect of supercoiling, was cloned in pBR322. A fragment of approximately 400 bp, which contains the GC sequence, is cut from the plasmid, labeled with ^{32}P and religated in vitro in the presence of variable amounts of ethidium bromide. The extraction of the ethium bromide after the ligation generates miniplasmids containing a

limited, quantized number of superhelical turns; each topoisomer has a distinctive electrophoretic mobility and is easily distinguishable. Topoisomers with 0 or -1 superhelical turns do not contain Z-DNA, topoisomers with -2 or -3 superhelical turns contain Z-DNA.

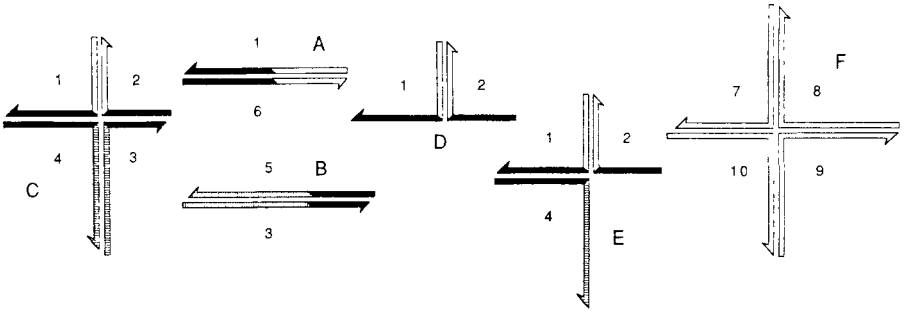
When exposed to a monoclonal antibody which recognizes Z-DNA, topoisomers -2 and -3 form protein-DNA complexes with a different electrophoretic mobility, topoisomers 0 and -1 are unaffected. We tested whether recA formed complexes with topoisomers -2 and -3, but failed to detect any. This result is at variance with a presumed general ability of recombinases to interact with Z-DNA, and can be explained in two ways. One possibility is that Z-DNA binding is a peculiar property of rec1, not shared by recA. The other possibility is that rec1 and all other recombinases do not recognize Z-DNA per se, but some other structural feature present in the Z-DNA molecules that were used in previous studies, such as bromine substituents or small regions of denatured DNA. In any event, binding to Z-DNA does not look like a viable assay for eukaryotic recombinases.

We also investigated whether recA can bind to triple-stranded DNA formed transiently during recombination. Reporter molecules containing triple-stranded DNA have been constructed as described by Francois et al. (Nucleic Acids Res. 16, 11431-1440). Preliminary results indicate that recA can bind to one such structure, but further verification with more triplexes and control DNA molecules is needed.

(ii) identification of rat liver proteins that recognize cruciform DNA (Holliday junctions)

The Holliday junction is the key intermediate in genetic recombination. Since it is formed by two interacting duplex DNA molecules, it comprises four DNA strands, that are arranged in a peculiar cruciform configuration. Symmetric cruciform DNA structures, as those formed during recombination, are inherently unstable. However, semi-stable cruciforms can be formed by palindromic sequences under the effect of supercoiling, and stable cruciforms can be constructed with oligonucleotides of appropriate sequence complementarity.

We produced artificial Holliday junctions by annealing four chemically synthesized oligonucleotides in four-way branched DNAs (molecules C and F, see figure). The artificial junction C was labeled with ^{32}P and used as probe in gel retardation experiments. We also built control double-stranded molecules (A and B) that contain the same DNA sequences present in the cruciform molecule, but are linear rather than cruciform, and other control molecules (D and E).



A nuclear extract obtained from rat liver was tested for binding to the labeled Holliday junction C 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 was competed by cold cruciform DNAs C and F, 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 of type D (see figure), while it binds weakly to molecules of type E, which resemble more closely complete Holliday junctions. The rat liver cruciform binding protein could also bind to cruciform structures formed by palindromes in supercoiled natural plasmids like ColE1 or pPS11.

The cruciform binding protein was purified to physical homogeneity by affinity chromatography on cruciform molecules (F) immobilized onto a sepharose matrix. We obtained two polypeptides of 23 and 21 kD that showed strong cruciform-binding activity. The smaller polypeptide appeared to be a degradation product of the larger one. The larger polypeptide was partially hydrolysed with trypsin, and four of the resulting oligopeptides were sequenced with a gas-phase sequenator. Each of the four sequences corresponded to stretches of the previously determined sequence of rat High Mobility Group 1 protein (HMG1). To confirm this identification, we established that antibodies raised against HMG1 protein reacted with the protein contained in the complex with cruciform DNA, resolved by electrophoresis. Finally, we transcribed and translated in vitro the cDNA clone for rat HMG1, and verified that the protein thus synthesized did bind to cruciform DNA, but not to the linear control duplex DNAs.

HMG1 has no detectable enzymatic activity: its function could then be similar to that of sequence-specific DNA binding proteins, which have no activity by themselves but modify the activity of other enzymes (for example RNA polymerases). To test this hypothesis, we tried to identify possible protein:DNA and protein:protein interaction domains in HMG1. Proteins truncated at their N-terminal or C-terminal end were translated in vitro and tested for their binding to cruciform DNA. The somewhat surprising result is that HMG1 has three domains (A, B, C), and that the DNA binding site is actually duplicated. Domains A and B are both slightly basic, and have similar sequences (29% identity at the amino acid level). Both of them can bind independently to cruciform DNA, but with reduced structural selectivity. The third domain, C, is composed of an uninterrupted stretch of 30 Glu and Asp residues, and is not required for DNA binding. To test whether it represents the putative protein:protein domain, we plan to immobilize large amounts of HMG1 on a solid matrix and try to isolate interacting proteins by affinity chromatography. To this end, HMG1 has now been cloned in an expression system in *E.coli* and overproduced.

DISCUSSION

Our results provide the first demonstration of specific binding to cruciform DNA (Holliday junctions) 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 of unrelated sequence.

Only one class of proteins specific towards cruciform DNA had been characterized so far: all of them are endonucleases, which 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, and represents a whole new class of DNA binding proteins. Other proteins of the same specificity can be identified and cloned by the method we have developed.

We have demonstrated conclusively that the rat protein which binds specifically to cruciform DNA is HMG1. HMG1-like proteins are present in the nuclei of all eukaryotes and are apparently essential for cell viability, but their function has not been identified unequivocally. Our results imply that whenever DNA adopts a cruciform conformation, the high concentration of HMG1 in the

nucleus can drive the equilibrium towards the formation of a protein-DNA complex. The physiological significance of HMG1 binding to cruciform DNA is at present unknown, but the domain structure of the protein suggests that other enzymes will be recruited by HMG1 bound to Holliday junctions or palindromic DNA. Profounds effects on recombination, replication and transcription are expected.

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

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V Publications

1. Publications in scientific journals

Bianchi M.E.
Interaction of a protein from rat liver nuclei with cruciform DNA.
EMBO J. **7**, 843-849 (1988).

Bianchi M.E., Beltrame M. and Paonessa G.
Specific recognition of cruciform DNA by HMG1 protein.
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2. Short communications and theses

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Erasmus University Rotterdam
Dept. of Cell Biology and Genetics
P.O. Box 1738
NL-3000 DR Rotterdam

Contract no.: B16-E-141-NL

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

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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:

Prof.Dr. D. Bootsma, Dr. J.H.J Hoeijmakers, Dr. A. Westerveld.

Scientific staff.

Dr. M. van Duin, Dr. N.G.J. Jaspers, Dr. W.J. Kleijer, Drs. M. Koken, Drs C. Troelstra, H Odijk, J. de Wit, W. Keijzer, W. Vermeulen.

I Objectives of the project

1) The isolation, collection 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, or by evolutionary walking procedures. Functional characterization of the isolated genes.
3) Comparison of DNA repair genes in different species by
   a) introduction of E.coli, yeast and Drosophila repair genes into mammalian cells and
   b) analysis of homology at DNA and protein level between different organisms, based on sequence conservation during evolution.

II. Objectives for the reporting period.

1. 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 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 Schizosaccharomyces pombe, Drosophila and human 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 79 patients and characteristic biochemical or cytogenetical abnormalities were demonstrated for xeroderma pigmentosum (12 cases), ataxia telangiectasia (23 cases), trichothiodystrophy (1 case), the Nijmegen breakage syndrome (1 case), Fanconi's anemia (4 cases). Prenatal diagnostic tests using chorionic villi and/or amniotic fluid cells were performed in pregnancies at risk of xeroderma pigmentosum, ataxia telangiectasia and Cockayne syndrome (total of 10 cases).

For a genetic survey by complementation analysis the collection of ionizing-radiation sensitive human mutant cells was further expanded (in collaboration with Dr. R.A. Gatti, University of California, Los Angeles). 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. On the basis of these results the localization on human chromosome 11q22-23 could be established for the ataxia telangiectasia gene from complementation group AB. This is the first chromosomal assignment of a human inherited disease with hypersensitivity to radiation.

A permanent cell line (XP44RO(MEL)) was established from a metastatic melanoma from an XP-C patient. As far as we know this is the first established line derived from an XP-tumor. The tumorigenic properties were related to an N-ras oncogene, activated in codon 61 by a T $\longrightarrow$ A or G transversion at a potential site for dimer formation. This cell line should prove useful for studying the mechanism of excision repair, and the relationship between the XP defect and carcinogenesis.

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 respects to DNA synthesis inhibition after X-irradiation.

2. Isolation of repair genes by DNA transfection.

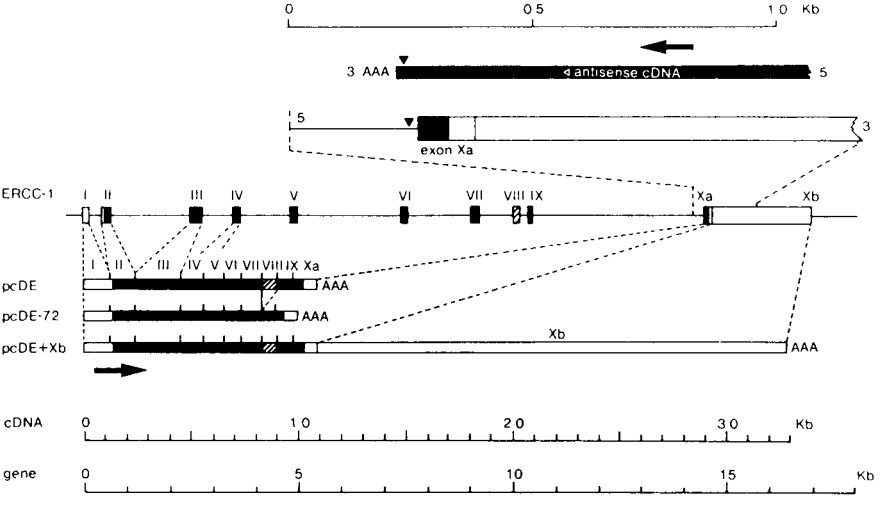
It is our main interest to try to unravel the molecular intricacies of the excision pathway in humans, its involvement in (the prevention of) carcinogenesis and the primary defect in XP. To that aim we have primarily concentrated on the isolation of genes controlling nucleotide excision. ERCC-1 was the first of a series of such genes, that we have isolated.

a. The human excision repair gene ERCC-1

ERCC-1 (excision repair cross complementing rodent repair deficiency) corrects excision deficient Chinese Hamster Ovary (CHO) mutants of complementation group 1. Mutants of this group are very sensitive to UV-light, carcinogens causing bulky adducts and even more

to cross-linking agents such as mitomycin-C. Transfection of the ERCC-1 gene fully compensates for the wide spectrum of impaired repair functions of the mutant including sensitivity to DNA damaging agents, incision of damaged DNA, lesion removal, repair synthesis, damage induced mutagenesis and chromosomal aberrations and preferential repair of the transcribed strand of active genes. Furthermore, ERCC-1 corrects only mutants of complementation group 1 and not of the other groups. This indicates that ERCC-1 represents the human counterpart of the mutated gene in group 1 mutants. We have investigated whether ERCC-1 is coincidently also involved in the human repair syndromes XP, and Cockayne's Syndrome (CS). From these experiments (which include Southern and Northern blot analysis of the ERCC-1 gene in representative cell lines of each complementation group, as well as transfection or microinjection of the cloned ERCC-1 gene or cDNA into the same cells) we conclude that ERCC-1 is very likely not (yet) encountered as a mutated gene in the in total 10 complementation groups of XP and CS patients.

The molecular architecture of the ERCC-1 gene and its expression have been elucidated in great detail and is summarized in Figure 1.



Legend to Figure 1:
Architecture of the ERCC-1 locus.

Lower part: ERCC-1 transcripts pcDE, pcDE-72 (lacking exon VIII) and pcDE+Xb (alternatively polyadenylated). Upper part: enlargement of the ERCC-1 exon X region and position of the partial ASE-1 antisense cDNA. Filled boxes: coding (parts of) exons; open boxes: non-coding (part of) exons; hatched box: the alternatively spliced exon VIII. Filled triangle: polyadenylation signal of the ASE-1 antisense RNAs. Arrows indicate the 5' to 3' direction. Note the difference in scale for the gene and transcripts.

The gene spans a region of 15-17 kb on chromosome 19q13.2 and is composed of 10 exons. ERCC-1 transcripts are found at a low, basal level in all mouse tissues and stages of embryogenesis analyzed, and its expression does not seem to be induced upon UV-irradiation in HeLa cells.

The 1.1 kb ERCC-1 mRNA encodes a protein of 297 amino acids. Comparison with consensus sequences of functional protein domains has pointed to the presence of a potential nuclear location signal (NLS) and a 'helix-turn-helix' DNA binding motive. Antibodies raised against an oligopeptide with the amino acid sequence of the putative 'ERCC-1-NLS' specifically react with nuclear proteins in immuno-fluorescence preparations of fixed human fibroblasts, consistent with the idea that this region specifies a NLS also present in other nuclear proteins. However, definite proof for the existence of this and other postulated domains awaits verification at the protein level.

Computer comparison of the ERCC-1 amino acid sequence with known repair proteins of lower organisms revealed striking homology with the predicted amino acid sequence of the yeast excision repair protein RAD10. This finding suggests that ERCC-1 and RAD10 are descendants of the same ancestral gene and, hence, have analogous functions. The only, major difference between the two proteins is the fact that ERCC-1 is longer than RAD10. At the position where the homology with RAD10 stops a stretch of amino acids begins, with significant similarity with part of the E.coli excision repair protein uvrA. Intriguingly, at the point where this homology terminates again another region of similarity turns up: this time between the carboxyl terminus of ERCC-1 and that of the E.coli uvrC protein. Therefore, it appears that ERCC-1 encodes a mosaic polypeptide composed by reshuffling of domains present in different repair proteins of lower organisms. Mutation studies have shown that the C-terminal 'extension' of ERCC-1 -not found in RAD10- is essential for its function.

From detailed analysis of the 3' region of the ERCC-1 gene it became apparent that this part of ERCC-1 is used at the same time by another gene (ASE-1). Sequence analysis of partial cDNA clones and hybridization with strand specific probes showed that this gene is transcribed from the opposite strand and that the 3' terminus of its 2.6 kb mRNA overlaps with exon 10 and terminates within intron 9 of ERCC-1 (see Figure 1). Also the mouse ERCC-1 locus appears to harbour such an antisense gene. Independently, Prakash and coworkers (Rochester) discovered that the yeast RAD10 gene region too contains a 3' overlapping, antisense gene. The occurrence of this type of gene configuration is rare. To our knowledge ERCC-1/ASE-1 represents the first case in the human genome. The evolutionary conservation of this exceptional type of gene configuration between the human ERCC-1 and its yeast cognate RAD10 strongly suggests that it has an important biological function. At present this function is unknown.

b. Isolation and characterization of the ERCC-3 gene (in collaboration with G. Weeda and A.J. van der Eb, Leiden University).

In addition to ERCC-1 we have isolated also the human excision repair genes ERCC-3. The ERCC-3 gene which has been mainly characterized in the laboratory of Molecular Carcinogenesis (State University Leiden, Prof. A.J. van der Eb) is approximately 40 kb and located on chromosome 2q2.1. It corrects specifically the excision repair defect of the rodent mutants of complementation group 3. The predicted 782 amino acid ERCC-3 protein contains putative domains for nucleotide, DNA

and chromatin binding as well as a nuclear location signal. Its amino acid sequence bears no significant homology to known repair genes of yeast and E.coli. The functional cDNA was transfected into the XP groups of which suitable SV40 transformed cell lines are available (XP-A, C-G). No correction was found. For the remaining groups microinjection of the functional ERCC-3 cDNA was performed in the nucleus of primary fibroblasts and the level of UDS was determined to monitor the effect on the repair defect. No increase of UDS was registered after injection into cells of XP-D, G and H. However, a clear restoration of UDS was observed after injection into fibroblasts of the sole patient comprising XP-B, which in addition to the clinical hallmarks of XP also exhibited the symptoms of Cockayne Syndrome. Presently, the nucleotide sequence of the XP-B ERCC-3 mRNA is determined to pinpoint the expected (point)mutation. These findings indicate for the first time overlap between the rodent and human excision deficient mutants.

c. Isolation and characterization of the ERCC-6 gene.

The ERCC-6 gene was isolated by DNA mediated gene transfer to a UV-sensitive mutant of CHO complementation group 6. The preliminary characterized gene appears to have a - for transfection cloning respectable - size of >100 kb, is located on chromosome 10q1 1 and encodes 2 lowly expressed mRNAs of 6.5 and 8.5 kb.

3. Isolation of repair genes by sequence homology.

The basic assumption for this part of the project is that at least a number of genes involved in DNA repair processes is strongly conserved in evolution. This was based on our initial observation that the human ERCC-1 and yeast RAD10 excision repair proteins display significant amino acid sequence homology. Further evidence, supporting this hypothesis came from the analysis of the ERCC-3 gene.

a. Isolation of the yeast homolog of ERCC-3.

The first indication that the ERCC-3 gene was also strongly conserved in evolution came from ZOO-blot analysis, which revealed cross-hybridizing sequences in all vertebrates and even in Drosophila DNA under stringent hybridization conditions. Using a strategy involving identification of "junction" clones and defined hybridization conditions we were able to isolate cross-hybridizing genomic DNA clones from S.cerevisiae, Ss.pombe and Drosophila melanogaster. Partial sequence analysis confirmed that in all cases the sequences were derived from the ERCC-3 homologs in the various species and revealed 50-75% sequence identity to the human protein. The cloning of the ERCC-3 versions of lower species provides valuable information about the evolution of this gene and opens the possibility to utilize the specific advantages of the Drosophila and yeast systems to define the ERCC-3 function.

In terms of sequence evolution of repair genes these data demonstrate that nucleotide sequence homology of at least a number of repair genes is sufficiently high to cover the evolutionary distance from man to yeast in one step. As expected, our plans to jump in the reverse direction proved to be more difficult, but not impossible, as shown below.

b. Isolation of human genes by sequence homology to cloned yeast repair genes.

A number of genes from the yeast (S.cerevisiae) RAD3 epistatis group (generously provided by Dr. S. and L. Prakash, Rochester) were tested for their degree of sequence conservation as determined by hybridi-zation under varying conditions of stringency to DNA from evolutionary distant organisms such as Ss.pombe, D.melanogaster, D.hydei. The RAD2 and RAD7 probes did not yield specific hybridizing fragments on Southern blots nor clones on library filters. For RAD23 we found recently weakly hybridizing 'junction' bands in Drosophila DNA, which suggests they are derived from a homologous Drosophila gene The RAD1 gene cloned in our laboratory by A. Yasui gave many and some very clear cross-hybridizing bands in Drosophila DNA digests. The most prominently hybridizing sequences were cloned first, but sequence analyses revealed that they were derived from genes which shared only homology limited to specific domains in the RAD1 protein. However, when these sequences, which apparently occur frequently elsewhere in the Drosophila genome, were omitted from the probe we were able to identify Drosophila genomic clones which exhibit hybridization on more than one position in the insert with the yeast RAD1 probe. Sequence analysis of these very promising clones is in progress.

The yeast phr-1 gene appeared to be sufficiently conserved to visualize homologous sequences in many different yeast species but did not display specific hybridization in Drosophila.

We initiated also experiments using the yeast RAD6 gene. Rad6 mutants are characterized by a high degree of sensitivity to a variety of damaging agents. These include UV, 4NQO, ϑ-rays, alkylating and cross-linking agents. The pleiotropic phenotype of rad6 mutants shows deficiencies in post replication repair and damage induced mutagenesis. The RAD6 gene, cloned by Prakash and coworkers encodes a globular protein with an extended, acidic c-terminus. Jentsch et al. 1987 did the surprising discovery that the RAD6 protein is in fact a ubiquitin conjugating enzyme specific for histones 2A and 2B. This suggests that RAD6 is involved in chromatin remodelling necessary for repair and recombination The RAD6 probe corresponding with the globular protein part yielded specific hybridization to Ss.pombe and Drosophila DNA. Subsequently, the hybridizing fragments were cloned from a Ss.pombe library and sequence analysis verified the RAD6 identity of the clones. The Ss.pombe protein shares 76% sequence identity with the S.cerevisiae cognate including the cysteine residue used for ubiquitin attachment, but completely lacked the acidic tail. By gene disruption Prakash and coworkers have generated a 'RAD6' Ss.pombe mutant, which is at present being characterized. The Ss pombe and S.cerevisiae probes were used in turn to identify the Drosophila RAD6 gene, which exhibited 67% and 65% amino acid sequence homology to the Ss.pombe and S.cerevisiae genes, respectively. Finally, mainly with the aid of the probe of Drosophila, which is evolutionary more related to man than either of the two yeast species, we succeeded in cloning the human RAD6 cDNA. Sequence analysis of the central, most conserved part of the gene showed that it is 90% identical to the Drosophila gene. Since RAD6 mutants are impaired in the process of post replication repair thought to be involved in XP-variant patients it will be of interest to examine the functioning of this gene in cells of these patients.

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

- the Department of Molecular Carcinogenesis, University of Leiden (Prof.Dr. A.J. van der Eb)
- the Department of Molecular Genetics, University of Leiden (Prof.Dr. P. v.d Putte)
- the Department of Radiation Genetics and Environmental Mutagenesis, University of Leiden (Prof.Dr. P.H.M. Lohman, Dr. A.A. van Zeeland and Dr. J.W.I.M. Simons)
- the Medical Biological Laboratory TNO, Rijswijk (dr. G.P. v.d. Schans).
- the MRC Cell Mutation Unit, University of Sussec, England (Prof.Dr. B.A. Bridges)

V. Publications:

1. Publications in scientific journals, monographs, etc.

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Thesis, Erasmus University Rotterdam 1988.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Medical Research Council
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Contract no . BI6-E-142-UK

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

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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 A.M. Carr (from Mar 1989)
Dr J. Cole
Dr S.W. Dean (to Feb 1989)
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:

In order to understand the response of human cells to radiation damage, it is logical to follow the classical approach, which has been outstandingly successful in micro-organisms, of identifying mutant cell lines with an altered response to radiation, determining the nature of the aberrant response at the cellular level and ultimately cloning the normal gene defective in the radiation-sensitive mutants. Our approach has been to collect mutants represented in the human population in patients with certain genetic disorders and to characterise the nature of the molecular defect in these human mutants. Below we describe our increased collection of mutants, the cellular characterisation of a new radiosensitive individual, and of a new DNA repair disease trichothiodystrophy. We also describe methods of increased sensitivity for detecting radiosensitivity at the cellular level and finally we present our data on the cloning of DNA repair genes, making use of fission yeast as a stepping-stone to cloning human DNA repair genes.

III. Progress achieved:

Methodology

(1) Response of human cells to radiation

Procedures for the measurement of cell survival of both fibroblasts and T-lymphocytes and many parameters of DNA repair have been established and used in this laboratory for many years. The methodology for measuring clastogenic damage using the micronucleus assay has been developed and refined. Improvements have included the use of cytochalasin B to discriminate between cells in the first and subsequent cycles following irradiation, and employment of the Crest antibody to distinguish between acentric micronuclei and those containing centromeres.

(2) Immortalisation of human fibroblasts

Cells were transfected with the large T-antigen-containing plasmids pSV3*neo* or pSV3*gpt* followed by selection for the dominant marker (*neo* or *gpt*) to remove the non-transfected population. The resulting transformed cells were subcultured until they senesced and entered "crisis". The cells were then treated with great care during the crisis period, until a rapidly growing immortal line grew out.

(3) Gene transfer

Immortalised human fibroblasts were transfected using the calcium phosphate precipitation procedure. Various combinations of plasmids and human DNAs were transfected into the recipient cells and selection was applied for the dominant marker. Transfected clones were expanded and the structures of the transfecting DNA in the recipient cells were analysed by Southern blotting and hybridisation techniques.

(4) Cloning of S.pombe repair genes

The *leu1.32*$^{-}$ genetic marker was crossed into various *Schizosaccharomyces pombe* repair-deficient mutants. The *rad4* and *rad9* mutants were transformed with an *S.pombe* genomic library constructed in a plasmid containing the *Saccharomyces cerevisiae leu2* marker. This was followed by selection first for the *leu2* marker, then for temperature-resistance (*rad4*) or radiation-resistance (*rad9*). Plasmids were recovered from the resistant yeast transformants and transferred into *E.coli* where they were analysed using standard molecular biological techniques.

Results

1. Cell culture collection

Over the period of the contract our culture collection has been expanded with the cell strains shown in Table 1. Ninety one primary fibroblast cultures have been initiated from skin biopsies, 80 fibroblast cell cultures have been obtained from other laboratories, 9 amniocyte cultures and 3 villus cell cultures have been accessed to our collection. Five SV40-transformed fibroblast cultures have also been accessed. Much of this material has been sent to us for cellular diagnosis and some remains unclassified. The overall distribution of types is summarised in Table 1.

Mononuclear cells have been separated from some 350 human blood samples during the period under review and some of these cultures have been used to study the radiation response of T-lymphocytes. Our collection is recognised internationally as an important resource. During the contracting period a total of over 300 fibroblast cultures were dispatched to Europe, the USA and Japan.

2. Immortalisation of human fibroblasts

Primary human fibroblasts provide an excellent source of material for genetic and biochemical characterisation of DNA-repair-deficient mutants, but their limited lifespan in culture renders them inadequate for gene cloning experiments, for enzymological studies and for detailed analysis of mutagenesis. Some years ago we initiated a programme to immortalise some of our more relevant cell strains. A list of cultures immortalised by our procedure is shown in Table 2.

Table 1. Fibroblast cultures acquired during the reporting period.

Type		*Type*	
Normal	13	Unspecified "sunsensitives"	4
Xeroderma pigmentosum (XP)		Ataxia-telangiectasia (A-T)	14
(i) Excision defective	3	A-T heterozygotes	6
(ii) Variant	5	"Radiation sensitives"	3
(iii) Heterozygotes	2	Multiple tumours	2
(iv) Putative XP	18	Gorlin's syndrome	4
Cockayne's syndrome (CS)	8	Hypogammaglobulinaemia	1
CS heterozygotes	4	Poikiloderma	1
Putative CS	18	Porokeratosis of Mibelli	2
Bloom's syndrome	5	Cataracts	26
Trichothiodystrophy (TTD)	9	Miscellaneous	25
TTD heterozygotes	9		
Putative TTD	2		186
Dyskeratosis congenita	2		

Table 2. Immortalised cultures.

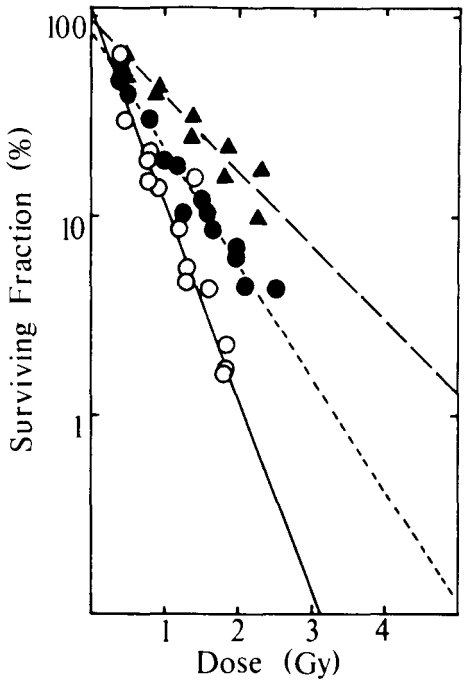
Culture	*DNA/Selection*	*Comment*
1BR.3	gpt	Normal
1BR.3	gpt/neo	"
1BR.3	neo	"
48BR	neo	"
142BR	neo	"
AT4BI	gpt	A-T
ATHM4BI	neo	Mother of AT4BI
AT1BR	gpt	A-T
108BR	gpt	Parent of AT1BR
109BR	gpt	" "
TTD2GL	gpt	TTD (type 2)
TTD1BI	gpt	TTD (type 3)
CS1AN	gpt	CS
CS3BE	gpt	CS
46BR	gpt	Bloom's variant

a Effect of transformation and immortalisation on the radiation response

In our previous programme, we reported that immortalised cells had increased resistance to the lethal effects of gamma-radiation. This was observed both for a normal cell line and a radiation-sensitive A-T line, so that the differential between these lines was maintained. We have now shown that this increased resistance on immortalisation is a general phenomenon, observed in all immortalised cells tested to date (Arlett et al 1988).

The immortalisation procedure involves two clearly defined steps. Initially, following transfection with the pSV3 plasmid containing the T-antigen gene, the transfected cells in the culture become morphologically transformed (step 1), forming foci, losing contact inhibition, adopting an epithelial-like structure and having an extended lifespan. At some point subsequently most of the cells senesce, but eventually (in step 2) an immortal line grows out of the senescent culture. We have addressed the question as to whether the increased radiation resistance occurs during the transformation step, or upon immortalisation, and our results clearly demonstrate that the resistance is associated with the transformation step (Fig. 1). This has been found in a total of 11 transformed cultures. Fig 1 shows our results for one ataxia-telangiectasia strain.

Fig. 1. Survival curves for the ataxia-telanglectasia primary fibroblast strain AT4BI and for its immortal and pre-crisis derivatives. o —— o primary cells; ● --- ● immortal cells; ▲ pre-crisis cells



(b) Transformation and the Mex phenotype

Following immortalisation of cells with SV40 T-antigen some cell lines lose the ability to repair O^6-methylguanine in their DNA (the Mex^- phenotype). Since we have Mex^- immortalised cells frozen down at various stages along the immortalisation pathway, we have determined the point at which the cells lose their Mex^+ phenotype. In contrast to the result with ionising radiation, we have found that there is no fixed stage at which methyltransferase activity is lost, and that in at least one instance the change in a bulk population was very abrupt. This investigation has been carried out in collaboration with Dr. P. Karran (ICRF) (Green *et al.*, 1990).

3. Inhibition of DNA replication by ionising radiation is mediated by a trans-acting factor

In project 2 we describe experiments utilising Epstein-Barr virus (EBV) vectors transfected into human cells. In this work we generated cell lines which contain extrachromosomal EBV-based vectors. With these cell lines we have been able to investigate the mechanism of the radiation-induced inhibition of DNA synthesis (which does not occur in A-T cells). Gamma-irradiation of a cell line containing such an extrachromosomal plasmid results in inhibition of the replication of both genomic and plasmid DNA. This inhibition is observed at doses of radiation which produce an insignificant amount of damage in the plasmid DNA molecules. Thus radiation-induced inhibition of DNA replication appears to be mediated by a *trans*-acting factor (Lamb *et al.*, 1989).

4. The identification of a radiosensitive individual with a novel cellular radiation response

We have identified an individual (180BR) - recently deceased - whose fibroblasts show comparable radiosensitivity to A-T cells. However the characteristic lack of inhibition of DNA synthesis following radiation treatment of A-T cells is not observed in 180BR cells. A detailed study of these cells is in hand

5. Radiation sensitivity 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 were more radiosensitive than 4 bladder-derived cell lines. The bladder cells had radiation sensitivities (D_0 = 1.82 Gy) similar to that of an SV40-transformed normal cell line (MRC5VI). The testicular cells were hypersensitive (D_0 = 1.36 Gy) but not as sensitive as an SV40-transformed A-T cell line (AT5BIVA, D_0 = 0.98 Gy). 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 (Parris et al. 1988)..

6. Radiation sensitivity of T lymphocytes

The rapidity with which estimates of cellular radiosensitivity may be made with T-lymphocytes (9-14 days) may not only have important implications for radiation protection but may also allow an assessment of an individual's radiation response prior to a course of radiotherapy. A substantial validation study is in progress to establish the reproducibility of the assay and any correlation with the radiation sensitivity of other cell types. The T-lymphocytes have been studied as a population of non-dividing cells separated from whole blood. Lymphocytes from 9 normal individuals gave a mean D_0 value of 1.55 Gy but unlike fibroblasts they appear to have a genuine but small shoulder component in the survival curve. Seven A-T patients gave a mean D_0 value of 0.73 Gy and the heterozygotes 1.32 Gy (Figure 2). Cells derived from cord blood (8 bloods, mean D_0 1.24Gy) appear to be more radiosensitive than from normal adult donors. In addition T-lymphocyte lines have been established from 9 normal individuals and 2 A-T patients. These cells are more radiosensitive than the Go T-lymphocytes and show a reduction in the extent of shoulder. The distinction between A-T and normals is maintained. (Cole et al. 1988).

Fig. 2. The gamma radiation survival curve for nine ataxia-telangiectasia heterozygote G_0 T-lymphocyte cultures. + ——— + individual points and best fit line for heterozygotes; — — — best fit line for data from eight normal G_0 T-lymphocyte cultures; ._._. best fit line for data from seven ataxia-telangiectasia G_0 T-lymphocyte cultures.

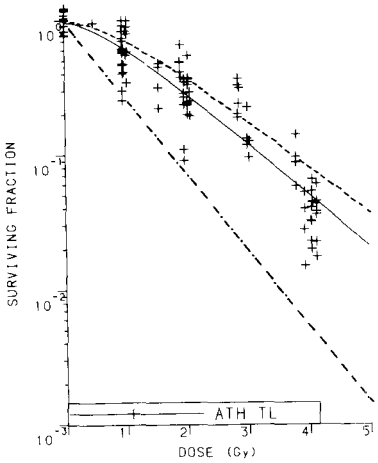
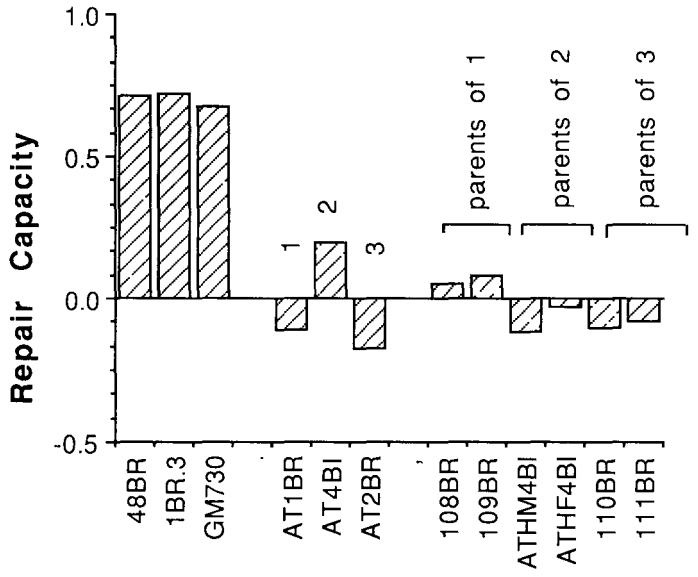


Fig 3. Repair of potentially clastogenic damage in three ataxia-telangiectasia families. Repair of potential damage is expressed as the ratio of micronuclei in dividing versus resting cells following ionising radiation minus one.



7. A-T heterozygotes

Ataxia-telangiectasia heterozygotes continue to be a subject of interest since there are substantial claims for an increased incidence of cancer, particularly early breast cancer, in such individuals, who may represent 5% of the population at large (Pippard *et al.*, 1988). In addition there are a number of reports (including our own) that their cells are also slightly more radiosensitive than normal individuals not heterozygous for the gene. We have examined the repair of potentially clastogenic damage in the heterozygotes by comparing the induction of micronuclei in cytochalasin B treated fibroblasts following gamma-irradiation of dividing or resting cells.

The results of such a study are summarised in Fig. 3. Here three A-T families (proband plus both parents) were investigated with a control. The results show that the heterozygotes behave like the probands rather than the control normals and are defective in the repair of potentially clastogenic damage.

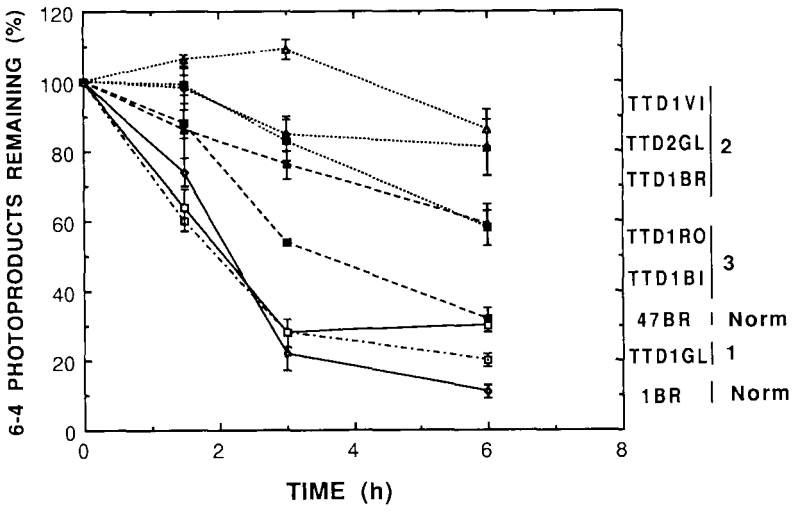
8. Trichothiodystrophy: a new repair-deficient disorder

Trichothiodystrophy is a genetic disorder characterised by sulphur-deficient brittle hair, physical and mental retardation and in some instances photosensitivity. In collaboration with Dr. M. Stefanini (Pavia) and Dr. D. Mitchell (Texas) we have carried out a detailed biochemical and genetic analysis of DNA repair and radiation sensitivity in seven patients (Lehmann et al., 1988; Broughton et al., 1990). Three categories have been identified: (1) Cells with a totally normal response to UV-irradiation; (2) Cells very defective in excision repair of UV-damage with properties indistinguishable from those of cells from patients with xeroderma pigmentosum. Cell fusion experiments indicate that these TTD cell strains are in the same complementation group as XP group D; (3) Cells with a normal response to the lethal and mutagenic effects of UV-irradiation, but nevertheless having a moderate deficiency

in excision-repair, which we have identified as a specific defect in the ability to remove (6-4) photoproducts from DNA (Fig. 4). In contrast these cells are proficient at removing cyclobutane dimers (Broughton et al., 1990).

The paradox that TTD cells in Category (2) and XP-D cells appear to have the same molecular defect but give rise to quite different clinical entities has important implications for the relationship between DNA repair and carcinogenesis, and has led us to propose a model which reevaluates some widely accepted tenets (Lehmann and Norris, 1989).

Fig. 4. Percentage of 6-4 photoproducts remaining in trichothiodystrophy fibroblasts.



9. Cloning of DNA repair genes

The difficulties experienced world-wide in some 10-20 laboratories in attempting to correct the defect in radiation sensitive mutant human fibroblasts, such as XP and A-T cells, led us to search for the underlying reason for the refractoriness of SV40-transformed human fibroblasts to DNA-mediated gene transfer. We have transfected eight of our SV40-transformed normal and repair-deficient cell lines with DNA containing two selectable markers separated by 0-50Kb of random human DNA sequences. If selection is applied for one marker but not for the other we have found in all cell lines that the selected marker is maintained at 1-4 copies per cell. In contrast the unselected marker is initially integrated into the genome of the recipient cell, but is subsequently lost, so that after one month's growth of the culture, on average only 0.1 copies remain per cell. The implication of these findings is that even though the transfection frequencies of these cell lines are high the amount of unselected DNA stably integrated into the genome of recipient cells is very small (<50Kb) (Mayne et al., 1988). This explains the poor success rate observed when using human cell lines as recipients in gene transfer experiments and contrasts with the dramatic success obtained in comparable experiments with rodent cell lines which can integrate and stably maintain several megabases of DNA. Our collaborators in Rotterdam have reached a similar conclusion using a somewhat different approach (Hoeijmakers et al., Expl.Cell Res., **169**, 111-

119, 1987).

We have evaluated, as an alternative transfection strategy, the use of vectors based on Epstein-Barr virus (EBV), which can be maintained extrachromosomally in human cells. These vectors circumvent the necessity to maintain large tracts of DNA stably integrated into the genome of the recipient cells. We found that although human cells were indeed able to maintain the EBV-based vectors extrachromosomally, in the majority of cases the human inserts in the vectors were deleted or underwent gross rearrangements (Dean et al., 1989).

We have therefore adopted a totally different approach using the fission yeast *Schizosaccharomyces pombe* as a tool for cloning human DNA repair genes. Two approaches are being adopted:

1. Many radiation-sensitive mutants of *S.pombe* were isolated in the 1970s. They fall into 22 different complementation groups. We have begun a programme to clone the genes which are deficient in these mutants by transfection of radiation-sensitive mutants with an *S.pombe* genomic library followed by selection for radiation resistance. Complementing plasmids are isolated from the resistant transfectants, and analysed in detail. We have cloned and sequenced the *rad4* and *rad9* genes. The *rad4* gene codes for a 579 amino acid neutral protein, which has a basic C-terminus. It is expressed at very low levels in *S.pombe*. The C-terminal 40 amino acids are not essential for conferring radiation resistance on *rad4* mutants. *Rad4* mutants are temperature-sensitive for growth, suggesting that the gene is essential in *S.pombe*.

The *rad9* gene codes for a slightly acidic 368 amino acid protein. From the gene sequence we predict the presence of two short introns. Like the *rad4* gene, it is expressed only at very low levels.

Following cloning of the *S.pombe* genes, we propose either to identify from computer searching of the data bases, or to isolate and sequence, the genes from *Saccharomyces cerevisiae* which are homologous to our *S.pombe* genes. Since *S.cerevisiae* and *S.pombe* are evolutionarily very distant, any conserved sequences are likely to be important for gene function and therefore they are also likely to be conserved in higher organisms. We therefore propose to design oligonucleotides for use as probes to identify homologous genes in human cells. Any gene that we clone and sequence will also be compared immediately with repair genes being cloned and sequenced using alternative techniques by our colleagues in Rotterdam and Leiden.

2. Our second approach involves attempting directly to clone human genes by their ability to complement the defects in *S.pombe* mutants. A human gene bank is transfected into the *S.pombe* mutants and resistant derivatives are isolated. Such an approach enabled Lee and Nurse to clone a human gene controlling the cell cycle.

Discussion

Our collection of human radiation-sensitive mutant cell strains provides the necessary starting material for understanding the response of humans to radiation. Only by dissection of the response using defective mutants and comparison with normal donors can we begin to understand the response of cells to radiation-induced damage. We have thus generated a large data base for human cellular radiosensitivity and during the period under review, we have identified an individual with a unique cellular radiation response. We have now extended the usefulness of some of our most important cell lines by immortalisation, thus providing a potentially inexhaustible amount of material from these cells. We discovered that during the transformation step of the immortalisation procedure the response of all cells to radiation was modulated, and in addition we have studied the loss of methyltransferase activity which occurs in some of these cultures. Our culture collection provides an internationally recognised resource not only for our own work but for that in many other laboratories both inside and outside the EC.

Complete understanding of the response of human cells to radiation will only be achieved when the genes controlling this response have been cloned and their gene products characterised. We have succeeded in cloning two such genes from the fission yeast, *S.pombe*, and we hope that this approach will lead us to the corresponding human genes.

The development of a procedure for estimating cellular radiosensitivity in T lymphocytes may provide a practical procedure for population monitoring for radiosensitivity, since with a small sample of blood, results can be obtained within two weeks. This work together with our measurements of micronuclei in fibroblasts continue to suggest that, in general, the cancer-prone A-T heterozygotes are radiation-sensitive and that they may therefore be identified as an at-risk population using a cellular test. Since these individuals may comprise up to 5% of the human population, the importance of identifying them cannot be overestimated in the context of radiation protection.

IV. 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.

V. Publications:

Are listed under Project 2

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) to evaluate the utility of three shuttle vector systems by the production and analysis of spontaneous and radiation-induced mutants in:

i) an integrated bacterial gene system (*gpt*) in normal human cells.

ii) the *lacZ* gene in Epstein-Barr virus shuttle vectors in normal and repair-deficient human cell lines.

(iii) the *supF* gene of the SV40-based shuttle vector pZ189 during passage through normal and repair-deficient human cell lines.

B) to exploit the human endogenous *hprt* gene mutation system based upon selection of 6-thioguanine (TG) resistance in both circulating human T-lymphocytes (using new culture methods) and SV40-transformed fibroblasts (using conventional methods). This requires:

(i) the development of techniques to select and expand in culture TG resistant and sensitive T-lymphocytes from blood samples from normal and repair-defective individuals.

(ii) molecular analysis of *hprt* mutants by measurement of HPRT enzyme activity and *hprt* mRNA activity, Southern analysis of the *hprt* gene and analysis of point mutations using the polymerase chain reaction.

(iii) molecular analysis of T-cell receptor (Ti) beta and gamma chain genes in mutant phenotypes.

Methodology

Shuttle vectors-integrated genes

The *hprt*$^-$ cell lines C10 or E2 containing a single copy of the *gpt* gene were plated in the presence of 6-thioguanine to generate Gpt$^-$ derivatives. The revertibility of these lines to Gpt$^+$ was assessed either following treatment with 5-azacytidine, or following growth for one month in the absence of selection. Revertants were identified by their ability to grow in the presence of mycophenolic acid.

Shuttle vectors in Epstein-Barr virus (EBV)-based plasmids

The plasmids p205z and p220z contained a part of the *lacZ* gene cloned into a vector containing the hygromycin-resistance gene, and the EBNA-1 and *oriP* regions of EBV. These sequences are necessary and sufficient for extrachromosomal maintenance of these plasmids. Human cells were transfected with the plasmids and plasmid-containing lines were selected with hygromycin. Extrachromosomal DNA was extracted from the cells by an alkaline lysis procedure, and used to transform highly competent *E.coli*. When plated on appropriate indicator plates, bacteria containing plasmids with a mutated *lacZ* gene gave white colonies against a background of wild-type blue colonies.

Shuttle vectors - extrachromosomal SV40-based plasmid pZ189

The plasmid pZ189 contains the bacterial *supF* gene cloned into an SV40-based vector. Following UV-irradiation of the plasmid (0 - 1000Jm^{-2}) it was transfected into human cells. Two days later replicated plasmid was recovered from the cells using the Hirt procedure and used to transform host bacteria in which a mutated *supF* gene could be detected as a white colony against a blue background. Plasmid DNA was extracted from mutant bacteria and analysed on agarose gels to detect large deletions, or sequenced to detect point mutations.

Mutations in the human hprt gene

(a) Lymphocytes. Mutant T-lymphocytes resistant to $5x10^{-6}$M 6-thioguanine (TG) were cloned directly from the mononuclear fraction of venous blood from donors. These clones were expanded and treated as for fibroblasts (see below).

(b) Fibroblasts. Cells were UV-irradiated and plated in $5x10^{-6}$ M TG, after different expression times. Resistant clones were expanded in the presence of TG.

DNA was extracted, digested with PstI and the structure of the *hprt* gene was analysed by Southern blotting to detect large deletions or rearrangements. For point mutations RNA was extracted and reverse-transcribed into cDNA. This was used as substrate for the polymerase chain reaction (PCR) using oligonucleotides at either end of the coding region of the *hprt* gene as primers. 25 cycles of PCR were carried out and the product run on low melting point agarose gel. The 750 bp *hprt* band was excised from the gel and a fraction of it used for a second round of 25 cycles of PCR. The final product was purified using Geneclean and sequenced directly using the Sanger procedure with 4 separate primers for different parts of the *hprt* gene.

In the case of the lymphocyte mutants Southern blots were also hybridised with T-cell receptor Beta and Gamma chain probes.

Results

Shuttle vector systems

(1) Use of an integrated gene (gpt).

We have isolated two cell lines (C10 and E2) containing single integrated copies of the bacterial *gpt* gene in an SV40-transformed human fibroblast line. Spontaneous Gpt$^-$ derivatives of these lines have been obtained and the state of the *gpt* gene has been analysed using molecular techniques (Table 1) (Gebara et al., 1987). In a subsequent series of experiments, we carried out a similar analysis of 41 UV-induced and 18 EMS-induced Gpt$^-$ derivatives. In many cases inactivation of the gene resulted from alterations of gene expression (see Table 2). This occurred either by methylation or by an ill-characterised phenomenon termed phenotypic switching (Lehmann et al., 1989a). Very few spontaneous or induced Gpt$^-$ derivatives resulted from point mutations. This integrated gene system seems particularly susceptible to carcinogen-induced epigenetic events.

Table 1. Alterations in Spontaneous Gpt⁻ Derivatives of E2 and C10.

	% of derivatives with alteration[a]	
	E2	C10
Deletion/rearrangement[b]	74	12
Methylation[c]	5	79
Phenotypic switching[d]	15	0
No detectable change	5	8

[a] A total of 21 Gpt^- derivatives of E2 and 24 derivatives of C10 were analysed.
[b] Alterations detected on Southern blots of EcoRI-digested DNA.
[c] Reversion of Gpt^- to Gpt^+ phenotype induced by 5-azacytidine.
[d] Reversion of Gpt^- to Gpt^+ phenotype resulting from 1 month's growth in the absence of selection.

Table 2. Revertibility of Gpt⁻ Derivatives.

	Spontaneous	UV-induced	EMS-induced
Non-revertible	2	2	0
Azacytidine-revertible	3	10	4
Switchable	1	0	2

The table shows the number of derivatives in each class.

(2) Epstein-Barr virus (EBV)-based shuttle vectors

We have established derivatives of normal, XP, A-T and CS cell lines which maintain the plasmids extrachromosomally. In all lines from which we have recovered plasmid into bacteria, the spontaneous mutation frequency was less than 10^{-5}. Three UV-induced mutants were recovered from a normal cell line and these were analysed by sequencing. Surprisingly, all three contained deletions (Lehmann et al. 1989b).

Although the EBV-based shuttle vectors fulfilled the criteria of being a stable system and having a low spontaneous mutation frequency, it became apparent that the effort involved in generating induced mutations was enormous, and that this system could hardly be described as rapid. At the same time, more efficient shuttle vector systems had been developed, and more importantly, the application of the polymerase chain reaction enabled mutations to be analysed in endogenous human genes, so that many, but not all features, of shuttle vectors were becoming obsolete. Our results with these more recent systems are described below.

(3) The SV40-based plasmid pZ189

pZ189 is an SV40-based shuttle vector which replicates to a high copy number in SV40-transformed human cells. Although this is a transient system, the spontaneous mutation frequency is less than 10^{-4} and mutant plasmids can easily be induced and recovered after passage through normal, A-T or CS cells. We have sequenced 74 UV-induced mutants from A-T cells and 43 mutants from CS cells. The mutational spectra from both these cell lines were similar to that reported in the literature for normal cell lines. Thus in this respect the UV-sensitive CS cells give a response similar to that of normal cells, in contrast to the findings of Kraemer and colleagues with XP cells which showed a significantly different mutation spectrum (Proc.Nat.Acad.Sci. **83**, 8273-8277, 1986) .

Analysis of mutations in the* hprt *gene in human cells

(1) T-lymphocytes

(i) Measurement of mutant frequency in normal and repair-deficient syndrome

We have now generated a substantial data base for the frequency of 6-thioguanine-resistant mutants in circulating T-lymphocytes from normal donors, xeroderma pigmentosum

and ataxia-telangiectasia patients and individuals heterozygous for these two autosomal recessive cancer prone diseases. (Figs. 1 and 2). In normals there is about a 10-fold increase in mutant frequency between birth and 20 years and a subsequent slower increase during adult life which we estimate as approximately 1.4% per annum. For the sun sensitive xeroderma pigmentosum patients there is a clear enhancement of mutant frequency (Fig. 1). The heterozygotes are indistinguishable from normals. With the ionising radiation sensitive ataxia-telangiectasia patients there is again a dramatic increase in mutant frequency in comparison with most normals.

Fig. 1. Frequency of 6TG-resistant mutants in circulating T-lymphocytes: XP and XP heterozygotes.

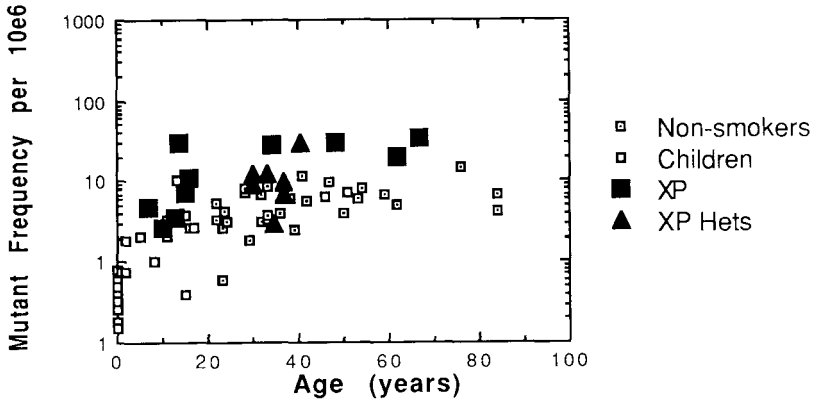
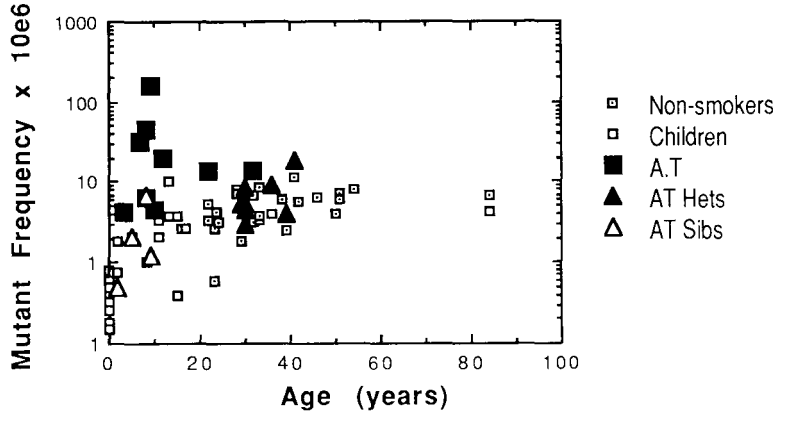


Fig. 2. Frequency of 6TG-resistant mutants in circulating T-lymphocytes: A-T and A-T heterozygotes



(ii) Molecular analysis of mutations

Mutant cells derived from normal donors or from XP and A-T patients have been analysed to identify the nature of the mutations. Southern analysis of the DNA revealed very few deletions in the *hprt* gene. In collaboration with our partners in Leiden we have developed a technique for analysing point mutations in the *hprt* gene. RNA extraction, cDNA synthesis followed by PCR and sequence analysis of the *hprt* amplified product has revealed a wide variety of alterations in the *hprt* gene in mutant lymphocytes. Out of 41 thioguanine-resistant mutants analysed, 17 had no detectable alteration. Some but not all of these may be altered in a gene other than *hprt*. Of the remaining 24, 11 had single base changes (both transitions and transversions), 4 had small deletions, 1 contained a single-base insertion, and 8 resulted in aberrant splicing of the RNA with loss of an exon.

When Southern blots of DNA from mutants were probed for the T-cell receptor (Ti) beta and gamma chain genes different rearrangements were found for most of the clones analysed to date. This indicates that the mutant clones from any one donor have, in general, arisen from independent mutations and do not represent clonal expansions of a single mutant cell.

(2) Cultured fibroblasts

We have carried out similar analyses on spontaneous and UV-induced mutations at the *hprt* locus in SV40-transformed normal and XP fibroblasts.

In a normal cell line MRC5VI, 64 spontaneous mutants were picked, representing at least 11 independent mutational events. Most of these were large deletions. In 12 UV-induced mutants representing at least 5 independent events, again most of the mutations were deletions.

In an XP cell line from complementation group A, 46 induced mutants have been sequenced representing at least 28 independent events. 6 had no change, there were 9 single and 2 tandem base changes. Seventeen mutations resulted in aberrant splicing with either loss of exons or transcription of parts of introns. Of the 13 base changes 6 were GC → A-T transitions and 6 were transversions, and 9 out of the 11 point mutations can be attributed to ultraviolet damage on the transcribed strand. Our data provide a preliminary suggestion that the nature of the UV-induced mutations in normal and XP cells might be different. If confirmed, this would support the data obtained for shuttle vectors mentioned above. Our data provide considerable information on the origin of mutations in the widely used *hprt* gene.

Discussion

In the period under review we had two main objectives: (i) to relate DNA repair to mutagenesis, (ii) to develop new mutagenesis systems.

Three shuttle vector systems have been examined in detail. The first, based upon the use of a bacterial (*gpt*) gene stably integrated in low copy number into the genome of a *hprt*$^-$ SV40-transformed normal cell line, has generated a large volume of data and much molecular analysis. We observed that few induced or spontaneously derived variants (*gpt*$^-$) arose from genuine mutational events. Their origin was largely based upon alterations in gene expression. We concluded that although we had generated a novel system to study the properties of human cells, this system was more appropriate to the study of gene expression rather than the mechanisms of radiation induced mutation. The second system which utilised an Epstein-Barr virus-based vector which was maintained extrachromosomally had a more acceptably low and stable frequency of spontaneous mutation. Here, however, induced mutants were very difficult to recover in numbers which might provide any meaningful analysis. The third system, which superseded the others was the SV40 virus-based plasmid pZ189. To date this system has been utilised to generate mutants in SV40-transformed normal, A-T and CS cells. We have undertaken a molecular analysis of mutants from A-T and CS cells. We conclude that the pZ189 system represents the only shuttle vector of practical utility available to us.

Developments in the use of the polymerase chain reaction, coupled with the complete sequencing of the human *hprt* gene together with our proven ability to clone human T lymphocytes has meant that we have now, in collaboration with the Leiden group,

concentrated our efforts on the more relevant endogenous human gene. We have collected many mutants from normal, XP, A-T and CS donors and are now in the process of compiling a catalogue of mutant characteristics by molecular analysis. It is already becoming clear that high mutant frequency is unlikely to be a consequence of clonal expansion and that, perhaps surprisingly, mutants are rarely the consequence of large deletions. It is anticipated that such a catalogue, supplemented with data from fibroblasts will assist in our understanding of the mechanism of mutation.

Studies of mutagenesis in human cells are still at a relatively early stage of development. Ultimately, however, the information they provide may throw light on the contribution of environmental mutagens such as ionising radiation to human mutation rates.

IV. 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.
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V. Publications:

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.. BI6-E-294-UK

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Title of the research contract:

Molecular mechanisms of radiation damage to chromosomes of human and rodent cells.

List of projects:

Molecular mechanisms of radiation damage to chromosomes of human and rodent cells.

Title of the project no.: B16-E-294-UK

Molecular mechanisms of radiation damage to chromosomes of human and rodent cells.

Head(s) of project:

Dr P.E. Bryant

Scientific staff

I Objectives of the project:

To gain an understanding of the biochemical mechanisms involved in the induction of deletion and exchange chromosomal aberrations in cells exposed to ionizing radiations.

II. Objectives for the reporting period:

To establish conditions for the maintenance of human and rodent lines in the G_2 phase. To study the kinetics of chromosomal aberrations (deletions and exchanges) particularly in the G2 phase of the cell cycle of human and rodent cells. To study kinetics of aberrations in cells treated with inhibitors of DNA double-strand break repair. To extend the G2 phase of the cell cycle using lowered temperature in order to increase the time to investigate the kinetics of aberration induction and repair in detail. Measurement of the length of G2 phase at various temperatures using the labelled mitosis method. Also to study the frequencies of aberrations in the CHO double-strand break repair defective strain xrs 5 at lowered temperature (extended G2 phase). Measurement of DNA double-strand break repair in CHO cells under reduced temperature using neutral filter elution.

III. Progress achieved:

1. Methodology

1.1 ***Measurements of kinetics of chromatid aberrations in human lymphocytes and in normal and AT fibroblasts.***

Peripheral blood was taken from healthy normal donors and cultured in RPM1 (1640) medium supplemented with 15% FCS and buffered with HEPES (20 mmol/l). Lymphocytes were stimulated with PHA and used after 72 h in culture. G_2 cells were scored by harvesting at between 1.5 and 4.5 hours following X-irradiation. Cells were irradiated in medium with X-rays (250 Kv, 14 mA, 0.5 mm Cu) at a dose-rate of 0.8 Gy/min. The X-ray dose in all experiments was 0.75 Gy. Parallel cultures were treated with ara A and ara C (Sigma) added to various final concentrations. Cells were arrested with colcemid and metaphase spreads made by standard cytogenetic procedures. A series of seven experiments have so far been completed. Fibroblasts were cultured as previously described (Mozdarani and Bryant, 1989, Int. J. Radiat. Biol., 55, 71) and the kinetics of aberrations examined in G_2 cells as described above, in the presence or absence of ara C at between 1.5 and 3.5 h before fixation. Irradiation was as above with a dose of 1 Gy.

1.2 ***Chinese Hamster cell culture***

Monolayer cultures of CHO K1 cells were routinely passaged in Eagle's modification of minimal essential medium (GIBCO, Paisley, Scotland) supplemented with 10% calf serum to which $FeCl_3,6H_2O$ (2.7mg/ml) had been previously added.

1.3 ***Measurement of the effect of temperature on DNA repair as measured by neutral filter elution.***

Neutral filter elution has been used to measure repair of DNA dsb in ^{3}H-TdR labelled CHO cells. Labelling was carried out for 48 hrs in the presence of 3.7 kBq/ml tritiated thymidine. After trypsinization aliquots of $5x10^5$ cells were resuspended in 3 ml MEM and transferred to water baths at $37^o/33^o/29^oC$ for 30 to 60 minutes prior to irradiation (30 Gy at a dose-rate of 5.8 Gy/min). After irradiation at room temperature, tubes containing cells were returned to the appropriate water bath for up to 2 h. Irradiations were staggered to facilitate simultaneous harvest. To halt repair, samples were chilled by the addition of 7 ml ice-cold phosphate buffered saline (PBS). Elution was performed as per Bradley and Kohn at pH 9.6. Except that before elution was begun, cell lysates were treated with proteinase K or pronase for 1h at 60^oC. for 1 h.

1.4 ***Effect of temperature on the length of G2 phase measured using the labelled mitoses method.***

The length of G_2 phase was measured in CHO cells using ^{3}H-TdR labelling and scoring frequencies of labelled mitoses. Samples were grown overnight at 37°C from $2x10^5$ cells per 25 cm^2 flasks and then duplicate samples shifted to either 33°C or 29°C for 3 h before addition of ^{3}H-TdR (5 mCi/ml). Cells were harvested at intervals of up to 7 h, washed free of label and fixed by standard procedures. Slides were prepared and dipped in autoradiographic emulsion (Ilford K2), dried and held in the dark for 4 days. Slides were then developed, stained in Giemsa and the frequencies of labelled mitoses scored. Mitotic figures were scored as labelled if they contained 20 or more grains.

1.5 ***Transient hypothermia treatment***

Cultures of cells were grown to equal density at 37°C and then transferred to either 33°C or 29°C for the period of the experiment. The use of only a short (transient) hypothermia was necessary to obtain cultures of equal density . In the case of experiments at 29°C the transient hypothermia was unavoidable because steady-state growth was not possible at that temperature

Total hypothermic exposure times (including colcemid arrest) were 10 hours for the 33°C experiments and 9.5 hours (with the colcemid arrest at 37°C) for the 29°C experiments (insufficient good quality metaphases were present after a 29°C arrest). Sampling times were varied from 1 hour to 9 hours. During transport to and from the X-ray set culture flasks were held in insulated containers and jacketed by flasks filled with water at the appropriate temperatures.

1.6 ***Cytogenetic preparations of CHO cells***

Metaphase cells were harvested by the mitotic shake-off technique, following a 30 minute colcemid (0 04 mg/ml) arrest of log phase cultures (this rapid harvest technique was intended to minimise heterogeneity within mitotic samples which might have occured as a result of diversity in cell-cycle progression at the time of irradiation. An interval of 30 minutes was left after the final irradiation before addition of colcemid to minimise inclusion of cells which had been in mitosis at the time of irradiation. Cells were harvested and prepared for scoring using standard procedures.

2. Results

2.1 ***Kinetics of G_2 chromosomal aberrations in human cells***

The kinetics of G_2 aberrations was studied in both transformed human fibroblasts and in stimulated peripheral blood lymphocytes, treated with the double-strand break repair inhibitors ara A (9-B-D-arabinofuranosyladenine) and ara C (1-B-D-arabinofuranosyladenine). The results for human fibroblasts treated

with the inhibitor ara C after X-irradiation showed that frequencies of deletions increased with time in the presence of the drug (Figure 1). This was interpreted as an interaction between a lesion induced by ara C with X-ray induced lesions. This was in contrast to results with ara A which had previously been shown to yield a constant level of deletions in X-irradiated cells.

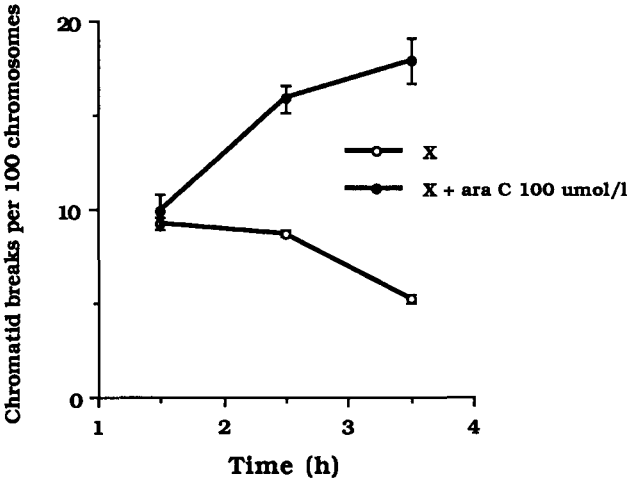

Figure 1 Frequencies of chromatid aberrations in X-irradiated (1 Gy) G_2 normal (MRC5SV1) fibroblasts in the presence or absence of ara C. Errors are standard errors of mean values of 3 experiments.

Figure 2 shows results obtained with X-irradiated human lymphocytes treated with ara A. Previous results with human fibroblasts (Mozdarani and Bryant,1989, Int.J.Radiat.Biol., 55, 71) showed a strong inhibition by ara A of disappearance of chromatid breaks in G_2 cells. However, in human peripheral blood lymphocytes (Figure 2) the first-order rate of disappearance of deletions observed in controls was also found for treatment with 100 or 200 µmol/l, concentrations previously shown in fibroblasts to give a level response (indicating total inhibition of 'repair' of lesions). At 800 µmol/l, a strong inhibition of disappearance of breaks was observed. in lymphocytes. This result may be indicative of a powerful deaminase activity in lymphocytes which degrades ara A. However other possible explanations are that the DNA polymerases in lymphocytes are less sensitive to ara A than those in fibroblasts or that the drug is less well taken up by lymphocytes.

Another strongly contrasting result was that the rate of disappearance of deletions (interpreted as reflecting repair of underlying dsb) was twice the rate observed in fibroblasts (t1/2 for lymphocytes 50 min; for fibroblasts 2 h). Also the frequencies of exchanges induced by X-rays were much lower in lymphocytes. As shown for fibroblasts (Mozdarani and Bryant, 1989), treatment with ara A after X-ray exposure led to an increase in the frequencies of exchanges; however, also in contrast to data for fibroblasts where a 3-fold increase in exchanges occured in the apparent absence of repair of deletions, the increase in exchanges caused by ara A in lymphocytes occured in the **absence** of any apparent inhibitory effect of ara A (at 100 μmol/l) on the rate of disappearance of deletions (Figure 2).

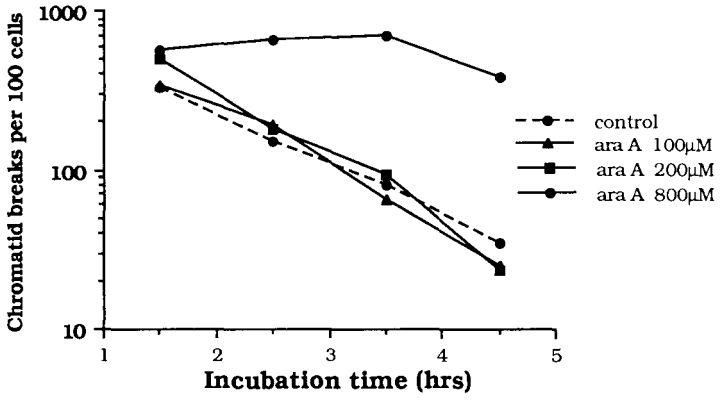

Figure 2 Frequencies of chromatid breaks in cultured human peripheral lymphocytes as a function of time between X-irradiation and sampling of metaphse cells using colcemid block. Samples were treated with ara A for 30 mins before and during the interval between X-irradiation and sampling. Pooled results of seven experiments. Error bars are omitted for clarity.

Treatment of X-irradiated lymphocytes with ara C (at 50 μmol) similarly caused an inhibition of the disappearance of deletions (Figure 3). This is broadly in agreement with the result of Preston (1980, Mutat. Res. 69, 71), but differs from our own results for human fibroblasts (Figure 1) where a clear increase in breaks was observed during treatment with ara C.

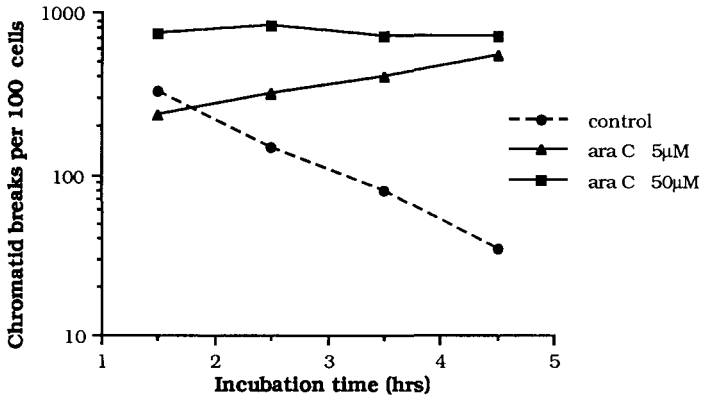

Figure 3 Frequencies of chromatid breaks in cultured human peripheral lymphocytes as a function of time between X-irradiation and sampling of metaphase cells using colcemid block. Samples were treated with ara C for 30 mins before and during the interval between X-irradiation and sampling. Pooled rsults of seven experiments. Error bars are omitted for clarity.

From Figures 2 and 3, it can be seen that the sensitivity of X-irradiated lymphocytes to ara C was much greater than for ara A; a concentration of even 5 μmol/l leading to an inhibition of repair. Very few exchanges were observed in any of the lymphocyte samples. It is clear from these results an those for fibroblasts previously reported (Mozdarani and Bryant 1989, Mutation Res. 226, 223) that ara C and ara A have somewhat different modes of action.

The data presented is not inconsistent with the notions 1) that the disappearance of deletions with time represents the repair of an underlying lesion (assumed to be DNA double-stand breaks or a subclass of dsb) and 2) that the mechanism of exchange aberrations is independent of that involved in rejoining of dsb, and possibly involves a simple ligation process. The evidence for this second notion are results in which the increase in exchanges can be uncoupled from that of the decrease in deletions. We shall see further evidence of this later.

2.2 ***Kinetics of induction and repair of G_2 chromatid breaks in CHO cells***

In recent work we have also attempted to study the kinetics of chromatid deletions and exchanges in CHO K1 cells. These cells have however only a very short G_2 phase (around 3 h) which is too short to obtain accurate kinetics of aberrations. We therefore used a procedure (see under 1.5 above) of transiently lowering

temperature in order to lengthen the G_2 phase. Results obtained using labelled mitoses showed that the G_2 phase increased from 2.8 h at 37°C to 5.7 h at 33°C and to 7.3 h at 29°C (Figure 4).

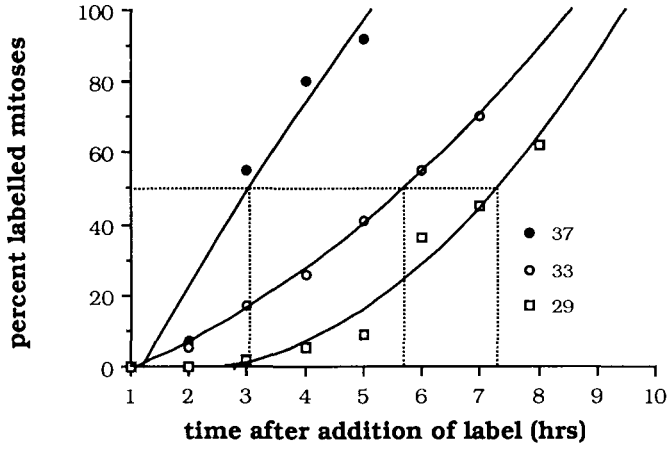

Figure 4 Labelled mitoses as a function of time after addition of ^{3}HTdR in CHO K1 cells held at various temperatures (37°C, 33°C and 29°C)

These values were also confirmed in independent experiments (results not shown) in which cells were treated with ara A for various times at the three different temperatures - the onset of aberrations caused by ara A was indicative of the tail end of S phase.

Chromatid break data obtained as a function of time for X-irradiated cells incubated at the three temperatures are shown in Figure 5. The kinetics were followed over a 9 h period at the two lower temperatures , in the case of 33°C without any apparent alteration in the first order disappearance of breaks at later times (t1/2=1.5 - 2 h) which might have signalled the onset of S-phase. The surprising result was that the kinetics of decrease in breaks with time was not substantially altered at 33°C, and with the exception of an apparent 'shoulder' region at 29°C, was similar at this lower temperature. As we argue later, the similar rate of disappearance of breaks at lowered temperature in a lengthened G_2 phase indicated that the disappearance of breaks is indeed the result of the repair of some underlying lesion in DNA. A variable chromosome sensitivity through G2 seems unlikely since the large alteration in length of G_2 should have led to a changed kinetic of breaks at lowered temperature.

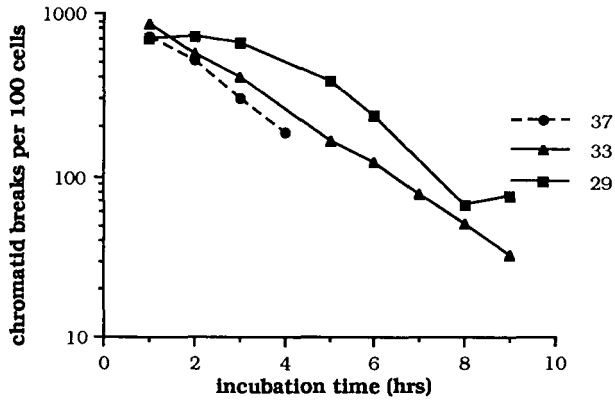

Figure 5 Frequencies of chromatid breaks in CHO K1 cells held at 37°C, 33°C or 29°C as a function of time between X-irradiation (1.5 Gy) and sampling. Pooled results of 3 experiments.

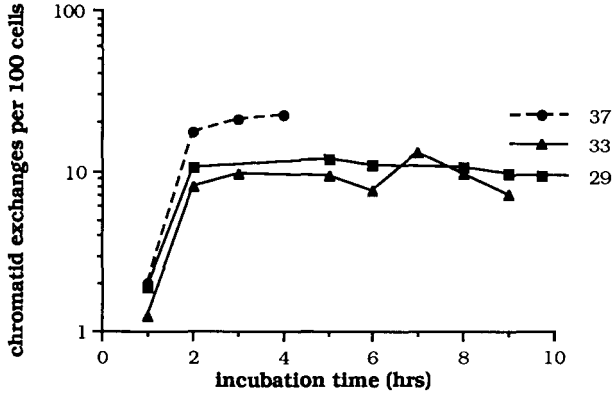

Figure 6 Frequencies of chromatid exchanges in CHO K1 cells held at 37°C, 33°C or 29°C as a function of time between X-irradiation (1.5 Gy) and sampling. Pooled results of 3 experiments.

2.3 ***Chromatid exchanges in G_2 CHO cells***

The frequencies of exchanges in CHO cells induced during incubation at the three temperatures was lower than that of deletions and increased with time, eventually levelling off after 2-9 h incubation (Figure 6). This kinetic did not resemble to that of the disappearance of breaks, again indicating the uncoupled nature of the two processes.

2.4 ***Measurement of kinetics of dsb in CHO cells at various temperatures***

Experiments using the neutral filter elution technique showed that the rate of disappearance of DNA double-strand breaks in CHO cells was not significantly altered by shifting cells to 33°C and only a slight change was observed at 29°C (Figure 7). The rate of repair of dsb measured by the neutral filter elution technique (t1/2=10-20 min) was quite different from that of the disappearance of chromatid breaks (t1/2=1.5 h at 37°C). The rate of disappearance of chromatid breaks is more similar to the kinetics of dsb repair measured by neutral velocity sedimentation in mouse Ehrlich Ascites tumour cells (Bryant and Blocher 1980, Int. J. Radiat. Biol. 38, 335). The reasons for this are not understood, however the results of the neutral elution experiments indicate that DNA strand break repair is not markedly affected by a reduction in temperature to 33°C or 29°C.

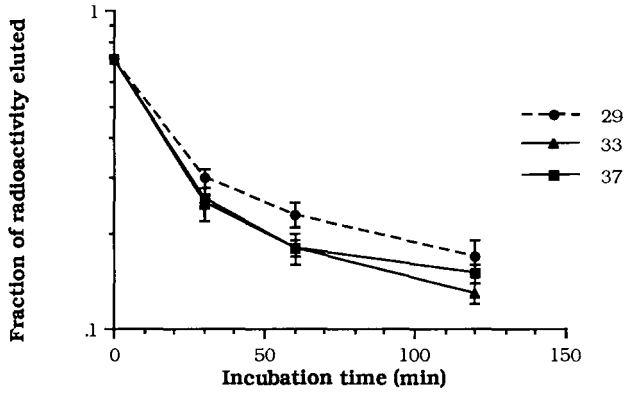

Figure 7 Fraction of radioactivity (DNA) eluted (asumed to represent DNA double-strand breaks) in CHO K1 cells held at various temperatures (37°C, 33°C, 29°C) during post-irradiation incubation The dose was 30 Gy. Errors are standard errors of mean values of two experiments.

2.5 ***Kinetics of chromatid aberrations in X-ray sensitive (xrs 5) mutant cells***

We also carried out experiments to study the kinetics of repair of chromatid aberrations in the X-ray sensitive and dsb repair defective line xrs 5 derived from a CHO K1 parent (Jeggo and Kemp 1983, Mutation Res. 112, 313). Xrs 5 cells were X-irradiated (with an equiclastogenic dose to that in CHO K1 cells) and incubated for various times at either 37°C or 33°C. The results are shown in Figure 8. These showed that there was no significant difference between the rate of disappearance of chromatid breaks in xrs 5 from that in the CHO K1 parent line.

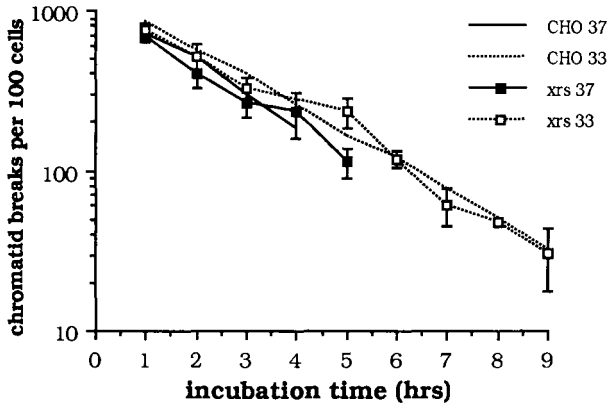

Figure 8 Frequencies of chromatid breaks in CHO K1 and xrs 5 cells incubated at either 37°C or 33°C following X-irradiation. Doses were: CHO K1 - 1.5 Gy; xrs 5 - 0.375 Gy. Errors are standard errors of mean values of 3 experiments.

Since these two cell lines had been irradiated at equiclastogenic doses, the results show that there was a fourfold difference in the dose required to induce the same number of chromatid breaks. This indicates a fourfold higher conversion of DNA damage into visible chromatid breaks in xrs 5 than in CHO K1. There was also an enhanced frequency of chromatid exchanges. A two-fold difference in exchanges in the xrs 5 line compared to CHO K1 was seen (Figure 9) since fewer exchanges were induced at a dose equally effective with respect to breaks.

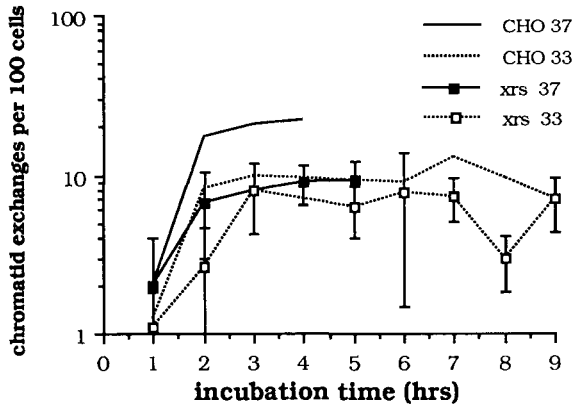

Figure 9 Frequencies of chromatid exchanges in CHO K1 and xrs 5 cells incubated at either 37°C or 33°C following X-irradiation. Doses were CHO K1- 1.5 Gy; xrs 5 - 0.375 Gy. Errors are standard errors of mean values of 3 experiments.

3. Discussion

The question arises as to whether the kinetics of disappearance of chromatid breaks represents a repair process. It might be argued that the kinetics of chromatid breaks reflect a changing sensitivity of cells to X-rays as they progress through the G_2 phase. This hypothesis appears however not to be supported by two strong lines of evidence. Firstly, the disappearance of chromatid breaks can be inhibited in human cells by known inhibitors of DNA dsb repair (ara A and ara C), and secondly that in Chinese hamster cells, lowering temperature from 37°C to 33°C increased the length of the G_2 phase (from 2.8 h to 5.7 h) but does not significantly alter the rate of disappearance of breaks. An alteration in frequencies of breaks would be expected, based on a changing sensitivity through the cell cycle. It is therefore concluded that the kinetics of breaks in both human and Chinese hamster cells may reflect an underlying repair of a DNA lesion which we presume to be double-strand breaks. It then seems to be paradoxical that the rate of repair of chromatid breaks observed in xrs 5 cells is essentially the same as that in CHO K1 cells, since the xrs 5 line is known to be dsb repair deficient (Kemp *et al*, 1984, Mutation Res. 132, 189). We cannot at this stage explain this paradox, however one possibility is that the results indicate the existence of a subclass of dsb which gives rise to chromatid aberrations and the repair of which is not altered in xrs 5. There is however clearly a higher frequency of conversion of dsb into visible chromatid breaks in xrs 5 as compared to that in CHO K1 cells. A similar high frequency of breaks is also

observed in AT cells (Mozdarani and Bryant, 1989) which may in part contribute to the hypersensitivity to X-rays of these mutant cell lines. However in this case the (AT) cells are known to be proficient in dsb repair (Lehmann and Stevens, 1977, Biochim. Biophys. Acta 474, 49). There must therefore be other reasons than the ability to repair dsb for the high frequency of conversion of dsb into chromatid breaks.

Our results showing that a different kinetic of exchange aberration induction from that of deletions appear to add further support for the notion that the misjoining mechanism leading to chromosomal exchanges is independent of that for joining breaks (presumed to be the same mechanism as that for repair of dsb or subclass of dsb). The separate nature of the chromosome joining and misjoining mechanisms is also strongly indicated by an earlier result of ours (Mozdarani and Bryant, 1987, Mutagenesis 2, 371) in which we show that the frequency of exchanges **increased** three-fold while the rejoining of breaks was inhibited.

Our results for X-irradiated human fibroblasts (Figure 1) treated with ara C after X-ray exposure suggest that this agent induces a type of lesion, not in itself manifest as a visible chromosomal aberration at metaphase, but nevertheless able to interact with X-ray induces lesions to increase the number of these giving rise to chromosomal aberrations. This result is supported by data shown here for human peripheral blood lymphocytes (Figure 3). In this respect the action of ara C appears to differ from that of ara A which seems to act solely as an inhibitor of repair.

These results throw some further light on the kinetics and mechanisms of induction of chromosomal aberrations and suggest future directions. Although we are as yet still far from understanding either the biochemical basis of repair of dsb, or the kinetics of chromatid breaks and exchanges, our results suggest that we should concentrate our effort into understanding the basis and mechanism of the conversion of dsb into chromosomal aberrations. Further experiments which seek to identify the enzymes involved in this pathway are planned.

Chromosomal aberrations appear to occupy a central role in radiation effects such as cell killing, and perhaps more importantly from a radiation protection point of view, genetic mutations and radiation-induced oncogenic transformation. In order to appreciate and evaluate risks of low level radiation to man it will be necessary to understand how these alterations in the genome occur. Mechanisms of chromosomal aberration induction appear to be the key to understanding these effects.

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

V Publications:

Mozdarani, H. and Bryant, P.E. (1989) Cytogenetic response of ataxia telangiectasia and normal human G2 cells exposed to X-rays and ara C. Mutation Research Letters, 226, 223-228.

Bryant, P.E. (1989) Molecular mechanisms of chromosomal aberration induction. In 'Low Dose Radiation: Biological Basis of Risk Assessment. Proceedings of the 14th L.H. Gray conference, New College Oxford, September 1988. Ed. K.F. Baverstock and J.W. Stather, Taylor and Francis Ltd., pp 455-462.

Costa, N., and Bryant, P.E. (1990) Neutral filter elution detects only limited inhibition of double-strand break repair by 9-B-D-arabinofuranosyladenine. Mutation Research, in the press.

MacLeod, R.A.F., Christie , A.F. and Bryant, P.E. (1990) Repair kinetics in CHO cells of X-ray induced DNA damage and chromatid aberrations during a cell cycle extended by transient hypothermia. Mutagenesis, in the press.

MacLeod, R.A.F. , and Bryant P.E. (1990) Is xrs 5 deficient in chromosome repair? Int. J. Radiat. Biol. , (abstract) in the press.

MacLeod, R.A.F. , Christie, A.F., Costa, N.D. and Bryant, P.E. (1990) Hypothermia resistant DNA and chromosome repair in Chinese Hamster Ovary cells. Int. J. Radiat. Biol., (absract) in the press.

McLeod, R.A.F., and Bryant, P.E. (1990) Kinetics of repair of chromatid aberrations in xrs 5 and CHO cells in the G2 phase extended by transient hypothermia. Mutagenesis, submitted for publication.

MacLeod, R.A.F., and Bryant, P.E., (1990) Repair of chromatid breaks in X-irradiated G2 human lymphocytes treated with ara A and ara C, in preparation.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Medical Research Council
20 Park Crescent
GB-London W1N 4AL

Contract no.: BI6-E-143-UK

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

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Radiobiology Unit, Genetics Div.
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Harwell, Didcot
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Telephone number: 0235-834393

Title of the research contract:

Mutation studies upon spermatogonial stem cells of mammals and genetic tests for non-disjunction in the mouse.

List of projects:

1. Experimental studies of non-disjunction in the mouse.
2. Factors affecting the yield of mutations from spermatogonial stem cells of mammals.

Title of the project no 1

Experimental studies on non-disjunction in the mouse

Head(s) of project: Dr. B.M. Cattanach F.R.S.

Scientific staff. Dr. B.M. Cattanach F.R.S.

I. Objectives of the project:

To develop genetic methods for detecting non-disjunction and chromosome loss in mice.

II. Objectives for the reporting period:

1. To test the effectiveness of a series of Robertsonian translocations, singly or in pairs, with one or both arms genetically marked for use in tester animals to detect non-disjunction and chromosome loss events in chromosomally normal mice.

2. To develop further tester stocks and investigate their potential for use as in 1. above.

III. Progress achieved:

Methods

1. Rb complementation method Numerous studies have shown that heterozygotes for certain Robertsonian translocations have high spontaneous rates of non-disjunction for the chromosomes involved. Assessment of the frequency of such non-disjunction can be obtained by intercrossing heterozygotes one of which has the chromosome of interest genetically marked. Disomy from one parent can then complement nullisomy from the other to give chromosomally balanced young which can be distinguished on the basis of the marker gene phenotypes. This test was used to identify potentially useful tester systems.

2. Rb tester method Robertsonian translocation heterozygotes can be used as tester animals to detect non-disjunction and chromosome loss events in chromosomally normal mice following irradiation (Cattanach et al., 1984). To detect non-disjunction events leading to chromosome gains, genetically marked normal mice are mated to unmarked tester animals; to detect losses, unmarked normal mice are mated to marked tester animals. The tester animals used carried either a single translocation or two translocations involving different chromosomes.

3. MBH method Mice doubly heterozygous for two Robertsonian translocations sharing a common chromosome arm (mono-brachial homology) exhibit very high levels (up to about 40%) of non-disjunction for the chromosome arm concerned (Gropp et al., 1975). Such animals can be used as tester animals for detecting non-disjunction and chromosome loss in normal mice using the same system of crosses as in 2. above (Cattanach et al., 1984).

Unless otherwise stated irradiations comprised 4 Gy X-rays. Treated animals were mated for only one week following irradiation, unless specified otherwise, and therefore the progeny scored derived from mature oocytes in females and mature spermatozoa in males.

Results

Expt. 1 Irradiated and unirradiated wild type females were mated with tester males doubly heterozygous for Rb(4.6)2Bnr and Rb(9.14)6Bnr and homozygous for brown (b) on chr. 4, waved-1 (wa-1) on chr. 6, dilute (d) and short-ear (se) on chr. 9 and recessive spotting (s) on chr. 14 (Rb tester method). The progeny were scored for the marker gene

phenotypes the occurrence of which represented maternal loss events. Despite the presence of the 2 translocations the fertility of the cross was acceptable (Table 1). No marked young were detected in the control group but 5 b and 3 s young were detected among the progeny of the treated females (Table 2). Potentially, these represented maternal losses of chrs. 4 and 14, respectively. However, subsequent breeding and cytological tests showed that one of the s young was a new mutation. A new d mutation (without se) was also found and this, too, would have been due to a mutation. Thus, while the use of 2 translocations and 4 chromosome markers provided a method that was of practical value for testing for non-disjunction the high radiation-induced mutation rate of some of the marker genes used invalidated the use of the test for detecting chromosome loss events.

Table 1

Productivity of animals used with tester systems

Expt.	Tested per cent	X-ray dose	Tester system	No. ♀♀ used	Mean litter size/fert ♀
1	♀	0	Rb2Bnr/+, Rb6Bnr/+	594	4.76
		4 Gy		594	4.23
2	♂	4 Gy	Rb2Bnr/+, Rb6Bnr/+	810	3.24
3	♀	0	Rb2Bnr/+, Rb6Bnr/+	576	3.78
		4 Gy		576	3.25
6	♀	4 Gy	Rb1Bnr/+	612	5.63
	♂	4 Gy		594	3.90
7	♀	4 Gy	Rb4Bnr/+	360	5.79
	♀	4 Gy	Rb4Bnr/Rb8Bnr	324	4.28
	♂	4 Gy	Rb4Bnr/+	306	3.38
	♂	4 Gy	Rb4Bnr/Rb8Bnr	328	4.35

Expt. 2 The equivalent test applied to females in Expt. 1 was applied to males. The tested males were, however, given only 3 Gy X-rays and were mated to a fresh set of females each week for 3 weeks to sample late and early spermatozoa and spermatids, respectively. The fertility of this cross (Table 1) was lower than that of Expt. 1 and this can be

attributed to the greater sensitivity of the male germ cell stages tested (e.g. mean litter sizes in wk 1 was 3.07; in wk 2, 3.24 and in wk 3, 1.8) to induced dominant lethality. One b offspring was detected in wk 1 and an s animal in wk 2 (Table 2). However, se and d young representing gene mutations were also found again pointing to the difficulties with the gene markers used.

Table 2

Detection of chromosomal loss and non-disjunctional (gain) events

Expt.	Chr./event scored	Total per cent	X-ray dose	Tester system	No. young	No. marked	% marked
1	loss; chr. 4	♀	4 Gy	Rb2Bnr/+, Rb6Bnr/+	1945	5	n.a.
	6					0	
	9					0	
	14					3	
	as above	♀	0	Rb2Bnr/+, Rb6Bnr/+	2342	0	0
2	loss; chr. 4	♂	3 Gy	Rb2Bnr/+, Rb6Bnr/+	1449	1	n.a.
	6					0	
	9					0	
	14					1	
3	gain; chrs. 4, 6, 9, 14	♀	4 Gy	Rb2Bnr/+, Rb6Bnr/+	1278	0	0
			0		1816	0	
6	loss; chr. 1	♀	4 Gy	Rb1Bnr/+	2750	6	0.22
	3				2687	0	0
	loss; chr. 1	♂	4 Gy	Rb1Bnr/+	1888	4	0.21
	3				1888	1	0.05
7	loss; chr. 11	♀	4 Gy	Rb4Bnr/+	1418	2	0.14
				Rb4Bnr/Rb8Bnr	731	2	0.27
	loss; chr. 11	♂	4 Gy	Rb4Bnr/+	459	0	0
				Rb4Bnr/Rb8Bnr	1145	1	0.09

n.a. not applicable; see text

Expt. 3 The reciprocal type of cross to that used in Expt. 1 was employed to screen for non-disjunctional (gain) events involving chrs. 4, 6, 9 and 14 in females. The fertility of these crosses was lower than

those of Expt. 1 (Table 1) and this can be attributed to lower productivity of the genetically marked strain of female. Potentially, this could be improved. No marked young were detected (Table 2) which is not unexpected in view of the rarity of non-disjunctional events. The validity of the test was established in the 'loss' experiments (Expts. 1 and 2) and in 'gain' experiment to follow.

Expt. 4 The test system of Expt. 3 (Table 1) was used to screen for an age-effect upon non-disjunction in unirradiated normal females. This comprised scoring progeny for the marker gene phenotype in life time breeding studies upon the females. One d se offspring was detected, representing a maternal non-disjunctional event involving chr. 9 but, as this animal was conceived early in the life of the mother, it does not support the concept of an increase in non-disjunction with maternal age. Nevertheless, it provides a further example of the extremely rare non-disjunction in normal mice (Cattanach et al., 1984).

Expt. 5 Because of the difficulties with the marker genes available for use with the Rb2Bnr and Rb6Bnr translocations and the rather low fertility of the crosses, attention were focussed on the use of a single Robertsonian translocation for the Rb tester method (Cattanach et al., 1984) but with both rather than only one arm marked. The chr. 3 marker, matted (ma) was therefore introduced into an Rb(1.3)1Bnr stock already marked with leaden (ln) on chr. 1 and the occurrence of non-disjunction in Rb1Bnr heterozygotes checked with the use of the Rb complementation method. From crosses of marked females with unmarked males 19 ln and 8 ma young were detected among 766 progeny. The estimated non-disjunction frequencies were 23.9 ± 2.2% for chr. 1 and 16.5 ± 2.5% for chr. 3. In the reciprocal cross, 14 ln and 15 ma young were detected among 788 young giving estimated non-disjunction frequencies of 20.7 ± 2.3% and 21.4 ± 2.2%, respectively; 22.4 ± 1.6% and 19.2%, overall. Clearly, the doubly marked Rb1Bnr system was appropriate for use with the Rb tester method to detect gain/loss events in normal mice.

Expt. 6 Irradiated wild type females were mated with tester Rb1Bnr heterozygotes homozygous for ln and ma and the progeny scored for the marker gene phenotypes to detect maternal chr. 1 and 3 losses. The fertility of the cross was acceptably high (Table 1) and ln young were detected with a frequency (Table 2) expected from earlier studies (Cattanach et al., 1985). However, no ma young were found, which was

surprising in view of the Rb complementation test data (Expt. 5). The difference between the ln and ma data is significant (P = 0.031).

The reciprocal cross of irradiated wild type males with marked tester females yielded similar results (Table 2). The expected frequency of ln young was again found but only 1 ma animal was detected. The shortage of ma could be interpreted to mean that chr. 3 is less sensitive to radiation damage than chr. 1. The fertility of this cross was lower (Table 1) than that of the reciprocal with irradiated females and, as suggested for the test with males in Expt. 2, may reflect the greater sensitivity of the male germ cell stage tested to dominant lethal induction.

Expt. 7 The efficiencies of the Rb tester and MBH methods for detecting chr. 11 loss following maternal or paternal irradiation were investigated using Rb(11.13)4Bnr heterozygotes for the former method and mice doubly heterozygous for Rb(11.13)4Bnr and Rb(10.11)8Bnr for the latter. The marker gene used was vestigial tail (vt) which can be identified at birth. The fertility of the various crosses were generally in accord with those in other comparable tests (Table 1) and the MBH method appeared to be the more effective of the two for detecting loss events involving a single chromosome (Table 2). The frequencies of marked young detected with the two methods were lower than might have been expected from the high non-disjunction frequencies known to occur in the two types of tester animal. As suggested for chr. 3, chr. 11 may be relatively insensitive to radiation-induced change.

Discussion

Each of the test systems investigated proved effective for detecting chromosome loss or non-disjunctional events, if not for all chromosomes. Generally, the procedures compare favourably with those for detecting sex chromosome loss (Russell and Russell, 1960). A minor difficulty in some crosses was low fertility, but this was most commoly associated with the use of small, inbred stocks carrying the marker genes, and therefore easily correctable. The high mutation rate of some of the markers also created problems in chromosome loss experiments but this would not affect investigation of non-disjunctional (gain) events in which the marked animals are treated.

Two other problems were encountered, both of which in themselves were significant:

First, the phenomenon known as chromosome imprinting was found in some of the tests and, for the chromosomes involved, invalidated the methods. It was found that a complete set of chromosomes is not in itself adequate for normal embryonic or foetal development; for some chromosomes parental origin is important. Thus, with 2 maternal or 2 paternal copies, abnormality or embyronic lethality may occur and differential survival rates, without obvious abnormality, of maternal and paternal disomies may be a further manifestation of the same problem. Imprinting lethality has been found to be one cause of the failure to detect maternal chr. 6 non-disjunction in Expts. 3 and 4. Imprinting abnormality also occurs with chr. 11 but does not interfere with functioning of the tester systems (Expts. 7). Chrs. 7 and 17 have also been shown to exhibit imprinting effects resulting in lethalities. The phenomenon of chromosome imprinting is proving of importance for understanding features of many abnormality syndromes in man and the occcurrence of certain sporadic cancers. As such it is probably the most significant product of the experiments here described.

Second, the frequency of radiation-induced loss events involving some chromosomes (e.g. chr. 3 in Expt. 6, and chr. 11 in Expt. 7) seemed low relative to others (e.g. chr. 1 in Expt. 6), when it is known that the non-disjunctional events in the tester parents are in all cases high. This raises the possibility that the chromosomes differ in their sensitivities to radiation damage that lead to chromosome loss and, clearly, this possibility merits further investigation.

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

V. Publications:

1. PUBLICATIONS IN SCIENTIFIC JOURNALS

1. CATTANACH, B.M. and KIRK, M. Chromosome 11 parental source effect upon foetal growth in mice. Genet. Res., Camb., 45: 220-221 (Abs.) (1985).
2. CATTANACH, B.M. and KIRK, M. Differential activity of maternally and paternally derived chromosome regions in mice. Nature, 315: 496-498 (1985).
3. TEASE, C. and CATTANACH, B.M. Mammalian cytogenetic and genetic tests for autosomal non-disjunction and chromosome loss in mice. Chemical Mutagens Vol. 10, Plenum Press, ed. Frederick J. der Serres, pp. 218-283 (1986).
4. CATTANACH, B.M. Parental origin effects in mice. J. Embryol. exp. Morph., 97: 137-150 (1986).
5. CATTANACH, B.M. and BEECHEY, C.V. Chromosome imprinting phenomena in mice and indications in man. Chromosomes Today, in press.

2. SHORT COMMUNICATIONS

1. CATTANACH, B.M. Imprinting phenomena involving chromosomes 2 and 11 of the mouse. Mouse News letter, 76: 47 (1986).
2. CATTANACH, B.M. Chromosomes 1 and 3 non-disjunction in Rb(1.3)1Bnr heterozygotes. Mouse News Letter, 81: 64 (1988).
3. CATTANACH, B.M. Chromosome imprinting in the mouse. Mouse News Letter, 82: 93 (1988).
4. CATTANACH, B.M. Imprinting of distal chromosome 2 and lack of imprinting with distal chromosome 8. Mouse News Letter, 83: 161-162 (1988).

5. BEECHEY, C.V., CATTANACH, B.M. and SEARLE, A.G. Chromosome 6 and genetic imprinting. Mouse News Letter, 83, 162-163 (1989).
6. BEECHEY, C.V. and CATTANACH, B.M. Chromosome 6 and genetic imprinting. Mouse News Letter, 84: 82-83 (1989).
7. BEECHEY, C.V., CATTANACH, B.M. and SEARLE, A.G. Chromosome 8 and 16 and genetic imprinting. Mouse News Letter, 84: 83 (1989).

Title of the project no.: 2

Factors affecting the yield of mutations from spermatogonial stem cells of mammals

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 the sensitivity of the spermatogonial stem cells of the 101/H mouse to killing and genetic damage by X-rays and screen other inbred strains for their radiation responses.

2. To investigate cell stage sensitivity differences of spermatogonial stem cells to specific locus mutation and translocation induction and to assess the influence of size of chemical priming dose upon X-ray-induced translocation yield in combined TEM-X-ray treatments with 24 h intervals.

III. Progress achieved:

Methodology

Duration of sterile period following X-irradiation and recovered testis weight following return to fertility were used as indicators of spermatogonial stem cell killing.

Translocations, detected in spermatocytes after the return to fertility were scored as one measure of radiation-induced genetic damage in stem cells. Specific locus mutations detected among the F_1 progeny of irradiated males in crosses with females homozygous for 7 marker genes provided a further measure of genetic damage. In the latter work, allelism tests were conducted on all mutants, and all dominant mutations were recorded and tested.

Results and Discussion

1. Studies with 101/H strain mice 101/H sterile periods were consistently longer after all X-ray doses than those with the hybrid (C3H/HeH x 101/H) and became evident at doses as low as 0.5 Gy. The plateau found with the hybrid at 6-8 Gy was evident but at the lower dose range of 3-5 Gy. 101/H recovered testis weights were also lower after all doses, with the most marked reductions occurring with doses above 6 Gy, as occurs with the hybrid only at doses over 8 Gy.

The translocation dose-response curve was humped, like that of the hybrid, but the peak yield occurred at 3-5 Gy, rather than at 5-6 Gy and the responses at all but the lowest doses were significantly smaller. A 1+5 Gy, 24 h fractionated dose significantly increased the yield above the maximum obtained with the single 6 Gy dose and, with fractionation intervals of 4 and 12 days the translocation yields were sub-additive, like those for the hybrid, and similarly increased to additive levels with 21 days between treatments.

Using Leenhouts and Chadwick's (1981) model of the spermatogonial stem cell system with their parameters influencing translocation yield, it was calculated that the differences between the 101/H and hybrid responses to single doses could be accounted for either by 101/H mice having a higher proportion of sensitive cells in the heterogenous stem cell populations or by a higher intrinsic sensitivity of the strain to radiation. The fractionation data suggests that the former is the

correct explanation, and this is being supported by the results of further investigations.

Thus; a) dominant lethal induction in 101/H spermatozoa was no higher than in the spermatozoa of the hybrid; b) 101/H females did not permit a higher realization of dominant lethals induced in hybrid males than did hybrid females, no difference in repair capacity was therefore indicated; c) spermatocytes were no more sensitive to killing, as judged by sperm counts and testis weight 4-5 weeks post-irradiation; d) concurrent 1+5 Gy, 24 h fractionated X-ray treatments gave similar translocation yields from 101/H and the hybrid spermatogonial stem cells. As are the stem cell populations that survive the initital priming dose relatively homogeneous, their similar response to the second larger dose suggests that the stem cells of 101/H and the hybrid mice do not differ in their radiosensitivity. It may be concluded from all these experiments that the difference between genetic damage and cell killing responses of 101/H and hybrid mouse stem cells may lie in the differing proportions of radiosensitive and radioresistant cells in their normally heterogeneous stem cell populations.

Specific locus mutation studies to investigate the 101/H mouse response to this category of mutation are in progress. To facilitate comparison with the Neuherberg work on other mouse strains the 3+3 Gy 24 h fractionated dose was used. Fertility problems with the 101/H mice were initially encountered but results are now forthcoming. To date the mutation frequency for the hybrid control is 31.4×10^{-5}/locus/gamete (n = 3643) and for the 101/H mice, 25.9×10^{-5} (n = 1656). Little difference is therefore indicated, which in fact is in accord with the similar 101/H and hybrid translocation response to the 1+5 Gy 24 h fractionated treatment. A difference may be expected with the single 6 Gy dose that is to be tested in the studies that are to follow.

2. Studies with other inbred strains Sterile period and recovered testis weight data have indicated large differences between the sensitivities of the spermatogonial stem cells of several inbred strains to radiation killing (6 Gy). Sensitivity was highest with JU/Ct and its sub-strain JU/Ct-$+^{a}+^{c}$. Less sensitive were 129/H and CBA/H and least sensitive were C3H/HeH and C57BL/6J. The 'sensitive' 101/H strain, previously studied, fitted only into the intermediate category (129/H, CBA/H) of this range of strains. All strains were more sensitive than

the 'standard' C3H/HeH x 101/H hybrid. Hybrids between the highly sensitive JU/Ct and intermediate 101/H or more resistant C3H/HeH strains were resistant like that of the standard hybrid. Studies with backcross animals are in progress.

The sensitivity of the C3H/HeH stem cells to radiation killing has been further investigated over a range of doses (1-7 Gy). At all doses killing was indicated to be lower than with the 101/H strain; at 1-2 Gy levels of killing approximated those of the standard hybrid but at higher doses C3H/HeH stem cells appeared more sensitive. The plateau in the C3H/HeH sterile period dose-response curve occurred at an intermediate position between that of the hybrid (6-8 Gy) and 101/H mice (3-5 Gy).

C3H/HeH mice gave markedly different sterile period responses to those of the standard hybrid to 24 h fractionated X-ray doses. At the 8 Gy dose, equal 4+4 Gy fractionation gave a sparing effect, rather than enhanced effect as found with the hybrid and, at the 6 Gy dose, equal 3+3 Gy fractionation gave a response similar to that with the single dose, whereas the hybrid shows a sparing effect. Unequal (1+5, 1+7 Gy) fractionations gave higher levels of killing in C3H/HeH mice than the single doses, as they do in the hybrid. The translocation responses to these treatments (3+3 Gy, 1+5 Gy, 4+4 Gy, 1+7 Gy, 6 Gy and 8 Gy) were unenlightening, 6.5%, 4.78%, 8.75%, 13.50% and 12.47%, but larger scale studies to investigate these further are in progress.

3. Germ cell stage sensitivity differences When split doses of radiation are given there is no clear way of ascertaining the relative contributions of the two treatments to the overall mutation yield. However, if the chemical, TEM, which is known to be capable of killing stem cells, is used as a priming dose the genetic effects of a subsequent radiation exposure can be assessed. This relies on the fact that, at the dose used (2.0 mg/kg), the chemical is ineffective in causing genetic damage. The genetic response to the combined treatment is therefore dependent only upon the X-irradiation and the sensitivity of the stem cell populations surviving the chemical treatment. HU at high doses (500 mg/kg) kills cells in the S phase. Therefore when 2 such treatments are given several hours apart most surviving cells will be in G_1. The genetic response of stem cells in this stage of mitosis to irradiation can then be determined. The specific locus mutation responses of both such modified cell populations were determined. Details of the

treatments and of the sterile period and mutation responses are shown in Table 1.

Table 1

Series	Treatment	Interval between treatments	Duration of sterile period (days)	Total progeny	Specific locus mutation frequency
	TEM	-	44	10,061	0
	X-rays	-	69	9,352	13.75×10^{-5}
I	TEM + X-rays	24 h	94	8,515	30.20×10^{-5}
	HU + HU + X-rays	3 h 3 h	95	8,034	42.6×10^{-5}
	HU + HU	3 h	43	7,382	0
	HU* + X-rays	$<^{1}/2$ h	89	6,908	10.3×10^{-5}
II	TEM + X-rays	3 h	80.5	6,711	6.39×10^{-5}
	HU + HU + X-rays	3 h	77	6,711	10.54×10^{-5}

TEM dose = 2.0 mg/kg; X-ray dose = 6 Gy; HU doses = 500 mg/kg;
HU* dose = 750 mg/kg

The TEM and HU treatments alone did not cause any clear sterile periods but it is evident that, when given in combination with X-rays, extended sterile periods indicating high levels of killing are brought about. When the TEM was given 24 h prior to the X-irradiation an augmented mutation frequency was obtained and this suggests that with split 24 h interval X-ray doses, most mutations derive from the second exposure. A reduced mutation response was however obtained when the 2 treatments were given only 3 h apart and this may mean that the TEM is killing the same cell populations and same cell stages as X-rays. HU given 3-6 h prior to the X-ray exposure also greatly enhanced the mutation response suggesting that stem cells in G_1 are the most sensitive to X-ray-induced mutation. Consistent with this interpretation HU treatment given at about the same time as the X-rays did not bring about this effect, nor was the HU treatment effective in enhancing the mutation response to X-irradiation 24 h later. Neither chemical alone induced any mutations.

The two effective combined treatments provided the highest mutation rates recorded per unit X-ray dose, and thus illustrate the very high

sensitivities of stem cells in specific stages of the mitotic cycle to radiation mutation. Other stages must therefore be much more resistant. Over 22 new dominant muations were detected in these experiments, several of which have proven to be large deletions. Allelism tests confirmed the identity of the specific locus mutations

To determine if the chemical pre-treatments modified the translocation response in the same way as they influence the specific locus mutation response, 4 groups of 10 mice were given the following treatments; 6 Gy X-rays, TEM + 6 Gy X-rays (^{1}/2 h interval); TEM + 6 Gy X-rays (24 h interval), and HU + HU (3 h interval) + 6 Gy X-rays (3 h interval). The TEM and HU doses were as described earlier. Recovered testis weights were 63.0 mg, 47.0 mg, 43.1 mg and 41.6 mg respectively, again indicating the greater stem cell killing of the combined treatments. The translocation yields were as follows, 16.56%, 10.00%, 19.14% and 20.47%, respectively. The 6 Gy response (16.5%) was remarkably high but, nevertheless, the translocation responses of the various treatments paralleled exactly the specific locus mutation responses. This suggests that the cell stages of the spermatogonial stem cell cycle most sensitive to specific locus mutation are also those most sensitive to translocation induction.

In a final unrelated experiment priming doses of TEM of different sizes (2.0 mg/kg, 3.,0 mg/kg and 4.0 mg/kg) were given 24 h prior to 6 Gy X-rays to determine if the X-ray induced yield of translocations is influenced by priming dose. Recovered testis weights were 78, 71 and 69 mg for the 3 combined treatments, and 119 mg and 99 mg for 4.0 mg/kg TEM alone and 6 Gy X-rays alone. There were therefore only minor changes in the levels of total cell killing with the increasing priming dose although even the lowest priming dose markedly enhanced the amount of killing over that of 6 Gy X-rays alone. Consistent with this, the translocation yields changed little with increasing priming dose (17.71%, 19.14% and 17.17%, respectively) although all three yields were much larger than the 6 Gy dose alone (9.33%). No translocations were found following the TEM treatment alone. The toxicity of the chemical limits testing with larger priming doses, but it would be useful to determine the lowest TEM priming dose that can enhance the response to the radiation given 24 h later.

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

V. Publications

1. PUBLICATIONS IN SCIENTIFIC JOURNALS

1. CATTANACH, B.M. and JONES, C., with appendix by PAPWORTH, D.G. Specific-locus mutation response to unequal, 1 + 9 Gy X-ray fractionations at 24-h and 4-day fraction intervals. Mutation Res., 149: 105-118 (1985).
2. CATTANACH, B.M. Translocation and specific locus mutation response of mouse spermatogonial stem cells to fractionated and combined X-ray chemical mutagen treatments. In, Progress in Clinical and Biological Research. Vol. 209B. Genetic Toxicology of Environmental Chemicals. Part B: Genetic Effects and Applied Mutagenesis, ed. C. Ramel, B. Lambert and J. Magnusson. Alan Liss Inc. New York. pp. 485-491 (1986).
3. CATTANACH, B.M. and KIRK, M.J. Enhanced spermatogonial stem cell killing and reduced translocation yield from X-irradiated 101/H mice. Mutat. Res., 176: 69-79 (1987).
4. CATTANACH, B.M., RASBERRY, C. and BEECHEY, C. Factors affecting mutation-induction by X-rays in the spermatogonial stem cells of 101/H mice. Banbury meeting report, in press.

2. SHORT COMMUNICATIONS

1. CATTANACH, B.M. and RASBERRY, C. High frequencies of X-ray induced mutations. Mouse News Letter, 75: 25-26 (1986).
2. CATTANACH, B.M. Enhanced spermatogonial stem cell sensitivity in 101/H mice. Mutagenesis, 1: 387 (Abs.) (1986).

3. CATTANACH, B.M. and RASBERRY, C. Genetical effects of combined chemical X-ray treatments in male mouse germ cells. Int. J. Radiat. Biol., 6: 985-996 (1987).
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5. CATTANACH, B.M. and RASBERRY, C. A new T allele. Mouse News Letter, 77: 122-123 (1987).
6. RASBERRY, C. and CATTANACH, B.M. A new polydactyly mutation. Mouse News Letter, 78: 50 (1987).
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11. EVANS, E.P., BURTENSHAW M.D. and CATTANACH, B.M. A large deletion at the Sl locus. Mouse News Letter, 81: 66 (1988).
12. JONES, J., PETERS, J., BALL, S., CATTANACH, B.M. and KWON, B.S. A viable deletion at albino. Mouse News Letter 82: 123 (1988).
13. CATTANACH, B.M., RASBERRY, C., EVANS, E.P. and BURTENSHAW, M. Ta^{25H}, a presumptive X chromosome deletion. Mouse News Letter 83: 160 (1989).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Universidad de Sevilla
c/ San Fernando s/n
E-41071 Sevilla

Contract no.: BI6-E-311-E

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

Dr. F. Cortes-Benavides
Departamento de Biologia Celular
Faculdad de Biologia
Avda. Reina Mercedes s/n
E-41012 Sevilla

Telephone number: 954 610261

Title of the research contract:

Adaptative response to radiation damage in human lymphocytes induced by hydrogen peroxide and its modulation by antioxidant agents

List of projects:

1. Adaptative response to radiation damage in human lymphocytes induced by hydrogen peroxide and its moodulation by antioxidant agents

Title of the project no.:

Adaptative response to radiation damage in human lymphocytes induced by hydrogen peroxide and its modulation by antioxidant agents

Head(s) of project.
Dr.F.Cortés-Benavides (Univ.of Sevilla,Spain)

Scientific staff: Dr.J.Piñero
Dr.T.Ortíz
Dr.P.Escalza
Dr.G.García-Herdugo
Dr.S.Mateos

I. Objectives of the project:

Oxygen radicals may be responsible,not only for induction of cancer,but also for other degenerative diseases and for aging. The formation of endogenous oxygen radicals,on the other hand, is an unavoidable natural process.Hydroxyl radicals are generated by ionizing radiation.Since it has been demonstrated that exposure to low doses of some mutagens are protective for the cells when they are posttreated with higher doses ("adaptive response") it is worth studying the response to ionizing radiation after exposure to hydrogen peroxide.

II. Objectives for the reporting period.

III Progress achieved:

1.Methodology

The experiments carried out consisted of a prior exposure of cultured human lymphocytes to "adapting" doses of hydrogen peroxide(H_2O_2),and a subsequent challenging of the cells with 1.5Gy of X^2 rays.
The cells were scored to see if the "conditioning" exposure to peroxide reduced the number of chromatid and isochromatid breaks induced by the challenge treatment with X rays.
Whole blood (0.5 ml) from 3 healthy donors was added to 4.5ml of RPMI 1640 medium containing 10 percent fetal calf serum,2mM L-glutamine,100 units/ml penicillin,100ug/ml streptomycin,and 2 percent phytohemagglutinin to stimulate G_0 lymphocytes.
The peroxide treatment was given as a single 30min pulse administered 24h after setting up the cultures or,alternatively, the cells received three pulses with H_2O_2 at 24,30,and 36h of culture.Challenge with X rays was always at 48h and the cultures were harvested 6h after the 1.5Gy exposure.
Since very few,if any,chromatid exchanges are found in metaphases treated this way and,on the other hand,the adaptive response is considered to be an inducible error-free repair pathway, only the numbers of chromatid and isochromatid breaks were compared statistically.
Cultures were done in duplicate and an equal number of cells was scored from replicate cultures for each treatment.Since there were no differences between replicates,the data were pooled.To determine if the number of breaks observed was significantly lower than expected,a one-tailed t test was used.

2.Results

2.1.Clastogenic effect of conditioning peroxide treatment

Preliminary experiments consisting on exposure of human lymphocytes to the conditioning doses of H_2O_2 ranging from 10uM to 250uM resulted in rather low aberration frequencies,with a maximum of 7.5 percent aberrations for the highest dose assayed. According to this result,doses of 25uM,75uM,and 250uM H_2O_2 were chosen to be given as conditioning treatments before the challenge with 1.5Gy of X rays in order to study the adaptive response to oxidative damage.

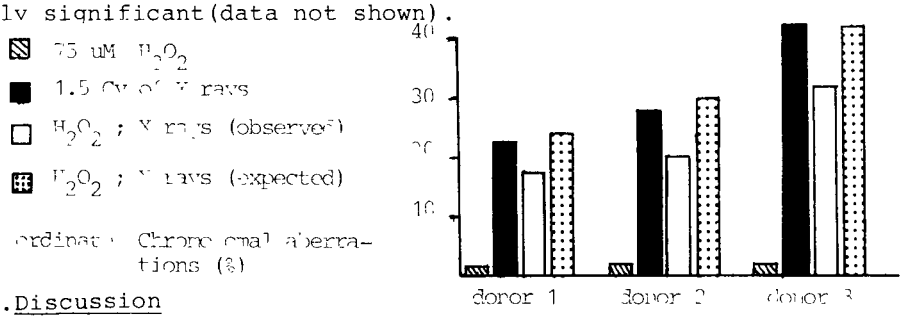
2.2.Adaptive response induced by a single pulse with H_2O_2

Fig.1 shows the total frequencies of chromatid and isochromatid breaks observed after conditioning treatment with H_2O_2,challenge treatment with 1.5 Gy of X rays,and consecutive conditioning and challenge treatments.As can be seen,pretreatment of human lymphocytes with H_2O_2 resulted in clearly diminished

aberration yields,as compared to those induced by X rays alone in the 3 donors.The difference between the expected and observed frequencies of chromosome aberrations was in all cases statistically significant,i.e. they showed the "adaptive response".

2.3.Effect of multiple pulses with H_2O_2 before X rays

When the cells were given 3 pulses with H_2O_2 at 6h intervals starting at 24h of culture and then challenged with 1.5Gy,the differences between the frequencies of aberrations expected and those observed for the combined treatment were not statistically significant(data not shown).



3.Discussion

Human lymphocytes pretreated with low doses of ionizing radiation either from incorporated tritium(Olivieri et al,1984) or from X rays(Shadley and Wolff,1987) become less susceptible to the chromosome damage induced by a subsequent irradiation with a higher dose of X rays.This phenomenon is reminiscent of the "adaptive response"to alkylation damage reported in E.coli(Samson and Cairns,1977).The response to alkylating agents,however, is known to be related to the induction of an alkyltransferase (Karran et al.,1979) which cannot be responsible for the repair of radiation damage.For Radiation Protection,a good knowledge of the levels of exposure of human population to ionizing radiation as well as the response of individuals to a given dose is necessary.Besides,exposure of cells to low doses of mutagens deserves special attention because it probably more appropriately reflects the situation in natural environments.

Peroxides yield transient radical species that can damage DNA. Such partially reduced oxygen species are also generated by lethal and mutagenic ionizing radiation.Low doses of H_2O_2 reduces the sensitivity of E.coli to a subsequent exposure to H_2O_2 or γ-rays(Demple and Halbrook,1983).Our results show the adaptive response after a single 30min pulse with H_2O_2.The conditioning doses induced only a low frequency of aberrations,if any,themselves.It therefore appears that low levels of oxidative radicals that are produced by H_2O_2,and might also be induced in water by low doses of X rays,can induce the adaptive response in human cells.On the other hand,an explanation for the lack of adaptive response when the cells received multiple pulses with H_2O_2 prior to X rays is not at hand,though the easiest explanation could be that the adaptive response becomes saturated after multiple conditioning insults.

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

Department of Radiation Genetics and Chemical Mutagenesis.State University of Leiden(The Netherlands).

Department of Genetics and Molecular Biology.University of Rome(Italy).

V. Publications:

Experimental work is still in progress.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor.

Centre National de la
Recherche Scientifique
15, Quai A. France
F-75700 Paris

Contract no · BI6-D-145-F

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

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Section de Radiobiologie Cellulaire
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Telephone number. 69-82.34.72

Title of the research contract

Induction of SOS functions from prokaryotes to higher eukaryotes.

List of projects·

1. Induction of SOS functions from prokaryotes to higher eukaryotes.

Title of the project no..

Induction of SOS functions along the evolution from procaryotes to higher eucaryotes.

Head(s) of project·

Raymond DEVORET

Scientific staff.

R. Devoret, A. Bailone, P. L. Moreau, J. Angulo, J. Célérier, S. Sommer, A.-M. Dri, M. Pierre, D. Bouillon, G. Coste.

I. Objectives of the project:

A) Since, during this contract, we have demonstrated that mouse embryos possess an analog of the RecA protein found in bacteria (Angulo et al 1989), our plan was to identify in mammalian cells the proteins which share or mimick the functions of the procaryotic RecA protein.

B) As we also found that a high cellular level of RecA protein protects chromosomal DNA against damage by physical and chemical carcinogens, we wanted to fully characterize the regulation of the various RecA protein activities in (i) the amplification of RecA protein itself, (ii) the induction of recombinational repair, (iii) mutagenesis, and (iv) the nature of the SOS signal causing the induction of the SOS pathway by the activation of RecA protein.

C) During this contract, we discovered a natural inhibitor of RecA protein and we wanted to characterize its action at the molecular level.

II. Objectives for the reporting period:

Our objectives were:

1) to identify the Kin protein and clone its cDNA from mouse embryos, coding for the functional analog of RecA protein in various mammalian cells.

2) to isolate new recA mutations that, instead of the classical mutations, would not be pleiotropic but would display only one functional deficiency, namely, in the mutagenic function of RecA protein.

3) to determine how PsiB polypeptide acts as an antagonist of RecA protein in preventing the activation of RecA protein to a coprotease.

III. Progress achieved:

Cloning of cDNA of mouse embryos that code for an analog of RecA protein.
(Angulo, Rouer, Benarous, and Devoret)

1. Methodology

We used antibodies raised against RecA protein of E. coli to identify proteins expressed by DNA fragments of mouse embryos cloned into lambda gt11.

2. Results

We have found 5 stable clones, out of which two, KIN2 and KIN17, have been sequences. KIN2 shares a large portion of its sequence with the N-terminal portion of RecA protein of bacteria from $Gram^-$ as well as $Gram^+$. KIN17, which expresses the most immuno-reactive polypeptide, shares a sequence with the carboxy-terminal end of RecA protein.

3. Discussion

Proteins involved in DNA transactions are now found to be common to procaryotes and eucaryotes. Isolation of the KIN polypeptide may help us to find the molecular basis of the SOS functions in mammalian cells, namely, the mechanism of mutagenesis.

The essential role of RecA protein in mutagenesis.
(Dutreix, Bailone, Moreau, Galibert, Battista, Walker and Devoret)

1. Methodology

In order to locate the diverse enzymatic activities of RecA protein that determine the coprotease and recombinase functions, we set to isolate new non-pleiotropic recA mutants. The recA gene of Escherichia coli was cloned into miniF plasmid. The advantage of using miniF plasmid as a vector is that the recA gene dosage is about one per chromosome, miniF plasmid producing a normal cellular level of RecA protein. We isolated the recA mutations by localized mutagenesis. All the mutations obtained were sequenced.

2. Results

We obtained a few recA mutants displaying a split phenotype, each mutant being still able to support one function of RecA protein. One mutation, recA1730, totally prevents mutagenesis. This mutation is dominant even in a wild type cell which expresses the wild type protein.

3. Discussion

We have provided evidence that RecA protein has an essential role in mutagenesis. One transversion is enough to confer to RecA protein an inhibitory action even as a heterodimeric protein. Two mechanisms of action of RecA protein may be suggested. (i) RecA protein acts at the replication fork to interact with some part of the replisome and blocks a mutator protein, or (ii) RecA protein must cleave a third protein that is involved in the by-pass of the lesion by the replisome. A unified theory may be that, in the recA1730 mutant, the UmuD protein is cleaved but the UmuCDD' complex is trapped on the mutant RecA protein and not released, preventing mutagenesis to occur.

Plasmid PsiB protein prevents cleavage of LexA protein, the repressor of SOS genes.
(Célérier, Sassanfar, Bailone, Bagdasarian, and Devoret)

1. Methodology

To show that the PsiB protein, discovered a few years ago, was really inhibiting the activation of RecA protein, we set to demonstrate the action of this protein on a reaction which is at the heart of the SOS pathway: the cleavage of LexA protein. Since Sassanfar and Roberts had isolated and purified anti-LexA antibodies, we decided to monitor the cellular level of LexA protein in cells irradiated with UV-light and carrying or not PsiB protein.

2. Results

We found that PsiB protein prevents the cleavage of LexA protein after UV-irradiation, indicating that RecA protein activation is inhibited.

3. Discussion

The PsiB protein may bind to RecA protein or may compete with it at a replication fork with DNA damage.

We have demonstrated the action of PsiB protein on the cleavage of LexA protein, a reaction that switches on the SOS pathway. Yet, we do not know if PsiB acts directly on RecA protein or indirectly by interfering with the damaged DNA structure,thus preventing the activation of RecA protein.

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

Drs. M. and M. Bagdasarian, Michigan Biotech. Inst., LANSING, MI 48909.
Dr. R. Benarous, Institut de Pathologie Mol., 75014 PARIS.
Dr. C. M. Radding, Yale Medical School, NEW HAVEN, CT 06510.

V. Publications:

1986

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

1987

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Sommer S (1987) Induction des fonctions SOS par introduction d'ADN exogène. Thèse de doctorat ès Sciences, Orsay pp 1-132

1988

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Devoret R, Bailone A, Dutreix M, Moreau PL, Sommer S, Bagdasarian M. (1988) Regulation of activation of RecA protein in *E. coli*. In: Friedberg EC, Hanawalt PC (eds) UCLA Symposia on Molecular and Cellular Biology: Mechanisms and Consequences of DNA Damage Processing vol. 83, Alan R. Liss, Inc. New York pp 437-443

Dri AM (1988) Rôle des protéines RecA, SSB et RecF dans la réparation par recombinaison chez *Escherichia coli*. Diplôme d'Etudes Approfondies, Compiègne pp 1-50

Dutreix M (1988) Caractérisation des activités de la protéine RecA impliquées dans la réparation de l'ADN et la mutagénèse. Thèse de Doctorat ès Sciences Orsay pp 1-147

Dutreix M, Bäckman A, Célérier J, Bagdasarian MM, Sommer S, Bailone A, Devoret R, Bagdasarian M (1988) Identification of *psiB* gene of plasmids F and R6-5 and molecular basis for the enhanced *psiB* expression in plasmid R6-5. Nucleic Acids Res. 16:10669-10679

Golub E, Bailone A, Devoret R (1988) A gene encoding an SOS inhibitor is present in different conjugative plasmids. J. Bacteriol. 170:4392-4394

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1989

Angulo JF, Moreau PL, Maunoury R, Laporte J, Hill AM, Bertolotti R, Devoret R (1989) KIN, a mammalian nuclear protein immunologically related to *E. coli* RecA protein. Mutation Res. 217:123-134

Arroub J (1989) Purification de la protéine RecA d'*Escherichia coli*. Diplôme d'Etudes Approfondies, Paris V pp 1-40

Célérier J (1989) La protéine PsiB, un inhibiteur de l'induction du système SOS chez *Escherichia coli*. Thèse de Doctorat ès Sciences, Paris XI pp 1-128

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Goguel V, Bailone A, Devoret R, Jacq C (1989) The bI4 RNA mitochondrial maturase of *Saccharomyces cerevisiae* can stimulate intra-chromosomal recombination in *Escherichia coli*. Molec. Gen. Genet. 216:70-74

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-E-147-F

Institut Curie
Section Biologie
Rue d'Ulm, 26
F-75231 Paris Cédex 05

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

Dr. B. Dutrillaux
Section Biologie
Institut Curie
Rue d'Ulm, 26
F-75231 Paris Cédex 05

Telephone number: 1-43.29.12.42 (Ext. 3350)

Title of the research contract:

Somatic cytogenetics of normal humans and people suspected of having a defect in the repair of DNA damage.

List of projects:

1. Study of the chromosomal constitution of normal humans and the effect of low doses of radiation.
2. Cytogenetic study of abnormal genomes particularly in cells carrying a suspected error in the repair of DNA.

Title of the project no.: 1

Study of chromosomal constitution of normal human and effect of the doses of radiation.

Head(s) of project:

B. DUTRILLAUX

Scientific staff:

AURIAS A.	MULERIS M.	FLURY-HERARD A.
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I. Objectives of 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:

Final report

III. Progress achieved:

Methodology

Estimate of the so-called spontaneous rate of chromosome anomalies :

Whole blood cultures from controls were performed for 48 and 72 h, using our usual method : cultures in TC 199 medium + 20 % human serum with phytohemagglutinin (PHA) stimulation. All mitoses were treated for R-banding (RHG). After photography, the chromosomes of each metaphase were identified and analyzed by two of us independently, and the karyotype was determined by cutting the chromosomes only when a structural rearrangement was suspected. The numbers of analyzed cells are given in the results. The following anomalies were scored for this study : chromatid breaks (ctb), chromosome gaps (csg), chromosome breaks (csb), chromatid exchanges (cte), branched chromosomes or triradials inversions (inv), reciprocal translocations (trcp), rings (r), dicentrics (dic), complex rearrangements (rea). Terminal deletions were classified into three categories : deletions without acentric fragment, deletions with acentric fragment, and deletions with isoacentric fragment.

Effect of low dose radiations :

The 10 donors used in this study did not have any pathological history and none of them were taking drugs. Blood was taken from 2 old adults (73 and 77 years old), 4 young adults (25-36 years old) and 5 newborns. Irradiations of whole blood were performed with doses of 0, 0.05, 0.1, 0.2 and 0.5 Gy using a cobalt-60 source at a dose rate of 0.5 Gy/min just before initiating the cultures. Metaphases were obtained from 48 h cultures in TC 199 medium supplemented with 20 % human serum and phytohemagglutinin. As demonstrated by bromodeoxyuridine (BrdU) incorporation

in non-irradiated cultures, more than 95 % of metaphases observed were first-generation mitoses.

Results

Spontaneous chromosome anomalies :

Chromosome lesions could be prospectively analyzed from 1000 lymphocyte karyotypes from each of the 8 controls. There was an obvious relationship between the rate of chromatid type lesions and chromosome gaps and aging. These breaks are not distributed at random in human karyotype. All controls were found carriers of fragile sites, the most frequent being located on 3p14 and 16q23. At least one fra(X)(q27) metaphase was found in each control. Radials (chromatid exchanges) were rare and were either mitotic chiasmata, i.e. occurring between homologous segments of homologous chromosomes or triradials (branched chromosomes). Thus, chromatid exchanges between non homologous chromosomes are very rare, and indicate either a recent exposure to mutagens or an abnormal constitution, especially Fanconi anemia (1). Most deletions occurred after breakage in either constitutive heterochromatin, or late replicating euchromatin. The 2 most frequent deletions are del(9)(q12) and del(1)(q12). A proportion of these deletions were associated with acentrics, which were occasionally duplicated, forming isoacentrics. These isoacentrics were formed only when the breakage occurred in a limited number of sites. Thus, a more extensive study was performed, including irradiated cells, which allowed us to analyse 100 isocentric (IA) or isocentric (IC) chromosomes. Both IA and IC were preferentially formed after breakage in heterochromatin. When euchromatin was affected, it could be shown that the sites of breakages corresponded to places where juxtacentromeric heterochromatin exists in other primate species. It is assumed that intercalary structures

conserving some properties of heterochromatin exist in human chromosomes. One of the best example is given by the excess of IA and even IC formation in 2q22 position, a band corresponding to a centromere in the chimpanzee (2).

Chromosome type rearrangements are differently distributed in newborns and adults (3). Almost all rearrangements involve bands 7p14, 7p35, 14q12 and 14q32 in newborns. They result in t(7;14), inv(14) and inv(7), and their frequency is surprinsingly higher in newborns (0.0043) than in adults (0.0024). These rearrangement seem to occur in utero, and may correspond to immunoglobulin family genes abnormal rearrangements. Conversely, all the other rearrangements are fairly rare in newborns, and have an increasing frequency with aging. In old adults, their frequency is about 0.04. This effect of aging must be kept in mind to study individuals exposed to mutagens. In particular, the effect of low dose radiation can be accurately studied in newborns, since the specific rearrangements involving chromosomes 7 and 14 are not radiation induced, whereas other rearrangements which can be radiation induced are rare at this age.

Effect of low dose radiations

The results described above were considered to develop a study on the effect of low doses radiation in relation to the age.

In old adults, no effect of radiations could be demonstrated for doses of 0.05, 0.1 and 0.2 Gy. In young adults, it was possible to find an effect for 0.2 Gy, but not for lower doses. Finally, in newborns, an effect could be observed for the lowest dose used. Indeed, the difficulties to demonstrate an effect of low doses in adult largely resulted from the background of preexisting chromosome lesions.

It was found that the types of aberrations induced largely depend on the dose. For the lowest doses (0.05, 0.1 and 0.2 Gy), deletions were efficiently induced, whereas the induction of dicentrics and translocations was detectable at 0.2 Gy and higher doses. Induced inversions were hardly detectable, even for 0.5 Gy dose.

Finally, a fairly good relationship between rearrangements and the received dose could be obtain only after substraction of the backgroung level of anomalies (4).

These results clearly point out the difficulties of chromosomal dosimetry at low doses of irradiation. Since our donors, although selected because they were healthy and non exposed to genotoxic agents, had an increase in the number of their chromosome aberrations with aging, it is likely that in dosimetry studies, confusions can be made between the effect of a natural process of aging and an exposure to mutagens.

Inversions

A large collaborative study, involving almost all French cytogenetics laboratories was developed to study pericentric inversions existing in human population (5). The whole sample is composed of 305 independent cases, most of which unpublished previously. Inv(2)(p1200q14.100) = 87 probands, inv(5)(p1400q1400) = 22 probands, inv(10)(p11.22q21.109) = 17 probands, and inv(10)(p1209q11.109) : 12 probands are the most recurrent. The risk of aneusomie de recombinaison varies from .00 to .10 in the progeny of inversion carriers, depending on the location of the breakpoints. The risk of other chromosome imbalances may be increased by a factor of 3, and that of abortions by a factor of 2. A reduction of fertility is likely to exist in male carriers, especially when large chromosomes are involved. In most instances, the apparent

preferential segregation of the inverted chromosome may be due to ascertainment biases, but such segregations may exist for some recurrent inversions. Endogany may also explain recurrence, such as that of inv(2) (p12q14.100) which is observed mostly in the Jewish communauty originating from Spain before inquisition time and from North Africa.

These inversions could be compared to those which are radiation induced and their non random occurrence was demonstrated (6).

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

V. Publications:

1. Marlhens F., Al Achkar W., Aurias A., Couturier J., Dutrillaux A.M., Gerbault-Seureau M., Hoffschir F., Lamoliatte E., Lefrançois D., Lombard M., Muleris M., Prieur M., Prod'homme M., Sabatier L., Viegas-Péquignot E., Volobouev V. and Dutrillaux B.
The rate of chromosome breakage is age dependent in lymphocytes of adult controls.
Hum. Genet., 73, 290-297 (1986).

2. Dutrillaux B., Al Achkar W., Aledo R., Aurias A., Couturier J., Dutrillaux A.M., Flüry-Hérard 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).

3. Prieur M., Al Achkar W., Aurias A., Couturier J., Dutrillaux A.M., Dutrillaux B., Flüry-Hérard A., Gerbault-Seureau M., Hoffschir F., Lamoliatte E., Lefrançois D., Lombard M., Muleris M., Ricoul M., Sabatier L. and Viegas-Péquignot E.
Acquired chromosome rearrangements in human lymphocytes : effect of aging.
Hum. Genet., 79, 147-150 (1988).

4. Lefrançois D., Al Achkar W., Aurias A., Couturier J., Dutrillaux A.M., Dutrillaux B., Flüry-Hérard A., Gerbault-Seureau M., Hoffschir F., Lamoliatte E., Lombard M., Muleris M., Prieur M., Ricoul M., Sabatier L. and Viegas-Péquignot E.
Chromosomal aberrations induced by low-dose γ-irradiation. Study of R-banded chromosomes of human lymphocytes.
Mutation Research, 212, 167-172 (1989).

5. Groupe de Cytogénéticiens Français
Pericentric inversions in man. A french collaboration study.
Ann. Génét., 29, 129-168 (1986).

6. Dutrillaux B., Prieur M. and Aurias A.
Theoretical study of inversions affecting human chromosomes.
Ann. Génét., 29, 184-188 (1986).

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.
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ZHANG F.

I. Objectives of the project:

Better knowledge of the chromosomal anomalies in genetic diseases which increase the risk of cancer. Improvement of the methods of diagnosis of the homozygote, and possibly heterozygote status.

II. Objectives for the reporting period:

Study of the karyotypes of patients affected by Ataxia telangiectasia, Fanconi anaemia, Xeroderma pigmentosum and Retinoblastoma. Research of chromosomal aberrations involved in clonal cells and characterization of the breakpoints observed in these aberrations. In situ hybridization of probes for immunoglobulin and related genes on metaphases carrying clonal rearrangements. Chromosome study in skin cancers from xeroderma pigmentosum patients.

III Progress achieved:

A number of studies were developed on various diseases predisposing to cancer. Cytogenetic and molecular biology methods were used, on blood lymphocytes, tumor cells, fibroblasts and established cell lines.

Methodology used and results obtained will be described for each disease independently.

I. Ataxia telangiectasia (AT)

Methodology

A first study was developed on PHA stimulated lymphocytes from a sample of 53 patients, which provided almost 10 000 metaphases, all analyzed using R-banding. "In situ" hybridization techniques were applied, using probes specific for the TCR α, TCR β, TCR γ constant and variable genes, nucleotide phosphorylase and IGH genes. To obtain clones with specific clonal anomalies, T-cell cultures were developed in different conditions.

Results

Clonal rearrangements involving chromosome 14 were analyzed, using high resolution chromosome banding. The wellknown t(14;14) was thus reinvestigated, and it could be proposed that it was not a simple translocation. It involves bands 14q11.1 or q11.2 and 14q32, and there is an excess of material suggesting that a duplication occurred. The same breakpoints are involved in the clonal inv(14). It was postulated that T-cell receptor and IgH genes were involved in these rearrangements (1).

In another study, all rearrangements observed in AT lymphocytes were analyzed. In addition to the overinvolvement of bands 7p14, 7q35, 14q11 and 14q32, that we demonstrated previously, it was found that some other bands (2p11, 2p12, 22q12, 22q13.2) were too frequently

affected. As for chromosomes 7 and 14, immunoglobulin superfamily genes are located in these regions, it was concluded that this family of genes were likely to be involved in the most recurrent chromosomal rearrangements of ataxia telangiectasia (2). To demonstrate this hypothesis, an "in situ" hybridization study was developed, using probes for a number of these genes. Indeed, it could be shown that rearrangements split the T-cell receptor α-chain gene, located in 14q11.2 (3).

Another information came from the comparison of the inversions affecting chromosome 14, in clones from AT and in malignant clones from T-cell lymphomas. R-banding indicated that the breakpoints might be different.

"In situ" hybridization techniques, using IGH and D14S1 probes showed that in inv(14) from AT, the breakpoint is not distal in band 14q32, and is probably located in 14q32.1. A putative oncogene may be located in this band (4,5).

Then, in collaboration with I. Kirsch laboratory, the anomalies affecting chromosome 7 were analyzed at the molecular level. The inv(7)(p14q35), which represents about half of the chromosomal rearrangements in AT was shown to result from the recombination between a TCR γ variable segment and a TCR β joining segment (6).

Xeroderma pigmentosum (XP)

Although this disease is a well-known "chromosome breakage syndrome", the cytogenetic information about an eventual specificity of breakages, occurrence of sister chromatid exchanges in relation to the type of repair deficiency were very scarce. Furthermore, information about cancer cells developed in these patients was still scarcer.

Methodology

A study was thus developed on blood lymphocytes, and in cutaneous neoplasms. 10 patients were studied for their chromosome breakage in lymphocytes. Unscheduled DNA synthesis (UDS) was studied in 9 of them. Sister chromatid exchanges were counted in a series of 15 patients, and 11 cutaneous neoplasms and two pre-tumoral lesions were cytogenetically analyzed, after short and long term cultures.

Results

The 10 patients studied for chromosome breakage were classified in relation to their UDS value : 6 were deficient and 4 proficient in DNA repair. The 4 UDS proficient patients had a rate of chromosome breakage very comparable to that of controles, whereas the 6 UDS deficient patients had a significant excess of chromosome anomalies.

This excess results from that of deletions, chromatid and chromosome gaps. These lesions were found to have a non random distribution, with an excess in G-band rich segments. This may be related to the fact that G-bands are richer in AT than R-bands, and may have a larger number of unrepaired thymidine dimers (7).

The distribution of spontaneous sister chromatid exchanges (SCEs) was studied in PHA-stimulated lymphocytes from 15 patients affected by xeroderma pigmentosum. The study of unscheduled DNA synthesis in twelve of these patients showed that seven were deficient and five proficient. The number of SCEs in XP patient cells was higher than in those of 19 controls, and the distributions of SCEs per cell were significantly different. However, the results varied when XP patients were considered in relation to their UDS : the group of XP patients with proficient UDS

did not differ, whereas the group of XP patients with deficient UDS was very significantly different from controls. The group not tested for UDS was similar to the deficient UDS group.

Since this result was published, other XP patients were studied. The study of the number of SCEs was confirmed to be a very efficient and easy method to differenciate those with or without UDS deficiency (8).

Tumor cells

On case of skin squamous cell carcinoma from a XP patient was studied at various passages of culture. Some rearrangement were observed recurrently. Dicentrics represented 53 % of all anomalies, and 67 % occurred after end-to-end association. In 38 % of dicentrics, the telomeric region of the long arm of chromosome 12 was affected. The short arm of chromosome 22 was the next most frequently rearranged. Chromosomes 12 and 22 were associated with many other chromosomes. These jumping translocations or dicentrics seem to characterize fairly early stages in tumor progression (9).

The same type of observation was performed on another case of skin tumors, in a series of eleven. On the whole, although no specific breakpoints could be detected, a clear overinvolment of centromeric and telomeric regions could be demonstrated (10).

Fanconi anemia (FA)

Methodology

PHA stimulated lymphocytes were cultured, and irradiated during S- or G2 phase, in presence or not of caffeine. Cell cycle was studied in these various conditions by adding BrdU immediatly after removal of a

thymidine block, a moment at which irradiations at 2 Gy, from a cobalt 60 source was performed.

Results

In Fanconi anemia (FA) cells, the duration of the G2 phase of the cell cycle is prolonged. Such a slowing of the G2 phase can be induced in normal cells by irradiation with γ rays during S phase, which also further increases the duration of G2 in FA cells. The addition of caffeine during the last 7 h of culture shortens the G2 phase in both nonirradiated and irradiated FA cells. In nonirradiated normal cells it may have no effect or may increase G2 phase duration, but in irradiated normal reduces the slowing of G2 induced by the radiation. This suggests that FA cells recognize and repair preexisting DNA lesions during G2 phase and that caffeine inhibits this process. The principal anomaly in FA may be a deficient repair during S phase, as manifest in the prolonged postreplication repair period during G2 phase required to repair the larger number of lesions passing through S phase.

Retinoblastoma and Wilm's tumor patients

Lymphocyte cultures from patients affected by retinoblastoma (Rb), with or without a microdeletion of chromosome 13, and Wilms tumor (WT), with a microdeletion of chromosome 11p where exposed to γ-ray radiation during S and G2 phase. Chromatid and chromosome lesions were scored and compared to those observed in controls. No significant differences were detected, neither between patients and controls, nor between patients carrying or not a microdeletion. This lack of difference was unexpected since the genes for catalase and esterase D, also called S-formyl glutathione hydrolase, which are two detoxication enzymes, are deleted in case of microdeletion of 11p and 13q, respectively.

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

V. Publications:

1. Aurias A., Croquette M.F., Nuyts J.P., Griscelli C. and Dutrillaux B.
New data on clonal anomalies of chromosome 14 in ataxia telangiectasia : tct(14;14) and inv(14).
Hum. Genet., 72, 22-24 (1986).

2. Aurias A. and Dutrillaux B.
Probable involvement of immunoglobulin superfamily genes in most recurrent chromosomal rearrangements from ataxia telangiectasia.
Hum. Genet., 72, 210-214 (1986).

3. Stern M.H., Zhang F., Griscelli C., Thomas G. and Aurias A.
Molecular characterization of different ataxia telangiectasia T-cell clones. I. A common breakpoint at the 14q11.2 band splits the T-cell receptor -chain gene.
Hum. Genet., 78, 33-36 (1988).

4. Zhang F., Stern M.H., Thomas G. and Aurias A.
Molecular characterization of ataxia telangiectasia T cell clones.II. The clonal inv(14) in ataxia telangiectasia differs from the inv(14) in T cell lymphoma.
Hum. Genet., 78, 316-319 (1988).

5. Stern M.H., Zhang F., Thomas G., Griscelli C. and Aurias A.
Molecular characterization of ataxia telangiectasia T cell clones.
Hum. Genet., 81, 18-22 (1988).

6. Stern M.H., Lipkowitz S., Aurias A., Griscelli C., Thomas G. and Kirsch I.R.
Inversion of chromosome 7 in ataxia telangiectasia is generated by a rearrangement between T-cell receptor and T-cell receptor Genes.
Blood, 74, 2076-2080 (1989).

7. Aledo R., Avril M.F., Dutrillaux B. and Aurias A.
Spontaneous chromosomal anomalies in lymphocytes from xeroderma pigmentosum. A study of ten patients.
Ann. Génét., 31, 211-215 (1988).

8. Aledo R., Renault G., Prieur M., Avril M.F., Chrétien B., Dutrillaux B. and Aurias A.
Increase of sister chromatid exchanges in excision repair deficient xeroderma pigmentosum.
Hum. Genet., 81, 221-225 (1989).

9. Aledo R., Aurias A., Chrétien B. and Dutrillaux B.
Jumping translocation of chromosome 14 in a skin squamous cell carcinoma from a xeroderma pigmentosum patient.
Cancer Genet. Cytogenet., 33, 29-33 (1988).

10. Aledo R., Dutrillaux B., Lombard M. and Aurias A.
Cytogenetic study on eleven cutaneous neoplasms and two pre-tumoral lesions from xeroderma pigmentosum patients.
In. J. Cancer, 44, 79-83 (1989).

11. Sabatier L. and Dutrillaux B.
Effect of caffeine in Fanconi anemia.I. Restoration of a normal duration of G2 phase.
Hum. Genet., 79, 242-244 (1988).

12. Sabatier L., Hoffschir F., Al Achkar W., Turleau C., de Grouchy J. and Dutrillaux B.
The decrease of catalase or esterase D activity in patients with micro-deletions of 11p or 13q does not increase their radiosensitivity.
Ann. Génét., 32, 144-148 (1989).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no : BI6-E-149-F

Commissariat à l'Energie Atomique
Institut de Protection et de
Sûreté Nucléaire
B.P. n° 6
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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 qualitative study of radiation-induced chromosomal breakage and development of a test for radiation sensitivity.

Title of the project no.:

Qualitative study of radiation-induced chromosomal breakage and development of a test for radiation sensitivity.

Head(s) of project.

B. DUTRILLAUX

Scientific staff:

AL ACHKAR W.
HOFFSCHIR F.
SABATIER L.

I. Objectives of the project:

1. Comparison of the types of chromosomal rearrangements induced by γ -rays and by heavy ions.
2. Comparison of the types of chromosomal rearrangements induced in various mammals, including man, selected for their karyotypic pecularities.
3. Assessment of the transmission of various chromosomal rearrangements through cell division.

II. Objectives for the reporting period:

Final report

III. Progress achieved:

Methodology

All experiments were performed on PHA stimulated peripheral lymphocytes obtained from human donors. For a single set of experiments, blood from a New World Monkey Ateles (Spider Monkey) was used.

The detail of the various culture conditions and drugs used will be given with the appropriate results.

All chromosome studies were performed either by R-banding or by BrdU incorporation followed by acridine orange or FPG staining.

Results

Specificity of radiation induced chromosome rearrangements :

The results obtained by studies performed during the previous contract indicated that chromosome rearrangements induced by ionizing radiations might be non random. The analyses of chromosome lesions were pursued in several conditions.

In a large survey of peri- and paracentric inversions, (see report contract BI6-E-149-F) it could be demonstrated that, in human populations, some inversions are very recurrent. A comparison of these inversions with those induced by γ -rays indicated that the same inversions were too frequently induced. This may indicate that after breakage, the numbers of reassociations leading to inversions, within a given chromosome are limited. This may correspond to homologous DNA sequences distributed along the chromosomes. In addition, it was shown that inversions are rarely induced for doses lower than .5 Gy, and that peri- are 3 to 4 times more frequent than paracentric inversions (1).

A new method of analysis was developed to identify hot and cold spots of chromosome damage. This method was applied on a study of 1598 breakpoints identified in a sample of 3264 metaphases from irradiated cultures. Indeed, this allowed us to identify a number of chromosome regions too frequently or too rarely affected (2).

A third approach to demonstrate that rearrangements do not occur at random consisted in irradiated cells from a species (Spider Monkey) with a fairly unusual karyotype, since it was composed of both very large and small chromosomes. Although anomalies of large chromosomes should have been easily detected, these chromosomes were found underaffected. In particular, they were very rarely involved in inversions. Obviously, for this karyotype, the probability of rearrangements was not related to the size of chromosomes. A possible interpretation of this finding is that large chromosome occupy large interphasic domains, which are relatively fixed. In these conditions, two breaks distantly located on the same chromosome have no opportunity to undergo a recombination (3). In addition to the role of chromosome size, it was found that one chromosome (n° 10) was much too frequently rearranged. This chromosome is equivalent to human chromosome 11, and it is noteworthy that, in the same conditions of irradiation, human chromosome 11 was also too frequently affected.

Comparison of the types of chromosomal rearrangements induced by γ-rays and by heavy ions

Chromosomal lesions, mitotic index and cell cycle progression delay induced by neutrons (protons 34 Mev on beryllium) and neon (250 Mev/u) particles were studied in human lymphocytes. The cell cycle progression was decreased at dose of 1 Gy, as well as mitotic indexes. By comparison to chromosome damages caused by γ-rays, it was found that the lesions observed after irradiation by neutrons and neon

particles are more complex on the average : the number of breaks is higher per abnormal metaphase and higher per rearrangement. This complexity is higher for neon ions than for neutron beams. Even at low doses, complex rearrangements can be formed by neon ions in some cells, indicating that they may be induced by a single particle (4).

Transmission of chromosomal rearrangements through cell divisions

A first study was developed on 2128 R-banded metaphases, obtained from γ -irradiated human lymphocytes after 48 to 96 h in culture. Depending on culture time, and possibly on the dose of radiation (1, 2 or 3 Gy) the most frequent aberrations were either dicentrics or translocations. The dose-response curve followed a quadratic function for dicentric aberration yields, but not for other rearrangements (5).

Then, a technique associating BrdU incorporation, denaturation, acridine orange staining and U.V. irradiation was applied to Go-irradiated cultures. This made possible to obtain an R-banding and to estimate the number of cell divisions undergone by each cell in metaphase since irradiation. Cell survival and slowing down of the cell cycle could be distinguished. The frequency of various types of rearrangements and their association was studied at each cell division. It could be shown that the loss of cells carrying chromosomal rearrangements is determined by several parameters such as the presence of dicentrics or multicentrics, and above all the presence of several rearrangements in the same cell (6).

The influence of time and cell cycle phase on radiation-induced chromosome lesions was studied by irradiation of lymphocytes with γ -rays every hour from 7 to 1 h before harvesting. BrdU was added to the cultures

immediatly after irradiation to estimate the cell cycle phase by studying replication band patterns for each analysed metaphase. The average number of lesions has been observed to be more related to the time elapsed between irradiation and harvesting than to the cell phase. However, the types of lesions i.e. chromatid, chromosome breaks and chromatid exchanges are closely dependent on the phase. Cells irradiated 2 h before harvesting exhibit 3 and 6 times more lesions than those irradiated 3 and 7 h before harvesting respectively. This high sensitivity of cells, 2 h before harvesting, is observed for cells irradiated during late S as well as during G2 phase.

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

V. Publications:

1. Dutrillaux B., Sabatier L., Al Achkar W., Muleris M., Aurias A., Couturier J., Dutrillaux A.M., Gerbault-Seureau M., Hoffschir F., Lamoliatte E., Lefrançois D., Lombard M., Marlhens F., Prieur M., Prod'homme M., Viegas-Péquignot E. and Volobouev V.
Radiation induced inversions in human somatic cells.
Ann. Génét., 29, 189-194 (1986).

2. Sabatier L., Muleris M., Prieur M., Al Achkar W., Hoffschir F., Prod'homme M., Gerbault-Seureau M., Viegas-Péquignot E. and Dutrillaux B.
Specific sites of chromosomal radiation-induced rearrangements.
New Trends In Genetic Risk Assessment, 212-224 (1989).

3. Hoffschir F., Prieur M. and Dutrillaux B.
Diagrammatic representation for chromosomal mutagenesis studies IV. Radiation-induced rearrangements in Ateles sp. (Primates, Platyrhini).
Mutation Research, 199, 103-110 (1988).

4. 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).

5. Dutrillaux B., Viegas-Péquignot E., Prod'homme M. and Sportes M.
Distribution of the various radiation-induced chromosomal rearrangements in relation to the dose and sampling time.
Mutation Research, 152, 197-203 (1985).

6. Al Achkar W., Sabatier L. and Dutrillaux B.
Transmission of radiation-induced rearrangements through cell divisions.
Mutation Research, 198, 191-198 (1988).

7. Al Achkar W., Sabatier L. and Dutrillaux B.
Influence of time and cell cycle phase on radiation-induced chromosome lesions.
Ann. Génét., 31, 87-90 (1988).

8. Al Achkar W., Sabatier L. and Dutrillaux B.
How are sticky chromosomes formed ?
Ann. Génét., 32, 10-15 (1989).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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und Umweltforschung mbH.
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Contract no.: BI6-E-156-D

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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) Extend the control sample size for dominant cataract mutations in the mouse.

b) The strain comparison of sensitivity to radiation-induced specific locus and dominant cataract mutations will be continued for inbred strain AKR.

c) An estimate of the induced mutation rate in oocytes of the mouse following 3 Gy will be continued.

d) The species comparison between golden hamster and the mouse for the radiation-induced mutation rate to dominant cataract alleles will be continued at 2+2 Gy.

e) Recovered dominant cataract mutations will be phenotypically and genetically characterized.

III. Progress achieved:

Estimates of genetic risk to man from exposure to ionizing radiation of necessity must be based on experimental results obtained in laboratory animals. Two sets of data are required for valid estimation of genetic risk to man from results in other species; (1) those based on a number of genetic endpoints, several of which can be applied in each species studied, and (2) those derived from a number of different species, strains or genotypes, based on one or more genetic endpoints. The key aims of the project are to provide a better basis for the extrapolation of animal data to man. With the completion of the present contract, experiments were undertaken to study the following assumptions required in procedures to estimate the radiation genetic risk in man from experimental data; (1) linear relationship of radiation dose and the induction of dominant mutations, (2) equality of the radiation doubling dose for different genetic mutational endpoints, (3) equality of the sensitivity to mutation induction of oocytes and spermatogonia, (4) that results for (102/ElxC3H/El)F_1 hybrid mice are representative in estimating the induced mutation rate in the mouse, and (5) the sensitivity to mutation induction by radiation is similar in the germ cells of different species of mammals.

METHODOLOGY

Dose response analysis in mice

Homozygous wildtype (102/ElxC3H/El)F_1 hybrid male mice were exposed to single, acute (0.75 Gy/min) gamma irradiation and mated to unexposed Tester stock females, homozygous recessive at seven specific loci controlling hair pigmentation and the size of the external ear (a, b, c, d, p, se, s). Offspring were screened for phenotypic variants indicative of recessive specific locus or dominant cataract mutations. When possible presumed mutations were genetically confirmed by breeding tests.

Oocyte experiments in mice

Female homozygous wildtype (102/ElxC3H/El)F_1 hybrid mice were exposed to single, acute (0.75 Gy/min) gamma irradiation and mated to untreated Tester stock males homozygous recessive at seven visible marker loci (a, b, c, d, p, se, s). Offspring were scored for induced recessive specific locus and dominant cataract mutations. All presumed mutations were genetically confirmed when possible.

Mouse strain comparisons

Male (102/E1xC3H/E1)F_1 hybrid, BALB/c, DBA/2 or AKR mice were exposed to 3+3 Gy (0.75 Gy/min; 24 h interval between exposures) and mated to untreated Tester stock females, homozygous recessive at seven visible marker loci (a, b, c, d, p, se, s). (102/E1xC3H/E1)F_1 hybrid mice are homozygous wildtype and induced mutations could be screened at all seven marker loci. Strain BALB/c is homozygous b/b, c/c and offspring could be screened at the five remaining marker loci (a, d, p, se, s). Strain DBA/2 is homozygous a/a, b/b, d/d and strain AKR is homozygous a/a, c/c, so that in these experimental groups specific locus mutations could be screened at the four (c, p, se, s) and five (b, d, p, se, s) marker loci, respectively. Offspring from all four treated genotypes were screened for dominant cataract mutations. When possible all presumed specific locus and dominant cataract mutations were genetically confirmed.

Mouse-hamster species comparison

Male golden hamster (Mesocricetus auratus) from a random bred stock maintained at Neuherberg or male (102/E1xC3H/E1)F_1 mice (Mus musculus) were exposed to a fractionated dose of acute (0.75 Gy/min; 24 h interval between exposures) gamma irradiation and mated to unexposed females. Offspring were screened for dominant cataract mutations and presumed mutations were genetically confirmed when possible.

RESULTS AND DISCUSSION

Dose response analysis in mice

The frequency of recovered specific locus and dominant cataract mutations is indicated in Table 1. It is evident that radiation increased the mutation rate for both genetic endpoints. Data were subjected to a regression analysis for the linear model, $y = a + bx$, where y is the per locus mutation rate, a is the spontaneous mutation rate, b is the radiation induced mutation rate per Gy, and x is the radiation dose in Gy.

TABLE 1: RECESSIVE SPECIFIC LOCUS AND DOMINANT CATARACT MUTATION RATES FOLLOWING ACUTE IRRADIATION

	SPECIFIC LOCUS			DOMINANT CATARACT		
DOSE	MUTANTS	OFFSPRING	MR*	MUTANTS	OFFSPRING	MR*
0	22(16)§	248 683	1.3	4	43 223	0.3
1.5	11	28 964	5.4	2	23 157	0.3
3.0	6	24 416	3.5	3	22 712	0.4
6.0	15	18 176	11.8	3	17 599	0.6

§ Independent mutational events given in parentheses.
* MR = mutation rate per locus $x10^{-5}$; for the dominant cataract results 30 mutable loci assumed (see Ehling, 1985).

For the specific locus mutations the best fit was

$y = (1.17 \times 10^{-5}) + (1.67 \times 10^{-5})x$. The best fit for the dominant cataract mutation data was

$y = (0.17 \times 10^{-5}) + (0.07 \times 10^{-5})x$. These results indicate that radiation induces per locus, by a factor of 24, more recessive specific locus mutations than dominant cataract mutations. We interpret this to be due to the quality of DNA damage ultimately resulting in a recessive or a dominant allele (Ehling and Favor, 1984; Ehling et al., 1982, 1985; Favor, 1983, 1986; Kacser and Burns, 1981; Kohn and Melvold, 1976). A recessive mutation represents a loss of functional gene product. Radiation, which mainly results in DNA deletions, would be expected to induce a large proportion of loss mutations. Dominant alleles, by comparison, mainly represent alterations of a functional gene product which in the heterozygote interfere with the normal gene product. These mutations would be expected to be more likely due to point mutations. Thus the greater likelihood that radiation induces recessive rather than dominant mutations.

The data may be analysed to estimate the radiation doubling dose for the induction of recessive specific locus or dominant cataract mutations. The doubling dose is defined as that dose which results in an induced mutation rate equal to the spontaneous mutation rate. It is calculated as the ratio of the fitted regression parameters, a/b. For the specific locus mutation results the doubling dose is estimated to be 0.7 Gy whereas for the dominant cataract mutation results the doubling dose is estimated to be 2.4 Gy. This observation is most important for two reasons. First, the doubling dose method of genetic risk estimation has assumed the doubling doses for different genetic endpoints to be the same and can be based upon the doubling dose estimated for the specific locus alleles (UNSCEAR, 1972, 1977, 1982, 1986). Our present results suggest that the different genetic endpoints may have different doubling doses, which violates the basic assumption of the doubling dose method as presently employed. Should the doubling dose method be used to estimate the genetic risk to man, an estimate of the doubling dose for each genetic endpoint would be required. Second, the radiation doubling dose has been estimated for induced mutations recovered in the offspring of the survivors of the Hiroshima and Nagasaki atomic bombings and it has been suggested that the doubling dose in humans is higher than that in the mouse (Schull et al., 1981). The relevance of the mouse experimental data for extrapolation to humans has been

questioned. However, the human doubling dose for dominant genetic traits has been compared with the mouse doubling dose for recessive specific locus mutations. Our present results suggest that the comparison is confounded by a difference in the genetic endpoints screened. Thus, differences observed can not be concluded to be species differences. In fact, the doubling dose which we estimate for dominant cataract mutations in the mouse is similar to the doubling dose for dominant genetic traits in humans (Neel et al., 1974; Schull et al., 1981), and supports the assumption that mouse germ cell mutagenicity data are the most relevant experimantal data for extrapolation to humans.

Oocyte experiments in mice

Table 2 presents the results of radiation induced dominant cataract and recessive specific locus mutations in exposed oocytes in which the

TABLE 2: COMPARATIVE MUTATION RATES IN DIFFERENT GERM CELLS OF THE MOUSE FOLLOWING IRRADIATION

DOSE	GERM CELL§	SPECIFIC LOCUS MUTANTS/OFFSPRING*	DOMINANT CATARACT MUTANTS/OFFSPRING*
3 Gy	oocytes	5/ 8 208 (8.7)	2/ 8 208 (0.8)
3 Gy	pg	12/ 6 572 (26.1)	4/ 6 465 (2.1)
3 Gy	g	6/24 416 (3.5)	3/22 712 (0.4)
6 Gy	oocytes	18/11 379 (22.6)	3/11 379 (0.9)
6 Gy	pg	2/ 1 509 (19.0)	0/ 1 487 (-)
6 Gy	g	16/18 176 (12.6)	3/17 599 (0.6)

§ pg = post spermatogonial stages; g = spermatogonia
* Mutation rate per locus x 10^{-5} given in parentheses; for dominant cataract mutations 30 mutable loci assumed (see Ehling, 1985).

interval between exposure and fertilization was one week or less. At intervals greater than one week at the doses employed cell killing was too extensive so that only a very small number of offspring were recovered and a meaningful estimate of the induced mutation rate was not possible. Radiation was effective in inducing dominant cataract as well as recessive specific locus mutations. Comparative results at the same doses for spermatogenic stages of the mouse are included and indicate the induced mutation rate in oocytes to be similar to the radiation induced mutation rate in spermatogonia. These results support an assumption to now required in the

estimation of the human radiation genetic risk (see Abrahamson, 1979, 1985; BEIR, 1972, 1980; Ehling, 1982, 1984a, 1984b, 1988; Lüning and Searle, 1971; Oftedal, 1984; Oftedal and Searle, 1980; Searle and Edwards, 1986; UNSCEAR, 1972, 1977, 1982, 1986), i.e. the sensitivity of oocytes to the induction of dominant mutations by radiation is similar to the sensitivity of spermatogonia.

Mouse strain comparison

The number of specific locus and dominant cataract mutations recovered in offspring of four treated genotypes are given in Table 3. All offspring resulted from 3+3 Gy irradiation of spermatogonia. The observed per locus mutation rate for the treated genotypes (102/ElxC3H/El)F_1, BALB/c, DBA/2

TABLE 3: SPECIFIC LOCUS AND DOMINANT CATARACT MUTATIONS INDUCED FOLLOWING 3+3 Gy SPERMATOGONIAL IRRADIATION IN THE MOUSE

GENOTYPE	OFFSPRING	a	b	c	d	p	se	s	CATARACT
Hybrid	18 139	1	4	3	3	7	3	8	3
BALB/c	14 132	1	-	-	5	1	2	8	4
DBA/2	15 931	-	-	2	-	2	1	15	12
AKR	13 954	-	0	-	1	1	1	3	5

and AKR for specific locus alleles was 22.8 x 10^{-5}, 24.1 x 10^{-5}, 31.4 x 10^{-5} and 8.6 x 10^{-5}, respectively. For the dominant cataract alleles the observed mutation rate in the four genotypes was 0.6 x 10^{-5}, 0.9 x 10^{-5}, 2.5 x 10^{-5}, and 1.2 x 10^{-5}, respectively. Results for specific locus alleles indicate the genotypes (102/ElxC3H/El)F_1, BALB/c and DBA/2 to have a similar mutation rate following irradiation while a lower mutation rate was observed in strain AKR. This is a first indication that the mutation rate to specific locus alleles following radiation is dependent upon the genotype of male treated. A similar observation was not observed for dominant cataract alleles. In fact the genotypes (102/ElxC3H/El)F_1, BALB/c and AKR were observed to be similar whereas strain DBA/2 was observed to have a higher mutation rate to dominant cataract alleles. Strains BALB/c and DBA/2 were chosen since they represent extremes in the sensitivity to radiation toxicity (Roderick, 1963) or the induction of dominant lethals (Storer,

1967) as well as the DNA repair capacity as measured by unscheduled DNA synthesis (Sega and Sotomayor, 1982). The sensitivity to mutation induction presently observed does not comply with our experimental hypothesis based on radiation toxicity, dominant lethals and DNA repair. Until these discrepancies can be clarified we maintain the cautious interpretation that no clear cut demonstration of an effect of genotype on the sensitivity to mutation induction in germ cells of the mouse was observed and assume results from (102/ElxC3H/El)F_1 hybrid mice are representative.

Mouse-hamster species comparison

The critical assumption in an extrapolation from experimental results in the mouse to a human genetic risk is a similarity of the sensitivity to mutation induction in germ cells of mammals. Since comparable experimental results for mouse and man are not possible, this assumption can only be tested in an array of mammalian laboratory species. Results for radiation induced dominant cataract alleles in the golden hamster and the mouse are given in Table 4. A species difference in radiation induced cell killing was observed. 3+3 Gy exposure resulted in over 90% sterility in the golden

TABLE 4: COMPARATIVE RADIATION INDUCED DOMINANT CATARACT MUTATION RATE IN THE GOLDEN HAMSTER AND THE MOUSE

SPECIES	DOSE	MUTANTS	OFFSPRING	MR*
Hamster	Control	0	5 562	-
	2+2 Gy	3	6 983	1.4
	3+3 Gy	0	110	-
Mouse	Control	4	43 223	0.3
	2+2 Gy	3	9 160	1.1
	3+3 Gy	2	6 050	1.1

* MR = mutation rate per locus x 10^{-5}; 30 mutable loci assumed (see Ehling, 1985).

hamster and so few progeny were produced that a meaningful estimate of the induced mutation rate was not possible. At a lower dose, 2+2 Gy, dominant cataract mutations were recovered in both the golden hamster and the mouse with an observed mutation rate of 1.4 x 10^{-5} and 1.1 x 10^{-5} in the hamster and mouse respectively. These first results for the induction of comparable mutations in the germ cells of different mammalian species indicate no ob-

servable difference in the radiation induced mutation rate which supports the basic assumption critical for an extrapolation of experimental results to man.

CONCLUSIONS

In an outbred, randomly breeding population, such as in humans, the risk posed by an increased mutation rate due to radiation exposure is from dominant deleterious alleles since by definition newly occurring dominant mutations will have an immediate phenotypic effect in carriers. There are presently two methods to estimate the radiation genetic risk in man based on experimental results in animals, the direct method and the doubling dose approach. Both methods extrapolate representative results in the mouse to humans based upon the assumption that mammalian species have similar sensitivities to the induction of germ cell mutations by radiation. The direct method of genetic risk estimation extrapolates the expected number of dominant deleterious mutations in humans based upon experimental results for dominant mutations in the mouse. In contrast, the doubling dose approach estimates the increased frequency of dominant deleterious mutations in humans following radiation exposure based upon experimental results in animals for recessive specific locus mutations. Our most important observation during the contract period is that the critical assumption in the doubling dose method of genetic risk estimation as presently employed, i.e. the radiation doubling dose is similar for all genetic endpoints, may not be correct. We suggest that extrapolation procedures to estimate dominant deleterious mutations in humans should be based on experimental results for similar genetic endpoints in animals.

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

V. Publications:

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Title of the project no.: 2.

Biochemical analysis of cataract mutants in mice.

Head(s) of project.

J. Graw

Scientific staff:

L. Coban

A. Liebstein

I. Objectives of the project:

a) The Cat-2^{no} (formerly: Nop) cataract of the mice is a nuclear opacity, which is inherited as an autosomal dominant gene. Among the proteins of the lens, gamma-crystallins were diminished as compared to the wild type. The reason for the decreased amount of these proteins in the Cat-2^{no} lenses was investigated.

b) The lenses of 10 dominant cataract mutants in mice were analyzed biochemically including protein analysis, determination of metabolite concentrations and of enzyme activities.

II. Objectives for the reporting period:

1. The sequence analysis of the gamma-crystallin genes in wild type was continued.

2. Experiments were initiated to study the regulation of transcription of gamma-crystallin genes in the wild type as well as in the Cat-2^{no} mice.

III. Progress achieved:

In the contract period a series of experiments were conducted to determine histological and biochemical alterations in some spontaneous and radiation-induced cataract mutations in rat and mice. The experiments were performed to find out if one or more of the different described mechanisms of cataractogenesis occur also in the newly detected mutants. Additionally, for the Cat-?^{no} mutants the relationship between the altered lenticular differentiation and the diminished gamma-crystallin gene expression was studied by investigating DNA-protein interactions.

METHODOLOGY

All investigations used lenses of three week old cataract mutants and the corresponding wild type littermates. For histological observations, bulbi were enucleated, fixed and embedded in methylmethacrylate or paraffin. 3/um sections were stained and examined microscopically. The concentration of ATP and oxidized glutathione (GSSG) in perchloric acid extracts of single lenses was determined fluorometrically 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. The protein content was determined and protein composition was alayzed by isoelectric focusing (PAGIF) and electrophoresis (PAGE). Cytoskeletal proteins were analyzed after PAGE using Western blot techniques with antibodies against vimentin.

Freshly prepared total lens RNA obtained from three week old mice was analyzed by Northern-blot hybridization for alpha-A-, beta-23-, and different gamma-crystallins (the corresponding plasmids were kindly provided by Dr. J. Piatigorsky, National Institutes of Health, Bethesda, Maryland, USA). Genomic DNA was digested by different restriction enzymes and analyzed by Southern blotting procedures utilizing cDNA probes specific for gamma-crystallin sequences. Genomic DNA-libraries of wild type and homozygous Cat-2no mice were established in EMBL-3A phages. Clones containing gamma-crystallin genes were isolated by screening the libraries with cDNA probes coding for gamma-crystallins. Restriction patterns were determined using synthetic oligonucleotides as hybridization probes after digestion of the isolated phage DNA with different restriction enzymes. To detect factors binding to the 5' upstream regions of gamma-crystallin genes, a 208 bp fragment was incubated with lens extracts from mice and rats as well as with bovine crystallin containing fractions. DNA-protein complexes were analyzed in PAGE under nondenaturating conditions. Binding of DNA to lens extracts was quantified using the binding of DNA-protein complexes to nitrocellulose filters. Molecular weight of the DNA-binding proteins was determined in a gel overlay assay.

RESULTS AND DISCUSSION

A set of spontaneous (Cat-2^{no}, formerly Nop: nuclear opacity; Cat-2^{sc}, formerly Scat: suture cataract) and radiation-induced cataract mutations in mice (Asc-1, Asc-2, Cat-2^{t}, Cat-3^{vao}, Coc, Pcs-2, and Tcm) and rat (cat) were investigated for biochemical alterations in the cataractous lenses. Mutants from the Cat-2 allelic group were selected for further analysis of their protein composition and also for histological examination.

To determine whether an unbalanced redox state might accompany the development of particular inherited mouse cataracts, the lenticular content of GSSG and the activity of SOD were chosen as markers. For wild-type lenses, an enhanced GSSG content could be observed in females as compared to males. Such a sex effect could not be detected for the SOD activity. In the mutants, GSSG content in cataractous lenses was found to be enhanced in 4 (Cat-2^{no}, Cat-2^{sc}, Cat-2^{t} and Tcm) of 10 cases; the increases in other mutants were not significant. A decrease of the SOD activity as discussed as a causative factor in a murine hereditary cataract (Bhuyan and Bhuyan, 1984) was not observed among the mutants analyzed.

Seven cataract mutations were investigated for effects on osmotic alterations in the lenses. The water content is enhanced only in lenses of homozygous mutants from the Cat-2^{no} and Cat-2^{sc} line. Additionally, it is enhanced both in the heterozygous and homozygous Cat-2^{t} and Cat-3^{vao} lenses. In all cases, when the water content is enhanced, there was an accompanied increase in the Na^{+}-K^{+}-ATPase activity and a decrease in the ATP concentration. In no mutant line investigated could a decreased Na^{+}-K^{+}-ATPase activity be found similar to the causative factor in the Nakano mutant line (Fukui et al., 1978) leading to an increase of lenticular water content and swelling of lens fiber cells (Iwata, 1980).

The mutants Cat-2^{no}, Cat-2^{sc} and Cat-2^{t} exhibit alterations in the lenticular protein composition. They were characterized in the distinct mutant lines in different ways: In PAGE the Cat-2^{t} mutants exhibit additional bands at higher molecular weight. Some of them could be demonstrated to be caused by an overrepresentation of vimentin, but cannot be explained by crosslinks, which may be formed by the enhanced lenticular TGase activity, observed in Cat-2^{no} and Cat-2^{t} mutants. However, in both mutant lines the TGase activity in liver remained unchanged. In contrast to the murine cataract mutants, the TGase activity of the dwarf cat-rat is enhanced both in liver and lens. Cataractogenesis in the cat-rat is part of an overall syn-

drome which includes dwarfism and reduced litter size. TGase may be a key enzyme in these various biological processes.

The variations concerning vimentin in the murine Cat-2^{t} mutants might indicate a changed lenticular development: the presence of vimentin in the water-soluble lens fraction can be explained by the destruction of the intermediate filament leading to the release of the corresponding proteins into the water soluble fraction. Destruction of filamentous structures might occur either by an unspecific proteolytic activity or by a directed process. However, proteolytic activities lead to degradation products of lower molecular weight. In contrast, PAGE and Western blot experiments indicate that the molecular size of vimentin remains unchanged suggesting a specific monomerization process of the filamentous proteins. Such processes have already been reported for vimentin filaments: evidence was obtained for site-specific, phosphorylation-dependent disassembly of the vimentin filaments (Inagaki et al., 1987). The phosphorylation of lens vimentin is increased by epinephrine. It demonstrates - at least in part - a regulation of lens fiber intermediate filament phosphorylation via a beta-adrenergic receptor coupled to c-AMP production (Ireland and Maisel, 1987) involved in gene expression and differentiation. Up to now, neither the c-AMP-system nor cytoskeletal proteins have been reported to be involved in hereditary cataracts. Recently it was demonstrated that overexpression of the vimentin gene in transgenic mice inhibits normal lens cell differentiation (Capetanaki et al., 1989).

The mutants derived from the different alleles of the Cat-2 locus were analyzed histologically. In sections of the Cat-2^{sc} mutants, a hydropic swelling of lens epithelium was observed in the heterozygotes, whereas in homozygous mutants interruptions and degeneration of lens fibers as well as clefts and folds of the capsule were observed. In the lenses of heterozygous and homozygous Cat-2^{t} mutants the epithelial and fiber cells were swollen and the lens capsule was ruptured. The histological analysis demonstrated a complete destruction of cellular organization of the lens. Cat-2^{no} was detected by slit lamp investigations and characterized as a nuclear opacity. Histological investigations confirmed these findings and revealed additionally polar cataracts with vacuolization. In contrast to wild-type lenses, the nuclei of the cortical cells could also be detected in the area of the lens nucleus in the Cat-2^{no} lenses. No other pathological alterations were found.

The most intensive studies among our different cataract mutant lines

have been performed concerning the Cat-2no mutants. At the protein level, the cataractous Cat-2no lenses exhibit a reduced content of gamma-crystallins as demonstrated by PAGIF. Northern blots probed with cDNA specific for alpha-, beta- and gamma-crystallin genes suggested a reduced transcription of the gamma-crystallin genes. In contrast, the transcription of alpha- and beta-crystallins appeared to be similar in wild type and the mutants. The selective reduction in the amount of gamma-crystallin specific RNA can be discussed as a biochemical indicator for the histologically observed changed differentiation in the cataractous Cat-2no lenses, since gamma-crystallins are fiber-cell specifically expressed (Papaconstantinou, 1967).

The murine gamma-E-crystallin gene was isolated from a genomic DNA library and sequenced from 650 bp in the 5' region down to the second exon. The classification as murine gamma-E-crystallin was confirmed by DNA sequence comparison with the already known rat and murine gamma-crystallin genes. The result presented here may settle the controversey about the murine gamma-2-crystallin classification (Bloemendal et al., 1989).

Factors from mouse and rat lenses were identified binding to a 208 bp AluI/NcoI-fragment of the 5' region from the murine gamma-E-crystallin gene. The fragment includes some regulatory elements (two TATA-boxes, a rudimentary CAAT-box) and the putative transcriptional and translational start site. Following phosphatase treatment the DNA-binding capacity of murine lens extracts is decreased by 90%. The DNA-binding factors can be observed also in isolated bovine beta-H-, beta-S-, and gamma-crystallin containing fractions. In contrast, alpha-crystallins as well as the beta-L-crystallin fraction do not bind to DNA. Evidence was obtained that the in vitro DNA-binding data might be of biological relevance: in lens extracts from murine cataract mutant lines Cat-2no the overall binding of lens extracts to the particular DNA fragment was diminshed to about 50%. The observed DNA-protein complexes are different from that observed in the wild types.

In cooperation with Prof. Vrensen (The Netherlands Ophthalmic Research Institute, Amsterdam) postnatal lens development was compared between wild-type mice and carriers of the dominant cataract mutation Cat-2no. At postnatal day 1, in wild-type mice the lens nuclear fibers were filled with large spherical electron dense bodies, which gradually become smaller with postnatal age and nearly disappear at day 21. The epithelium and cortex of the lens from mutants are hardly distinguishable from the wild type. How-

ever, the fiber remnants of the lens nucleus are filled with large irregular, electron dense material. The electron density of these structures is identical to the dense bodies in the day 1 old wild-type lenses. It indicates a failure in the late stages of denucleation in the cataractous lenses.

From dav 1 to day 21 old mice, concomitant to the synthesis of the lens fiber cell specific gamma-crystallins, a clear switching of factors was observed binding to the AluI/NcoI-fragment of the murine gamma-E-crystallin gene. In contrast, in lenses from Cat-2^{no} mutants exhibiting fewer gamma-crystallin content DNA binding factors could be observed only at day 1. The binding decreases very rapidly with age; in the homozygous mutants the DNA binding is much more reduced. From the temporally coincident occurrence of the factors in the wild-type lenses as well as from the inhibition of both processes in the cataractous lenses it is suggested that the factors binding to the 5' region of the gamma-E-crystallin gene are also involved in lens differentiation including its denucleation process.

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

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V. Publications:

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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:

- Some selected radiation-induced mutations with altered phosphoglycerate mutase or glutathione reductase activity will be characterized biochemically.
- The control sample size for enzyme-activity mutations in offspring from $(102/El \times C3H/El)F_1$ males will be extended.

III. Progress achieved:

Results obtained with the recessive specific locus test with mice have been used as a basis for the estimation of the genetic risk of man exposed to radiation. However, the number of loci screened with the specific locus test is a very small fraction of the total genome, the results represent only one class of mutations (recessive visibles), and large numbers of animals are required to screen mutations. These disadvantages can be partially overcome by combining the scoring of specific-locus mutations with the screening of dominant cataract mutations, protein-charge mutations and enzyme-activity mutations. The screening for enzyme-activity mutations was developed in our laboratory as a new method for the detection of biochemical mutations in germ cells of mice (Charles and Pretsch, 1982). The principle is based on the determination of specific activity in erythrocyte enzymes of offspring from treated animals. For the specific locus test defined strains of mice have to be used. It is known from other organisms that the mutation frequency is dependent on the genetic background. See, for example, reviews for Bacteria (Osanna et al., 1986), Neurospora (Schroeder and Olson, 1980), yeast (Lemontt, 1980), Drosophila (Smith et al., 1980) and humans (Hanawalt and Sarasin, 1986). Therefore, it is an essential advantage of our method that no special strains of mice are required.

It can be concluded that more studies on the genetic effect of radiations in mice are needed in order to (1) gather additional information for an improved comparison between mutation rates obtained with test systems screening different genetic endpoints, (2) investigate the influence of the genetic background of the experimental animal, (3) expand the data-base of radiation experiments with biochemical methods.

The objective of the programme was the determination of mutation rates in offspring of irradiated mice by detecting enzyme-activity mutants, in a combined experiment scoring simultaneously specific-locus mutants.

METHODOLOGY

Male mice were irradiated for different purposes: a) for establishing a dose-effect curve, (102/ElxC3H/El)F_1 males were exposed to 1.5, 3 or 6 Gy, respectively; b) for a strain comparison, AKR, BALB/c, (102/ElxC3H/El)F_1 or DBA/2 males were exposed to 3+3 Gy with a 24h fractionation interval (acute irradiation with 0.75 Gy/min). Immediately after treatment each male was mated with an untreated test-stock female, homozygous for 7 recessive loci

(a, b, c^{ch}, d, p, s, se). The offspring were screened for specific locus mutations. At 4 to 6 weeks of age, a subgroup of the offspring was examined for alteration of the erythrocyte enzyme activity of 10 enzymes: lactate dehydrogenase (LDH), triose phosphate isomerase (TPI), malate dehydrogenase (MDH), glucosephosphate isomerase (GPI), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGAM), glyceraldehydephosphate dehydrogenase (GAPDH), glucose-6-phosphate dehydrogenase (G6PD), pyruvate kinase (PK), and glutathione reductase (GR) (Charles and Pretsch, 1987). Individuals with specific activities (expressed as units per 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. The enzyme-activity mutations were maintained by backcrossing to inbred C3H/El mice. To determine homozygous viability, mutant heterozygotes were crossed inter se and offspring classified for enzyme activity. Individuals which expressed an enzyme-activity alteration more extreme than the heterozygotes were suspected of being homozygous for the mutant allele and genetically confirmed by outcrossing to strain C3H/El. If no offspring were recovered expressing an extreme phenotype from the heterozygote inter se crosses, the mutation was suspected to be either homozygous lethal or completely dominant. To distinguish between these 2 alternatives, 20 offspring expressing the mutant phenotype were recovered from the inter se crosses of heterozygotes and genetically tested to determine if they were homozygous or heterozygous. A mutation was concluded to be homozygous lethal if all 20 offspring genetically tested were shown to be heterozygous. Litter size at weaning and transmission ratio were determined for the mutations.

Two heterozygous mouse mutants with 50% and 150% of wild-type glutathione reductase (GR) activity in blood were obtained after spermatogonial treatment with 3+3 and 5.1+5.1 Gy X-irradiation. The mutant lines were designated GR 6263 and GR 33004, respectively. The genetical analysis of the mutations and the biochemical and physiological characterization of the heterozygous mutant offspring was done according to the procedure previously described (Charles and Pretsch, 1987; Merkle and Pretsch, 1989).

RESULTS AND DISCUSSION

Altogether 50 596 offspring from irradiated males have been screened for enzyme-activity alterations. The majority are offspring from treated spermatogonia and the results of the various radiation-induced mutation experiments are summarized in Table 5. For the calculation of the mutation frequencies, 12 loci were presumed for the 10 enzymes, based on the data of Green (1989). 12 loci, 10 structural, i.e. Ldh-1 (chromosome 7), Tpi-1 (chromosome 6), Mor-2, Gpi-1s (chromosome 7), Pgk (X-chromosome), Pgam-1 (chromosome 19), Gapd (chromosome 6), G6pd (X-chromosome), Pk-1, and Gr-1 (chromosome 8), and 2 regulatory, i.e. Ldr-1 (chromosome 6) and Gdr-1, were described.

TABLE 5: OBSERVED ENZYME-ACTIVITY MUTATIONS IN MICE DERIVED FROM IRRADIATED SPERMATOGONIA

DOSE (Gy)	IRRADIATED MALES	ENZYME-ACTIVITY MUTANTS/OFFSPRING	MR[a]
Control	(102/ElxC3H/El)F_1	0 / 3 610	-
1.5	(102/ElxC3H/El)F_1	0 / 6 476	-
3	(102/ElxC3H/El)F_1	1 / 7 048	1.2
6	(102/ElxC3H/El)F_1	1 / 7 539	1.1
3+3	AKR	6 / 5 608	8.9
3+3	BALB/c	2 / 3 407	4.9
3+3	(102/ElxC3H/El)F_1	1 / 3 388	2.5
3+3	DBA/2	1 / 6 415	1.3

[a] MR=mutation rate (mutations per offspring per locus x 10^5). For the enzyme-activity mutations 12 loci were assumed.

Dose-effect relationship

After exposure to 1.5 Gy, no enzyme-activity mutants were found. In the 3-Gy group and 6-Gy group in nearly the same number of offspring of treated spermatogonia 1 enzyme-activity mutant was detected, respectively (Table 5). Due to the small number of mutants the experimental variability is too large. Summing up, it may be said that in consideration of the present results a dose-effect relationship for enzyme-activity mutations is not given.

Strain dependence

The selection of tested loci is important for the level of mutation rates. For the specific-locus method there exists variability in mutation rates depending on the loci screened. When an alternate set of 6 loci (Harwell Test-Stock) was screened in a radiation-induced specific-locus experiment with the same mouse stock and the same radiation dose (Lyon and Morris, 1966), the mutation rate was about one third of that for the original 7 loci (Oak Ridge Test-Stock) (Russell, 1963). Similarly the mutation frequency of the various specific loci can vary considerably. Russell (1964) observed in radiation experiments 36 times more s than se mutations. Strain differences in the sensitivity to radiation-induced cell killing,

toxicity or dominant lethals (Ehling, 1964, 1968; Roderick, 1963; Storer, 1967) may indicate an influence of the genetic background of the test animal on mutation rates.

The aspect of the genetic background influence of the test-animal on the radiation-induced germ cell mutation rate has been examined by the detection of enzyme-activity mutants (for results of other genetic endpoints see project no. 1).

In the offspring of treated spermatogonial cells of AKR, BALB/c, (102/ElxC3H/El)F_1 or DBA/2 mice mutants were observed in different frequencies. A comparison of the mutation frequencies indicates no statistically significant differences (P=0.16). Therefore, results for enzyme-activity mutations suggests no dependence of the radiation-induced mutation rate on the genotype of the treated spermatogonia for the 4 tested. Results for specific-locus alleles indicate the genotype (102/ElxC3H/El)F_1, BALB/c and DBA/2 to have a similar mutation rate following irradiation while a lower mutation rate was observed in strain AKR. A similar result was not confirmed for dominant cataract alleles (see project no. 1). Therefore no clear cut demonstration of a genotype effect on the mutation sensitivity to induction in mouse germ cells can be stated.

Genetic analyses of mutants

Out of altogether 17 spermatogonial irradiation-induced enzyme-activity mutants, 10 mutations cause a decrease and 7 mutations cause an increase of enzyme activity (Table 6). Examining the spectrum of the obtained mutations it can be noticed that mutants were observed for 7 of the 10 screened enzymes, whereby PGAM and GR mutants occur more frequently than the others. In contrast, ethylnitrosourea-induced enzyme-activity mutations are distributed regularly among 9 of the 10 tested enzyme activities (Charles and Pretsch, 1987). No mutants have been detected for the enzyme PGK.

Genetic analyses revealed that all up to now tested mutants are homozygous lethal. This is consistent with the suggestion that radiation-induced mutations recovered in germ cells of the mouse are more likely deletions.

TABLE 6: DISTRIBUTION OF RADIATION-INDUCED ENZYME-ACTIVITY MUTATIONS AMONG THE 10 SCREENED ENZYMES

DOSE (Gy)	IRRADIATED MALES	ENZYME[a] LDH	TPI	MDH	GPI	PGK	PGAM	GAPDH	G6PD	PK	GR
3	(102/E1xC3H/E1)F_1						1d				
6	(102/E1xC3H/E1)F_1						1e				
3+3	AKR	1d					1d,2e				2d
3+3	BALB/c						1e				1d
3+3	(102/E1xC3H/E1)F_1						1e				
3+3	DBA/2										1d
5+5	(102/E1xC3H/E1)F_1							2d		1e	1d,1e
Total		1d					2d,5e	2d		1e	5d,1e

[a] d=mutant with decreased enzyme activity, e=mutant with elevated enzyme activity

Characterization of 2 radiation-induced mouse mutants (Table 7)

The determination of GR activity showed that in both mutant lines the activity alteration of approximately 50% and 150% of the wild-type value in blood is similarily expressed in plasma and several tissues studied of heterozygotes. Moreover, the comparison of the activity of nine other erythrocyte enzymes in blood lysate revealed no significant differences between mutants and wild types supporting the haematological findings which revealed no clues for altered red-cell populations in both mutant lines. Furthermore, the results obtained in the tissues suggest that the mutations affected the structural locus Gr-1.

The dominant mode of inheritance of the altered enzyme activity has

TABLE 7: CHARACTERISTICS OF 2 RADIATION-INDUCED GR MUTANTS

MUTANT LINE	WILD-TYPE ACTIVITY (%)	TYPE OF MATING[a]	LITTER SIZE (x + SD)	GENOTYPE[b] +/+	+/+*	+*/+*	RATIO +/+ : +/+*
Control	100	C	6.4 ± 0.5	162			
GR 33004	150	B	5.5 ± 0.4	256	204	-	1 : 0.80
		I	4.3 ± 0.5	66	87	0	1 : 1.32
GR 6263	50	B	5.3 ± 0.4	166	88	-	1 : 0.53
		I	4.4 ± 0.3	44	51	0	1 : 1.16

[a] C: controlcrosses (+/+)x(+/+); B: backcrosses (+/+)x(+/+*); I: intercrosses (+/+*)x(+/+*)

[b] +/+: wild types; +/+* heterozygous mutants; +*/+*: homozyougus mutants

been demonstrated by backcrossing (B) with C3H/El. However, in both mutant lines there was a significantly reduced number of heterozygotes compared with Mendelian expectations. Together with the reduced litter size in backcrosses this result suggests a reduced viability and fitness of heterozygotes. In this context it seems interesting that the body weight of heterozygotes is also reduced by approximately 10-15% compared to the wild type. A reduction or elevation of GR activity by 50% is not expected to influence metabolism. Therefore the physiological effects of the mutations in heterozygous animals are not explainable by the altered enzyme activity suggesting chromosomal mutations i.e. large intergenic deletions or gene duplication.

The observed lethality of homozygous GR mutants is consistent with the assumed chromosomal nature of the mutation.

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

V. Publications:

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Pretsch, W., Charles, D.J., Merkle, S.: X-linked glucose-6- phosphate dehydrogenase deficiency in Mus musculus. Biochemical Genetics 26, 89-103 (1989)

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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Contract no.: BI6-E-205-I

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Title of the research contract:

Response to radiations of human cells modified by pR plasmid that confers radioresistance in bacteria.

List of projects:

1. Response to radiations of human cells modified by pR plasmid that confers radioresistance in bacteria.

Title of the project no..

RESPONSE TO RADIATIONS OF HUMAN CELLS MODIFIED BY pR PLASMID THAT CONFERS RADIORESISTANCE IN BACTERIA

Head(s) of project:

Prof. R. ELLI

Scientific staff:

F. Gigliani, L. Marcucci, A. Antonelli, P. Petrinelli, P.A. Battaglia, D. Kobal, E. Sporeno

I. Objectives of the project:

The increasing number of observed phenoma, including enhanced DNA repair, virus induction, induced cellular differentiation and neoplastic transformation, phenomena resulting from DNA damage or replication arrest, suggest that there is an SOS-like system in mammalian cells. The objective of this project is to ascertain in what measure this inducible system is correlated with mutagenesis and carcinogenesis. For this purpose it will be utilized the pR plasmid that, interacting with the inducible repair pathway, confers increased survival to UV and 4NQO in prokaryotic as in mammalian cells. This system has the following advantages: it permits the evaluation of inducible response to stress, directly in the damaged cells; it makes it possible to understand the molecular and genetic processes of the inducible response related to mutagenesis and carcinogenesis.

II. Objectives for the reporting period:

The purpose of this research program is to clarify the function of uvp1 and uvp2 (mucAB genes) regions of pR plasmid, the interaction between them and the possible correlation between their expression in normal and defective repair pathways in mammalian cells. The main objectives are:

1) molecular characterization of pR uvp1 region in terms of nucleotide sequences, and codified protein in bacterial cells.
2) analysis of pR product interactions with mutagenesis and different repair pathways in bacterial and in mammalian cells.
3) Characterization of the pR effects in radiosensitive mutant cells such as Ataxia Telangiectasia cells, through survival analysis and chromosome damage evaluation.

III. Progress achieved:

METHODOLOGY

- The electroporation method for transfecting mammalian cells was performed as described by G.Chu et al.(Nucleic Acids Research,15,1311,1987)
- Southern blot hybridization was performed as described by E.N.Southern (J.Mol.Biol., 98, 503,1975).
- Dot blot hybridization was performed as described by F.C.Kafatos et al. (Nucleic Acids Res.7,1541,1979).
- Mini-cells technique was performed as described by Rambach and Hoghness (Proc.Natl.Acad.Sci.,74,5041,1977)
- Nucleotide sequence determination was performed by dideoxy-chain-termination procedure (Sanger et al.,Proc.Natl.Acad.Sci.,74,5463,1977).
- Metaphase spreads from mouse cell lines were obtained by standard methods after cell synchronization with BudR (17 hrs) followed by a 7hrs thymidine pulse. The preparations were stained by 33258 Hoechst + Giemsa or by Acridine Orange followed by CBG banding.
- Metaphases from human lymphoblastoid cells were obtained by standard method. To evaluate the induced chromosomal breakage rate, BLM(final concentration 10 or 30 µg/ml) was added to cultured cells 4hrs before harvesting.
- Clonigenicity and survival of mouse and human fibroblasts were determined as previously described by R.Elli et al.(Nucleic Acids Research,11,3679, 1983).

RESULTS

EXPRESSION OF pR PLASMID IN MOUSE LTA CELLS

The pR plasmid protects bacteria and eukaryotic cells against UV and 4-nitroquinoline -N-1-oxide (4NQO) damages through the function of two regions, uvp1 and uvp2 (mucAB genes) (Marcucci et al.,Mol.Cell.Biol.,6,586, 1986). Furthermore, we thought to test the cytotoxic effect of drugs such as bleomycin (BLM), cis-diamminedichloroplatinum (cisDDP), mitomycin (MMC), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (which are known to interact with DNA, causing different types of lesions) on cells transformed with w. t. pR plasmid (LA-D line)or plasmid pR69s mutated in mucAB genes (LA-690 line). For this pourpose the colony forming ability following 1 hour exposure to different concentration of each drug was measured. Moreover a line (LA-Dr2) was obtained by selection of LAD cells surviving at doses of 4NQO sufficient to reduce cell survival to approximately 0.1%. This line was also exposed to some of the previously mentioned drugs. LTA untransformed cells and/or ptk1 transformants were used as control. All the transformed lines used in the experiments of this work are described in table 1 and were routinely tested, for the presence of pR,by dot blot hybridization .

The analysis of the survival curves shows that LA-D cells are significatively more sensitive to the damage induced by BLM, cisDDP and MNNG than either untransformed cells or cells transformed by ptk1 or by the mutated plasmid pR69s (LA-690 line). This phenotype is in marked contrast to the greater survival rate exhibited by pR transformants following exposure to UV

light or 4NQO. Finally the presence of pR plasmid appears not to influence cell survival after treatment with MMC. These results were quatified by the Do values extrapolated from the exponential portion of the survival curves. LA-D cells resulted more sensitive to BLM (Do=7.0) and to cisDDP (Do=0.83) than the control lines (LTA Do values were respectively 20.9 and 2.64).

The BLM survival curves showed no shoulder which was present instead in cisDDP survival curves. The higher MNNG sensitivity of LA-D cells, respect to control cells, was shown both by the ratio between Do values (18.3/10.4) and by the different shoulder width of the survival curves. After MMC treatment the survival curves of all lines had about the same value (0.21). Finally, LA-Dr2 survival curves were indistinguishable from those of the parental LA-D line, further confirming the stability of the expression of the pR phenotype in transformed cells. After treatment with UV and 4NQO LA-690 cells showed survival curves not significantly different from the control ones. It seems therefore that the expression of pR mucAB genes (uvp2 region) is necessary both for the plasmid protecting effect against UV and 4NQO and for the sensitizing effect to some of the drugs used. These observations suggest an interaction between mucAB gene products and the different repair pathways involved (Elli et al.Mutat. Res.191,177,1987).

In order to investigate the function of other pR region uvpl the following different approaches were used.

A) Molecular characterization of the uvpl region. We constructed a new plasmid pL11.5 (uvpl-,mucAB+) and analysed its expression in UV repair and UV induced mutagenesis. The UV survival curve of E.Coli cells harbouring pL11.5 plasmid is superimposable on that of untransformed cells, while its ability to increase the mutation rate following UV irradiation is not different from that of the pR plasmid . We demonstrated also that the uvpl region encodes a protein (MW 20000)by comparing the electrophoretic pattern of the protein coded by the pLM54(uvpl+) plasmid with those coded by the pRES (uvpl-) plasmid. This datum confirms a previous identification of the same protein obtained with a pR mutant by Tn5 insertion in the uvpl region (Gigliani et al. Nucleic Acids Res. 9, 623,1981). The nucleotide sequence of 820 bp of uvpl region contains an open reading frame of 594 nucleotides that corresponds to the protein of 20kd. The uvpl gene cooperates with the muc genes in repairing UV damage without being involved in UV-induced mutagenesis. A function of this gene could be to enhance the recombination activity of the cells as shown at least in two different genetic system tested: i) recombination of phage arms using a new genetic element, the pR phasmid (Battaglia et al., Mol.Gen.Genet.,209,1987); ii) recombination rate in E.ColiKS391 which contains two copies of lac operon deleted in 2 non overlapping regions(Gigliani et al., Mol.Gen.Genet.218,18,1989).

B) Uvpl expression in bacterial and mammalian cells. The results obtained by analyzing the survival curves of E.Coli C600 transformed by pR w.t. and by pLM54 (a pR deleted in the uvp2 region)show that only the former protects against 4NQO and BLM damage but it has no effect on cisDDP survival. The BLM resistance conferred by pR plasmid is more apparent both when the drug concentration is increased and when time exposure is lengthened.

BLM seems to be the most informative of the drugs, considering the opposi

te effect it has on bacterial cells and on mammalian cells. Therefore we investigated the effect of BLM on mouse cell lines transformed by different pR mutants obtained by the Tn5 insertion in the uvp1 region (LA-760 line) or between these two regions (LA-542 line). The Tn5 transposon contains three antibiotic resistance genes (bleo, neo and str, respectively for bleomycin, neomycin/kanamycin and streptomycin) controlled by the same promoter. The BLM survival curves of the tested lines show that: the LA-D line (in which Tn5 transposon is not present) is more sensistive to the damage induced by BLM than either untransformed line or lines transformed by pR:: Tn5 plasmids. The lines transfected with pR::Tn5 plasmids show different BLM survival rates according to the expression of Tn 5 bleo gene that counterbalances the different expression of uvp1 and/or uvp2 regions. (work in preparation).

pR INTEGRATION IN MAMMALIAN GENOME

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 seuqences. 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 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, further restriction pattern analyses are necessary.

pR integration does not modify chromosome costitution. In order to ascertain if pR presence determines some variations in cell karyotype, one of the cotransformed line, the LAD line, has been cytogenetically characterized and compared with the untransformed LTA line, with clonal line derived from LTA and with LA-TKO line (transformed only with the tK gene).
The karyotypes of the analysed lines show small variations in the distribution of chromosome number and structural differences that could be due to the effect of cloning and/or to the different selective pressures of the growth conditions. Heterochromatin variations have been also observed in two chromosomes of the transformed line that are interesting in view of the observation that the integration of transforming DNA could occur at or near the sites of gross chromosomal rearrangements or into DNA repetitive sequences. (Petrinelli et al. Cytotecnology,1,73,1987).

EXPRESSION OF pR IN ATAXIA TELANGIECTASIA CELLS

The significant differences in BLM response between pR transformed LTA mouse cells and the untransformed line suggest that pR determines an hypersensitivity to a specific type of damage (i.e. double strand breaks) in mam-

malian w.t. cells.
The main characteristics of cells derived from Ataxia Telangiectasia patients are hypersensitivity to ionizing radiations and radiomimetic drugs (such as BLM) but not to UV light, resistant DNA synthesis following ionizing radiations, low fidelity in repairing processes, hypersensitivity to topoisomerase II poisons (such as VP16). It has been therefore hypotesized that the principal biochemical problem for AT cells is DNA double strand breaks repair which is thought to require recombinative events. AT cells therefore represent an hypersensitive biological system to study the pR plasmid expression in human cells. For this purpose two AT lines (AT5BIVA fibroblasts and ATL6 lymphoblasts) immortalized by SV40 and Epstein Barr virus respectively have been transfected with pR plasmid. 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. We have previously observed that: 1) LTA mouse cells cotrasfected with pR and psv2neo plasmids show the same increase in 4NQO and UV survival observed with tK gene cotrasfection; 2) wild type human lymphoblasts cotransfected with pR and psv2neo plasmids show increased BLM sensitivity as expected. These results demonstrate that the pR effects do not depend on cells types or on the selection used. To overcome the problem of the low efficiency of transfection of AT cells we have used the electroporation technique, optimizing various parameters such as cell and DNA concentration and electric field setting. The AT lines cotransfected with pR and psv2neo, assayed for pR presence by dot blot hybridization, were tested for BLM and VP16 survival and for BLM induced chromosome breakage. The results show that pR plasmid stably expresses itself for about 70 cell passages in vitro and amplifies the BLM and VP16 sensitivity of both AT fibroblasts and lymphoblasts.

DISCUSSION

The experimental data obtained in E.Coli have clarified the function of uvp regions: the uvp1 region contains a gene coding for a protein of 198 aminoacids which increases generalized recombination in bacteria and partecipates in UV repair by cooperating with the uvp2 region (mucAB). Even if a regulative function of the uvp1 region has not yet been demonstrated in mammalian cells, all the Tn5 insertions in this region eliminate the ability of pR to increase UV resistance in tranfected mouse cells. The ability of pR plasmid to sensitize mammalian cells to BLM depends on uvp2 region integrity: in fact this ability is lost when the uvp2 region is mutated either by the insertion or by deletion. Furthermore the BLM sensitizing effect of the uvp2 region is not tightly dependent on uvp1 region, because it expresses this function also when uvp1 is mutated. However, the observation that uvp2 region expression is enhanced in presence of a functional uvp1 confirms that these two regions cooperate in mammalian cells also. The data concerning the sensitivity of pR transfected mammalian cells show that pR plasmid has a killing effect in transfected mammalian cells when treated with drugs that determine DNA breakage. In particular, the enhanced sensitivity to DNA-double-strand-breaking agents as BLM, VP16 and cisDDP seems to be a common

TABLE 1

PLASMIDS AND CELLS USED IN THE REPORTED EXPERIMENTS

TRANSFORMING DNA	MOUSE CELLS	HUMAN CELLS		
None	LTA	ATL6	NL1	AT5BIVA
pTK1+pR wild type	LA-D			
pTK1+pR 76S (uvp1-uvp2+)	LA-760			
pTK1+pR 69S (uvp1+uvp2-)	LA-690			
pTK1+pR 54R (uvp1+uvp2+)	LA-542			
pSV2neo	LG1	ATL6pneo	NL1pneo	AT5BIVApneo
psV2neo+pR wild type		ATL6pR	NL1pR	AT5BIVApR

TRANSFORMING DNA	E.COLI STRAINS
None	C600
pR w.t.	C600 plus pR
pLM54 (uvp1+uvp2-del)	C600 plus pLM54
pRES (uvp1-mucAB-)	C600 plus pRES
pL11.5 (uvp1-mucAB+)	C600 plus pL11.54

- pR (Ap+UV+) plasmid is an HindIII fragment (23kb pairs) of TP120 and it confers UV resistance both in procaryotic and eukaryotic cells (Gigliani et al., 1981)
- pR76S - pR69S - pR54R are mutants of pR plasmid obtained by Tn5 insertions
- pLM54 is a mutant of pR obtained by deletion of uvp2 region (Marcucci et al., 1986)
- pRES plasmid is a mutant of pLM54 obtained by deletion of uvp1 region
- pL11.5 plasmid is a derivative of pRES plasmid plus mucAB genes
- pTK1 (Ap+TK+) is derived from a 3.4 kb pairs fragment of HSV cloned into the BamH1 site of pBR322 and it contains a selectable TK+ marker (kindly supplied by S. Bacchetti)
- pSV2neo is a plasmid derived by SV40 hybrid plasmid vectors; it contains a bacterial gene (neo) conferring resistance to neomicyn kanamycin antibiotics (kindly sypplied by S. Bacchetti)

feature of pR transfected mammalian cells, both w.t. and radiosensitive mutant.

Therefore, pR transfected cells can be utilized as an intriguing hypersensitive experimental system to test the cytotoxicity of other radiomimetic agents that act via DNA breakage.

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

Silvia Bacchetti - Mc Master University.Dept. of Pathology, Hamilton Ontario, USA

Massimo Fiorilli - Ist. Immunologia Univ. Studi Roma

V. Publications:

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.

-F. Gigliani, E. Sporeno, S. Perri, P. Battaglia - The uvpl gene of plasmid pR cooperates with mucAB genes in DNA repair process. Mol.Gen. Genet.,218:18-24 (1989)

-A. Antonelli, R. Elli, P. Petrinelli, D. Kobal, E. Petrucci, R. Bosi, M. Proietti, L. Marcucci - Aumentata sensibilita' alla bleomicina indotta da un plasmide procariotico in cellule di Atassia Telangiectasia in:Genetica e Ritardo Mentale Ed.Monduzzi in press.

CONGRESS AND SHORT COMMUNICATIONS

-R. Elli, A. Antonelli, P. Petrinelli, L.Marcucci, M.Proietti, F.Gigliani - A mouse cell line (LA-D UV+) lets to discriminate among different damages induced by chemotherapeutic agents. European J. of cell Biol. Supplement 15, vol. 42 (1986)

-P.A.Battaglia, F.Gigliani, S.Perri, R.Elli, L.Marcucci - Regolazione della mutazione in E.Coli. III Congr.Soc.Ital.Microbiologia Generale e Biotecnologia Microbiche, 2-4 ottobre 1986.

-R. Elli, P. Petrinelli, L. Marcucci, M. Proietti, R. Bosi, A. Antonelli - Paradoxical effect of bleomycin on bacterial and eukaryotic cells transfected by a prokariotic plasmid. Cytotechnology suppl. p19, 1988

-P.Petrinelli, L.Marcucci, A. Antonelli, R.Bosi, M. Proietti, L.Verni, M.G.Giannantonio, R.Elli - Trasfezione mediante 'electroporation' di cellule di Atassia Telangiectasia con un plasmide procariotico che induce sensibilita' alla bleomicina. III Congr. FISME 1988

- P.A.Battaglia, S.Perri, E.Sporeno, F.Gigliani - A 'controlling element' in bacteria: the pR rep region. Proceeding of the Second Italy-Japan Joint-Seminar on Biological Science p.35-36, 1988

INTERNAL REPORTS AND THESIS

- R.Elli - Espressione del plasmide pR in cellule di mammifero
- L.Marcucci - Evoluzione dei sistemi di riparazione in procarioti ed eucarioti
- E.Sporeno - (thesis) Organizzazione strutturale ed espressione dei geni del plasmide pR coinvolti nel sistema di regolazione SOS. Referee R.Elli
- M.G.Giannantonio - (thesis) Caratterizzazione dell'instabilita' cromosomica di una linea linfoblastoide di Atassia Telangiectasia trasfettata con un plasmide implicato nei sistemi di riparo del DNA. Referee A. Antonelli
- M.Vinci - (thesis) Caratterizzazione a livello citogenetico e molecolare di una linea cellulare di topo trasformata da un plasmide batterico. Referee L. Marcucci

RADIATION PROTECTION PROGRAMME
Final Report

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)]:

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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 · 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 Wilms' tumour

Head(s) of project: Professor H J Evans

Scientific staff: Dr N Hastie, Dr Veronica van Heyningen, Dr D Porteous, Dr Wendy Bickmore, , Mrs Judy Fantes, Mr E Thomson (at one time Dr Karin Buckton)

I Objectives of the project:

The aim of the project was to examine the distribution, type and consequences of spontaneous and radiation induced deletions in the human chromosome complement with emphasis on the short arm of chromosome 11. Chromosome 11 was selected since it was known (i) to contain a number of genes involved in carcinogenesis (eg Wilms' tumour, H-*ras* 1, T cell acute leukaemia), (ii) to sustain a larger number of deletions than expected on chromosome length following exposure to ionising radiation (Buckton 1976 and unpublished), (iii) that constitutional deletions of the short arm of chromosome 11 were consistent with viability when heterozygous and that various sizes of deletions were available in the human population which were associated with specific disease states eg Wilms' tumour, aniridia, etc

II. Objectives for the reporting period:

As I 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 of 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

Lymphoblastoid cell lines were established from a series of patients constitutionally heterozygous for deletions of various sizes, or for translocations, involving regions of the short arm of chromosome 11. The cytological localisation of translocation breakpoints, and the extent of each of the individual deletions, were mapped using banded metaphase chromosome preparations. Most of the patients presented with Wilms' tumour, with or without aniridia, or with aniridia but without Wilms' tumour, and all deletions involved the 11p13 region of the chromosome. Patients with Wilms' tumour and aniridia frequently also have genito-urinary abnormalities and mental retardation (the WAGR syndrome) and there was other evidence (see below) indicating that the 11p13 region of the chromosome might contain a number of genes involved in the development of the urinary and gonadal systems.

DNA samples were obtained from all cell lines and where possible 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 or translocation) most probably originates in a parental germ cell. We therefore accumulated DNA probes that recognise polymorphic DNA sequences on chromosome 11 with the aim of identifying the parental origins of the mutated chromosomes. Our findings to date

complement those of others in indicating that the original mutation preferentially occurs in the paternal chromosome, and that the corresponding maternal sequences are lost in those cells that emerge as nephroblastoma

Our strategies for isolating genes in the WAGR region of chromosome 11 has involved producing hybridoma cells between a mouse fibroblast cell line and the lymphoblastoid cell lines derived from patients heterozygous for chromosome 11p13 rearrangements in order to segregate out the abnormal human chromosome 11 on a mouse background. We discovered that 2 cell surface antigens, MIC11 and MIC4 were frequently lost when the WAGR deletion chromosome was retained in any hybrid cell. This finding allowed us to use the fluorescence activated cell sorter to select out those cells retaining a single copy of a deleted 11p13 chromosome and these cells were then used as a source of DNA to develop probes specifically for the short arm of chromosome 11. Moreover, using this approach, it was possible to map the location of the 2 cell surface marker genes in relation to the other genes that we had already mapped in that region of the chromosome.

In parallel with the use of cell surface antigens as selection factors to detect the presence of genes on chromosome 11p we have made major use of the technique of chromosome mediated gene transfer (CMGT) for generating chromosome 11 human-mouse hybrid cells. Here we have employed the approach of selecting for the Harvey *ras* 1 gene located at chromosome 11p15 to select transformed mouse fibroblasts exposed to pools of chromosomes obtained from cultures of cells from human EJ bladder carcinoma cells which contain an activated H-*ras* gene We have shown that this procedure results in the selection of cells containing segments of human chromosome 11 with the H-*ras* 1 gene plus other linked loci on a mouse genomic background In most instances the flanking genes for catalase (CAT) and folicular stimulating hormone beta (FSHβ) are both retained with the H-*ras* 1 locus. Using such transformed mouse cells containing segments of human chromosome 11, we have isolated genomic libraries by hybridisation screening for human recombinants. These have then been sublocalised on the short arm of chromosome 11 by mapping to a panel of somatic cell hybrids prepared from our cell lines from patients having different sizes of deletions in different regions of the short arm of the chromosome. In this way we were able to build up a map of the 11p13 region of the chromosome and to isolate a number of highly polymorphic anonymous DNA fragments that map proximal to the aniridia and Wilms' tumour loci.

One particular Harvey *ras* 1-selected chromosome mediated gene transformant, E65-6, has proved to be particularly rewarding. Human recombinants isolated from E65-6 were mapped to a panel of 5 WAGR deletion hybrids and 2 clinically related translocations. We showed that E65-6 is enriched about 400 fold for 11p15.4-pter markers, and about 200 fold for 11p13

markers. The transformant contains around 10 Mbp of human DNA inserted at 3 sites on a mouse chromosome and from which we derived 19 cosmid and 35 lambda human DNA recombinants which were then localised and mapped to chromosome 11. A further 44 recombinants were derived from another transformant, E67-1 and shown to also be located in the chromosome 11p part of the human genome. Figure 1 shows the mapped location of the19 cosmid and 35 lambda human recombinants derived from the E65-6 transformant. Two of the 12 recombinant probes that locate to the 11p13 region of the human genome also show a significant cross-hybridisation to mouse DNA at high stringency and probably therefore identify coding sequences.

A general, and important, point of interest from our studies on chromosome mediated gene transfer as an enrichment cloning strategy for markers immediately flanking the locus under selection, is that our results suggest that even those transgenomes which have undergone extensive and complex rearrangement still consist largely, if not exclusively, of sequences syntenic with the locus under selection. There is therefore a strong tendency for markers tightly linked on the donor chromosome to cosegregate.

We have used the recombinant markers from our CMGT experiments to hitch-hike from the Harvey-*ras* 1 locus and this has allowed us to define 7 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 genito-urinary dysplasia, were shown to map within the smallest region of overlap as defined by our WAGR deletions, and within the region subtended by our isolated recombinants. Using pulse field gel electrophoresis we have now shown that the approximately 2 megabases of DNA encompassing that part of the chromosome between the gene for FSH beta and the sequence detected by our probe P411 contains 7 HTF islands (regions of hypomethylation that appear to mark the 5' end of genes). Moreover, we have found that the HTF islands are concentrated into the G-band negative11p13 region, the G-band positive 11p14 band containing few such islands. The breakpoint of the translocation associated with Potter facies and genito-urinary dysplasia, which maps within the smallest region of over lap as defined by our Wilms' tumour-aniridia-genito-urinary-mental retardation deletions, is located close to one of these 7 putative genes. Progress has been made in walking along the region of interest towards the translocation breakpoint and we have now cloned a new gene close to this breakpoint and are currently searching for sequence homologies. We further showed that the translocation breakpoint cluster at 11p13 which is well known to be associated with human T cell acute lymphocytic leukaemia is located near to, but is outwith, the Wilms' tumour locus.

Our own studies, and the work of others, have clearly shown that the 11p13 region of the

human chromosome complement, which is frequently subjected to spontaneous and indeed mutagen-induced chromosome breakage, contains a number of genes of importance. The presence of 7 putative genes clustered in the region associated with the Wilms' tumour gene and clearly containing a locus that is involveld in genito-urinary dysplasia, suggests a possible family of genes involved in normal kidney and uro-genital development

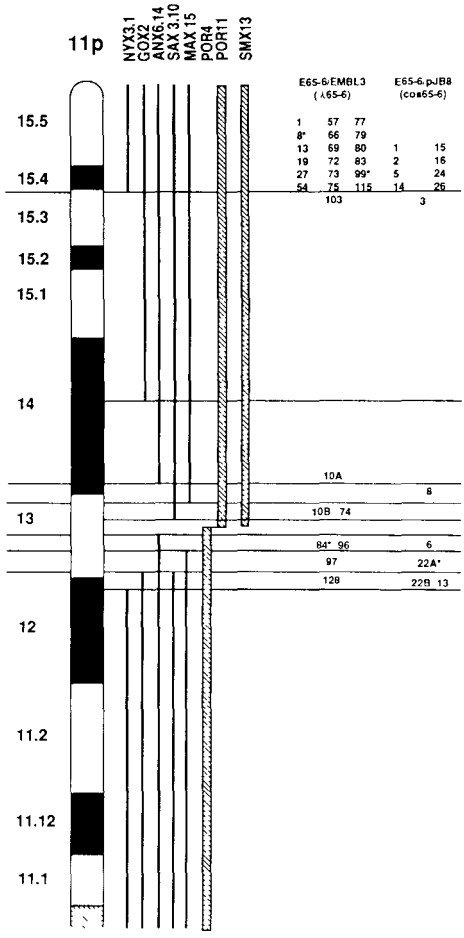

Figure 1 A physical map of the short arm of chromosome 11 and the WAGR region The figure summarises the mapping of 19 cosmid and 35 lambda human recombinants isolated from the HRAS1-selected CMGT E65-6 to 5 independent WAGR deletions and 2 WAGR associated translocations, as described in the text. Asterisked clones show significant cross-hybridisation to mouse DNA at high stringency and may therefore identify coding sequences

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

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Dr G Fekete, Semmelweis Medical School, Budapest, Hungary.
Dr Alan Balmain, Beatson Institute, Glasgow.
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Dr R Carachi, Royal Hospital for Sick Children, Yorkhill, Glasgow.
Dr A Pearson, Newcastle.
Dr E Stanbridge, Department of Microbiology and Molecular Medicine, University of California, Irvine, USA.
Dr T Rabbitts, MRC Laboratory of Molecular Biology, Cambridge.

V. 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 (1987) *Proc Natl Acad Sci USA* **84,** 5355-5359, 1987

Bickmore W, Christie S, van Heyningen V, Hastie N D and Porteous D J. (1988) *Nucleic Acids Res* **16,** 51-60

Hastie N D, Porteous D J, Bickmore W, Maule J and van Heyningen V (1988) **IN:** *Genetics of Immunological Diseases* (ed B Mock and M Potter), pp 41-46, Springer-Verlag, Berlin

Seawright A, Fletcher J M, Fantes J A, Morrison H, Porteous D J, Li S S-L, Hastie N D and van Heyningen V (1988) *Somat Cell Molec Genet* **14,** 21-30

Boyd P A, Christie S, Hastie N D and Porteous D J (1989) *Hum Genet* **81,** 349-352

Bickmore W A, Porteous D J, Christie S, Seawright A, Fletcher J M, Maule J C, Couillin P, Junien C, Hastie N D and van Heyningen V (1989) *Genomics* **5,** 685-693

Couillin P, Azoulay M, Henry I, Ravise N, Grisard M C, Jeanpierre C, Barichard F, Metezeau P, Candelier J J, Lewis W, van Heyningen V and Junien C (1989) *Hum Genet* **82,** 171-178

Bickmore W A and Hastie N D (1989) *Ophthal Paediat Genet* **10,** 229-248

Bickmore W A, Maule J C, van Heyningen V and Porteous D J (1989) *Somat Cell Molec Genet* **15,** 229-236

Hastie N D, Bickmore W A, Maule J G, Porteous D J and van Heyningen V (in press) **IN:** *Current Communications in Molecular Biology*

Hastie N D (in press) **IN:** *General Motors Cancer Research Foundation: Accomplishments in Cancer Research 1988* (ed J G Fortner and J E Rhoads), J B Lippincott Co, Philadelphia

van Heyningen V and Hastie N D (in press) *Trend Genet*

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
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Contract no.: BI6-E-159-D

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

Dr. D. Frankenberg
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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. M. Frankenberg-Schwager
Dr. H. Kühn

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 eukaryot 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:

RBE-values of ultrasoft characteristic X-rays for the induction of DNA double-strand breaks (DSB), for irreparable or misrepaired DSB, and for cell killing.
Dosimetry and spectroscopy of ultrasoft X-rays at the facility in Frankfurt.
Determination of the relative contributions of direct and $OH^{\cdot}$-mediated effects to the induction of DSB. Expression of gene conversion in dependence of DSB repair under different post- irradiation conditions.

III. Progress achieved:

1. Introduction

Ultrasoft and soft X-rays produce electrons of defined energy and short range and are therefore an excellent tool for probing the molecular mechanisms of radiation action and the role of the low-energy secondary electrons which are produced in abundance by most radiations. Carbon K, aluminium K and Wolfram L X-rays of photon energies of 0.278 keV, 1.5 keV and 10 keV generate secondary electrons of ranges of < 7 nm, < 70 nm and < 2 µm. The first range is only about three times the diameter of the DNA double helix.

DNA double-strand breaks (DSB) are suggested to play an important role in the production of chromosome aberrations (1,2,3,4) which in turn may lead to inactivation (5,6,7,8,9,10) and transformation of mammalian cells (11,12,13,14). Therefore the effectiveness of ultrasoft and soft X-rays at inducing DSB were determined in yeast because in this eukaryotic cell DSB can be measured over the same range of doses as cell inactivation.

For cell inactivation and transformation the irreparable or misrepaired DSB are relevant rather than initial DSB. Therefore, the RBE-values of ultrasoft and soft X-rays for cell inactivation after complete repair of DSB were evaluated.

The investigations with ultrasoft X-rays in the frame of this projekt show that low LET radiations induce DSB predominently by low energetic electrons of their secondary electron spectra. If there is any contribution of $OH^{\cdot}$ to DSB induction by sparsely ionizing radiation it must arise either from a mixed (direct and $OH^{\cdot}$-mediated) effect or by a totally $OH^{\cdot}$-mediated effect originating from the *same* electron. Using high concentrations of a specific $OH^{\cdot}$ scavenger the relative contributions of the totally direct and $OH^{\cdot}$-mediated effect can be determined.

Repair of DSB involves recombinational processes which may lead to gene conversion (intragenic recombination). Using the mutant rad 54-3/rad 54-3 which is deficient in DSB repair at 36°C and proficient at 23°C, gene conversion can be investigated in dependence of DSB repair under different post-irradiation conditions.

2. Material and Methods

2.1. Radiation sources and dosimetry

Ultrasoft X-rays (Al_K and C_K) were produced by bombarding 500-600 keV protons on appropriate targets. Two facilities were used: The facility at the MRC Radiobiology Unit at Harwell (England) in an experimental collaboration with Dr. D.T. Goodhead and the facility at the GSF Institute of Radiation Biophysics in Frankfurt Primary dosimetry was carried out with a small (0.1 cm^3) cylindrical ionization chamber in the position of the cell. Ionization chamber currents were converted to absorbed dose in biological tissue, using W-values in air, elemental composition of biological tissues and absorption coefficient in air and tissue as described by Goodhead and coworkers (15,16,17). The beams were of high purity consisting solely

of monoenergetic X-rays plus a very small (<< 1 per cent) contamination of bremsstrahlung as assessed by ionization chamber measurement of attenuation in air and Hostaphan (C_K) or by a calibrated proportional counter (Al_K). The dose rate at the entrance surface of the monolayered yeast cells was 10-24 Gy/min for Al_K and 8-12 Gy/min for C_K X-rays. Doses in the sample position with a diameter of 30 mm was uniform to better than 10 per cent for Al_K and C_K X-rays. W_L-irradiations were performed with a Dermopen X-ray facility (Siemens) at a dose rate of 20 Gy/min. ^{60}Co-gamma rays or 25 MeV electrons from a betatron served as reference radiations. For the studies on the direct and $OH^{\cdot}$-mediated effects of the induction of DSB and on gene conversion 25 MeV electrons were used.

2.2 Yeast strains

For DSB analyses use was made of the diploid yeast strain 211+B (ATCC 42607) whose nuclear DNA can be specifically labelled by ^3H-dTMP. For survival studies with and without repair of DSB, the radiosensitive temperature-conditional diploid yeast mutant rad 54-3was used , which is deficient in DSB repair at 36°C and proficient at 23°C. At both temperatures these cells form macrocolonies. For evaluation of the RBE-values of C_K and W_L characteristic X-rays for the production of irreparable or misrepaired DSB, the wild type strain 211 was used. The relative contributions of the direct and $OH^{\cdot}$-mediated effect to initial DSB were determined with haploid wild type yeast cells which in stationary phase are unable to repair DSB due to their unduplicated genome. For haploid yeast cells in stationary phase about one DSB per cell corresponds to one lethal event; therefore, the induction of DSB can be investigated by the survival assay. The investigations on gene conversion were performed with stationary cells of the diploid mutant rad 54-3 heterozygous for the his1 gene (his1-7/his1-1) at both the temperature permissive (23°C) and restrictive (36°C) for DSB repair.

2.3. DSB measurements

DSB induction was measured using the velocity sedimentation of liberated DNA in a neutral sucrose gradient in an ultracentrifuge for 21 h at about 9000 rpm. Details of the experimental procedure are given elesewhere (18). The average number of DSB per average molecular mass of DNA was calculated by computer simulation of random breakage as applied to the DNA of unirradiated cells and by fitting these curves to the DNA profiles obtained from irradiated cells (19,20).

2.4. Survival studies

For survival studies cells were grown at 30°C for 4 days and harvested from YPD agar (1 per cent yeast extract, 2 per cent peptone, 2 per cent glucose, 1.5 per cent agar). Cells in stationary phase were washed in 67 mM phosphate buffer, pH 7.0, and were mounted on filters for irradiation with ultrasoft and soft X-rays as well as for ^{60}Co gamma-rays, or suspended in phosphate buffer for irradiation with 25 MeV electrons. For the determination of the relative contributions of the direct and $OH^{\cdot}$-mediated effect, cells were incubated in 6 M glycerol 15 minutes

before irradiation under oxic or hypoxic (< 10 ppm O_2) conditions. Glycerol was washed out before irradiated cells were plated on nutrient agar. For evaluation of the RBE-values of C_K and W_L X-rays for the induction of irreparable or misrepaired DSB, irradiated cells of strain 211 were kept under nongrowth conditions for 72 h before plating on YPD agar, for the macrocolony assay. Cells were grown at 36°C (rad 54-3), 23°C (rad 54-3) or 30°C (211 and haploid cells) and macrocolonies were counted.

3. Results

3.1. Dosimetric comparison of the ultrasoft x-ray facilities in Harwell and Frankfurt

Exchange of the ionization chambers showed that the primary dosimetry of the two facilities was the same within 6 per cent for both Al_K and C_K X-rays. The survival curves of the rad 54-3 cells (36°C) for Al_K and C_K X-rays agreed to each other within 6 per cent. These agreements allow to compare the data obtained with both facilities.

3.2. Induction of DNA double-strand breaks

Induction of DSB in yeast (strain 211+B) as a function of dose is shown in figure 1 for ^{60}Co-gamma ray reference radiation and for Al_K and C_K ultrasoft X-rays. For

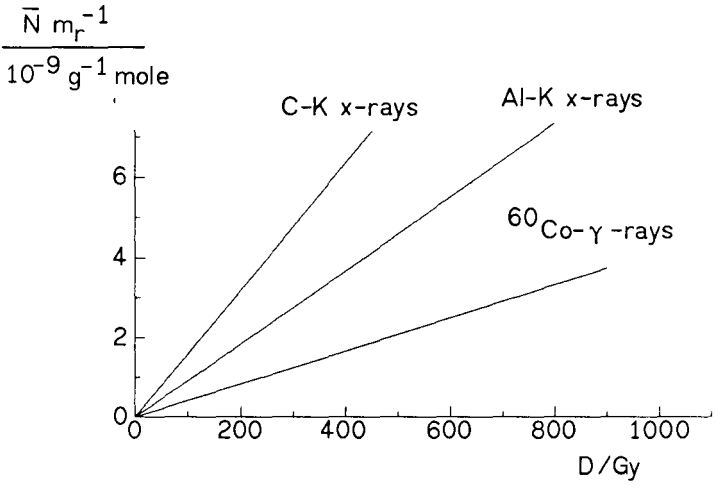

Fig. 1: The mean number $\bar{N}$ of radiation-induced DSB in yeast (strain 211$^+$B) per relative molecular mass m_r, $\bar{N}\, m_r^{-1}$, for C_K and Al_K X-rays and for ^{60}Co γ-rays as reference radiation. The lines are the best fits to the experimental data as obtained by regression analyses.

all three types of radiation, a linear relationship between the induction of DSB and absorbed dose was observed and fitted by regression analysis (18). The fitted frequencies of DSB induction are given in the 1st column of table 1..

Table 1: Average DSB induction frequencies $\bar{N}$ per relative molecular mass m_r per dose D, $\bar{N}\ m_r^{-1}\ D^{-1}$, in 211*B cells (1st column) and D_o-values of the exponential survival curves of the diploid mutant rad54-3 (36°C) for ^{60}Co γ-rays, Al_K and C_K X-rays (2nd column).

Radiation	$\frac{\bar{N}\ m_r^{-1}\ D^{-1}}{Gy^{-1}\ g^{-1}mol}$	$\frac{D_O}{Gy}$
^{60}Co γ-rays	4.23×10^{-12}	17.3
Al_K X-rays	9.32×10^{-12}	7.2
C_K X-rays	16×10^{-12}	6.7

3.3. Inactivation of a yeast mutant deficient in DSB repair

In order to determine the number of DSB per cell which correspond to a lethal event in a DSB repair deficient mutant, survival curves of diploid rad 54-3 cells were measured for the three types of radiation mentioned in 3.2.. Irradiated cells were plated on nutrient agar and incubated at the restrictive temperature for DSB repair for colony growth. For the three types of radiation used exponential survival curves were observed (fig.2) and fitted by regression analysis to the equation $S = \exp(-D/D_0)$ where S is the surviving fraction, D is the absorbed dose and D_0 is a fitted parameter giving the mean lethal dose (table 1, 2nd column)(18).

3.4. RBE-values and the average number of DSB per diploid rad 54-3 cell per lethal event

The relative biological effectivness (RBE) of each of the ultrasoft X-rays, relative to ^{60}Co gamma-rays, is shown in table 2 for the induction of DSB in yeast (RBE_{DSB}) and for inactivation of the diploid DSB repair deficient mutant rad 54-3 (RBE_S). Also shown in table 2 is the average number of DSB per rad 54-3 cell per lethal event for each radiation.

3.5. Relative contributions of the direct and OH'-mediated effect on the induction of DSB by 25 MeV electrons

Maximum protection of cells was achieved with 6 M glycerol. In figure 3 are shown the survival curves of haploid yeast cells in stationary phase, which are unable to repair DSB, with and without incubation in 6 M glycerol both under oxic or anoxic

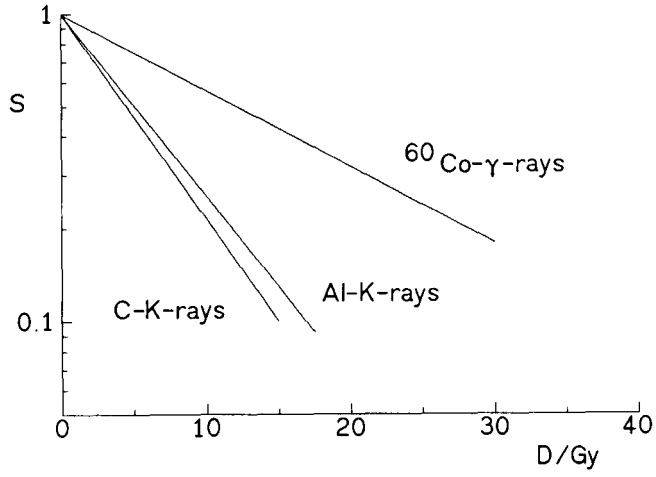

Fig. 2: Survival curves of the diploid mutant rad54-3 after irradiation with C_K and Al_K X-rays and with ^{60}Co γ-rays as reference radiation. The lines are the best fits to the experimental data as obtained by regression analyses.

Table 2: Experimental values of the RBE for the induction of DSB (RBE_{DSB}), for the inactivation of the diploid mutant rad54-3 (RBE_S) and the mean number of DSB per cell per lethal event ($\bar{n}_{D_o}$) for the radiations used.

Radiation	RBE_{DSB}	RBE_S	$\bar{n}_{D_o}$
^{60}Co γ-rays	1	1	1.32
Al_K X-rays	2.2	2.4	1.21
C_K X-rays	3.8	2.6	1.93

irradiation conditions. From the D_0-values of the exponential survival curves the fractions of the direct and OH'- mediated effect on the induction of DSB are calculated and given in table 3.

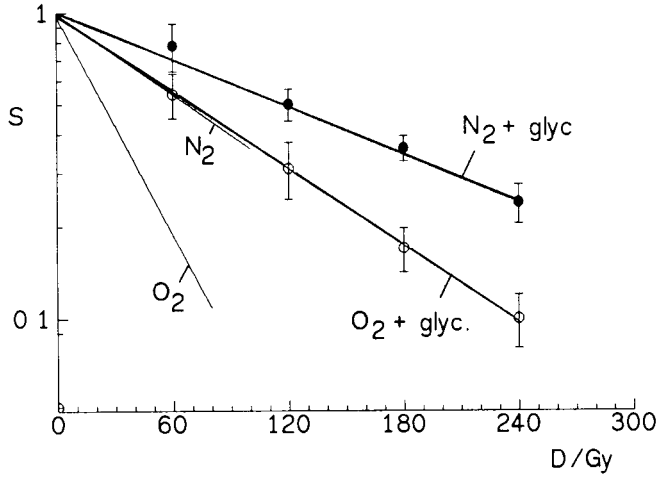

Fig. 3: Survival curves of wild type haploid yeast cells in stationary phase under oxic or anoxic irradiation conditions. Cells were incubated in the presence or absence of 6 M glycerol 15 min before 25 MeV electron exposure.

Table 3. D_0-values of the exponential survival curves, fractions of the direct (f_d) and OH'-mediated (f_i) effect on the induction of DSB, total OER (OER_t), OER of the direct (OER_d) and of the OH'-mediated (OER_i) effect observed for wild type haploid yeast cells irradiated in the stationary phase with 25 MeV electrons under oxic or anoxic conditions.

Gas	[Glycerol]	D_0/Gy	f_d	f_i	OER_t	OER_d	OER_i
N_2	—	97	0.58	0.42			
	6 M	166					
					2.7	1.6	4.2
O_2	—	35	0.34	0.66			
	6 M	102					

3.6. RBE-value of C_K and W_L X-rays for irreparable or misrepaired DSB

After repair of all reparable DSB, the inactivation of wild type diploid yeast cells is due to lethal lesions which are considered to be irreparable or misrepaired DSB (21). Figure 4 shows the survival curves of diploid wild type cells (strain 211) after irradiation with 25 MeV electrons, W_L and C_K X-rays , and , for comparision, with 3.5 MeV alpha particles . In table 4, RBE-values of W_L and C_K X-rays and , for comparison, of 3.5 MeV alpha particles at the surviving fraction of 0.9 are given.

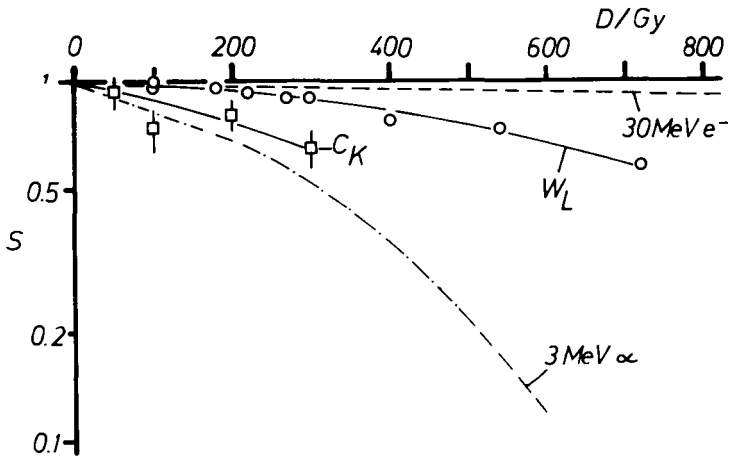

Fig. 4: Survival curves of diploid wild type yeast cells (strain 211) kept after irradiation with 25 MeV electrons, W_L or C_K X-rays under nongrowth conditions for 72 h before plating on nutrient agar. For comparison, the survival curve for α-particle (3.5 MeV) irradiation is included.

Tabie 4: Experimental values of the RBE of W_L and C_K X-rays and of 3.5 MeV α-particles for lethal lesions (irreparable or misrepaired DSB) at the surviving fraction 0.9.

radiation	RBE for DSB induction	RBE for irreparable or misrepaired DSB
25 MeV electrons	1	1
W_L X-rays	–	3
C_K X.-rays	2.6	9
3.5 MeV α-particles	2.8	15

3.7. Gene conversion under growth or non-growth conditions

In figure 5 is shown the gene conversion frequency of rad 54-3 cells in dependence of different post-irradiation conditions. It can be seen that gene conversion is negligible under conditions preventing the repair of DSB (i.e. at 36°C). In contrast, gene conversion is observed when cells are incubated at the temperature permissive for DSB repair (23°C). The yield of convertants depends strongly on the type of medium used: under growth conditions the frequency of gene conversion is about 5 times higher than under nongrowth conditions.

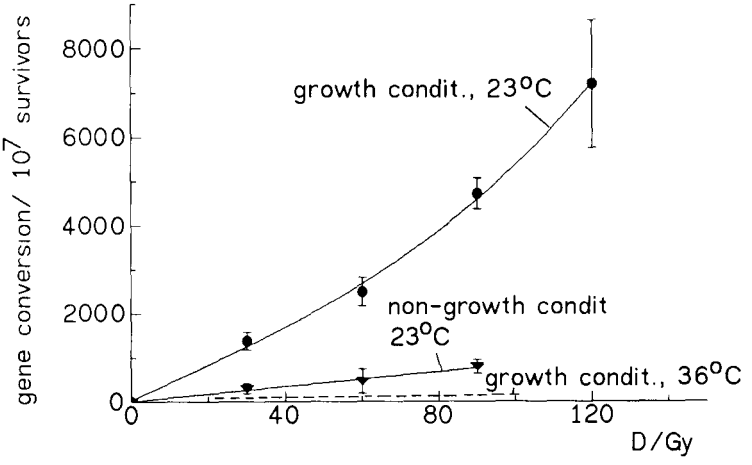

Fig. 5: The effect of DSB repair as the frequency of gene conversion at different post-irradiation conditions in the diploid mutant rad 54-3 after 25 MeV electron exposure under oxic conditions.

4. Discussion

4 1. Ultrasoft X-rays

Al_K and C_K X-rays are more effective per unit dose than ^{60}Co gamma-rays at inducing DSB (§ 3.2) and at inactivating rad 54-3/rad 54-3 cells (§ 3 3), with C_K X-rays being most effective. It is seen from table 3 that for ^{60}Co gamma-rays and Al_K X-rays, the mean number of DSB per cell per lethal event, n_{Do}, amounts to 1.3 and 1.2, respectively, whereas for C_K X-rays this value is 1.9. This apparent large excess of observed DSB in the case of C_K X-rays could indicate that this

radiation, in particular, induces many additional DNA lesions which do not lead to DSB in the cell, but which can be converted to DSB by shear forces during the treatment for DSB analysis.

The observed RBE-values of Al_K and C_K (see table 3) are in general agreement with RBE-values found for induction of inactivation, mutation and chromosome aberrations in different types of mammalian cells (17,22,23,24).

The linear dose-response curves obtained for the induction of DSB (figure 1) and the inactivation of DSB-repair deficient cells (figure 2) implies that the DSB is produced by a single radiation track. From the high RBE-value of C_K X-rays (table 3) it can be concluded that ^{60}Co gamma-rays, or another sparsely ionizing radiation, induces DSB predominently by the densely ionizing slow electrons (i.e. electrons with kinetic energies of some hundred eV) of the secondary electron spectra. Using the track entity model developed by Mozumer and Magee (25) in combination with the single collision energy loss distribution of fast electrons (26), the percentage of energy depositions up to about 60 eV, between 60 eV and 100 eV, and from 100 eV up to 500 eV can be estimated to be 55, 30 and 15 per cent, respectively. From the RBE_S-value of 2.6 for DSB induction by C_K photoelectrons, it may be concluded that electrons of energies between about 60 eV and 500 eV have to be considered responsible for the induction of DSB by low LET radiations. This conclusion is supported by the track structure calculations showing that the probability to find, for example, at least four ionisation within 1 nm around an arbitrary ionisation is highest for electrons with energies between 200 eV and 500 eV and decreases for lower and higher electron energies (27).

4.2. Direct and OH$^{\cdot}$-mediated effect of DSB induction

Considering the OH$^{\cdot}$ concentration being several 100 mM within energy depositions of slow electrons, the reaction rate between OH$^{\cdot}$ and DNA amounts to about 10^9 s^{-1}. Since the rate constant between OH$^{\cdot}$ and glycerol is 10^9 $M^{-1}s^{-1}$, a glycerol concentration of several M are needed to scavenge effectively most of the OH$^{\cdot}$ in those energy depositions.

The fraction f_i of the OH$^{\cdot}$-mediated induction of DSB under anoxic irradiation conditions is smaller by a factor of 1.6 relative to oxic conditions after exposure to 25 MeV electrons (table 3). This observation can be attributed to the chemical restitution of DNA radicals by endogenous sulphydryl compounds such as glutathione in the absence of oxygen.

The total OER (OER_t) for the induction of DSB can be split into an OER for the direct (OER_d) and for the OH$^{\cdot}$-mediated (OER_i) effect. The much smaller value for OER_d (1.6) compared with that for OER_i (4.2) might be due to a lower accessibility of sulphydryl compounds to the DNA radicals induced by the direct radiation action. Alternatively, the DSB pre-lesions induced by the direct radiation effect are of a non-radical type and cannot be restored by H-donation.

4.3. RBE-values of C_K and W_L X-rays for irreparable or misrepaired DSB

With ^{60}Co gamma-rays, the contribution to absorbed dose of electrons with several hundred eV is about 0.15 (see § 4.1). Considering the RBE-value of 9 of C_K X-rays for lethal lesions (irreparable or misrepaired DSB) (table 4), it may be concluded that mainly electrons with energies of several 100 eV produce irreparable or misrepaired DSB. In contrast, for the induction of DSB also electrons with lower energies (down to 60 eV) have to be considered (see § 4.1). However, these electons are much less effective at inducing irreparable or misrepaired DSB. The RBE-value of W_L X-rays at inducing irreparable or misrepaired DSB is less than that of C_K X-rays. This is in accordance with the smaller fraction of energy which is imparted to matter by electrons of several hundred eV.

4.4. Gene conversion

In yeast a recombinational process is required for the repair of DSB which may lead to intragenic recombination (gene conversion) between two alleles of the same gene (28), in our case the heterozygous his gene of the rad 54-3 mutant. Figure 5 clearly shows that gene conversion is correlated with the repair of DSB. Although DSB repair occurs at 23°C under both growth and nongrowth conditions, there is a 5 times higher yield of convertants observed when irradiated cells were incubated under growth compared to nongrowth conditions. This may be interpreted such, that the size of the heteroduplex (i.e. the probability of mismatched base pairs) is 5 times greater for stationary cells committed to grow.

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Dr. D.T. Goodhead , MRC Radiobiology Unit, Chilton, Didcot, GB.

V. Publications:

1. Publications in Scientific Journals:

Frankenberg, D., and Binder, A., RBE-values for the induction of DNA double-strand breaks as a function of photon energy. Radiat.Protect.Dosim. 13, 157-159 (1985)

Frankenberg, D., Goodhead, D.T., Frankenberg-Schwager, M., Harbich, R., Bance, D.A., and Wilkinson R.E., Effectivness of 1.5 keV aluminium K and 0.3 keV carbon K characteristic X-rays at inducing DNA double-strand breaks in yeast cells. Int.J.Radiat.Biol. 50, 727-741 (1986)

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RADIATION PROTECTION PROGRAMME
Final Report

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Contract no.. BI6-E-160-B

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Title of the research contract:

The role of recombination in yeast mitochondrial DNA repair. Influence of ionizing radiation.

List of projects

The role of recombination in yeast mitochondrial DNA repair.

Title of the project no.: Bio-E-160-B

The role of recombination in yeast mitochondrial DNA repair.

Head(s) of project: A. Goffeau
F. Foury

Scientific staff: F. Foury
A. Lahaye
E. Van Dyck

I. Objectives of the project:

a) Isolation of nuclear mutants defective in various aspects of mitochondrial DNA metabolism, specially in recombination.
b) Isolation of the genes, using yeast genomic libraries, by genetic complementation of the mutations.
c) Sequencing and characterization of the genes.
d) Overproduction of the gene products in order to determinate their function.

II Objectives for the reporting period:

a) Sequencing of the PIF1 gene an essential gene of the recombination/repair pathway of mitochondrial DNA.
b) Overexpression of PIF1 gene.
c) Assays for DNA helicase activity.
d) Cloning and sequencing of the MIP1 gene encoding the mitochondrial DNA polymerase in yeast.

III. Progress achieved:

Introduction

Mitochondria contain their own DNA. The mitochondrial genome is a multiple-copy system encoding a few polypeptides of the inner mitochondrial membrane which are essential for the respiration and oxidative phosphorylation. Most species require functional mitochondria in order to survive, and therefore, the study of the mitochondrial DNA (mtDNA) metabolism is extremely important, especially with respect to the injuries of the environment to the mtDNA (irradiations with gamma rays). The recent discovery that severe mitochondrial neuromyopathies are accompanied by mutations and deletions of the mtDNA still emphasize the importance of this study.

The objective of our research has been to initiate at the molecular level the study of the mtDNA metabolism in the yeast *Saccharomyces cerevisiae*. This yeast is a facultative aerobe and it is possible to isolate nuclear and mitochondrial respiratory-deficient mutants. In addition, this unicellular eukaryote is a valuable model for higher eukaryotes.

We have isolated a large number of nuclear mutants exhibiting various defects in the metabolism of mtDNA. In a second step, we have cloned and sequenced two important genes : the MIP1 gene encodes the replicative mitochondrial DNA polymerase and the PIF1 gene which encodes a DNA-helicase is an essential gene of the recombination/repair pathway of mtDNA.

Results

Mutators and antimutators of mitochondrial DNA.

We have isolated nuclear mutants falling into seven groups of complementation that exhibit a decreased spontaneous mutation rate of both mtDNA and nuclear DNA. In addition, these mutants exhibit an increase sensitivity to the lethal effect of ionizing radiations. The existence of these mutants indicate that there are common pathways in mitochondrial and nuclear DNA metabolism.

We have isolated specific mutators of mitochondrial DNA that define four complementation groups. Another mutant defining an additional complementation group only exhibits increased mutation rate of the mitochondrial E^R allele. This mutant is also sensitive to ionizing radiations.

The general mutators of mitochondrial DNA are of special interest since they could correspond to nuclear genes involved in mtDNA mismatch repair function or coding for a mitochondrial uracil-glycosylase.

mtDNA gamma-ray sensitive mutants

We have isolated four complementation groups of mutants whose induction of cytoplasmic petites (rho$^-$) is increased by gamma-rays at elevated temperature (36°C). The production of rho$^-$ at 36°C, in the absence of irradiation, is already significant in these mutants. It must be noted that in the wild-type strain rho$^-$ mutants are not induced by gamma rays.

In the mutant GA7-5, the degradation of the mitochondrial DNA in isolated mitochondria is slower at 36°C than in the parental strain. Moreover, the mitochondrial deoxyribonuclease measured at pH 5.4 is thermosensitive.

The PIF1 gene is an essential element of the recombination/repair pathway of mtDNA

1)Isolation of the mutants

Using the kar1 mutation to screen directly crosses from a large population of mutagenized cells, we have isolated three recessive allelic mutants that are affected in the recombination between rho$^+$ and rho$^-$ genomes. These mutants were assigned to the pif1 locus, on chromosome XIII.

In these mutants, the general recombination tested for several linked and unlinked alleles is normal. However, the recombination frequency between rho+ and specific rho$^-$ genomes is extremely decreased. Thus, the rho- genomes were classified into three categories.

In the first class, the recombination of the rho- genomes, in rho$^+$ x rho$^-$ crosses, depends upon the integrity of the PIF1 gene. The mitochondrial DNA exhibits either a direct tandem organization of the conserved sequence.

In the second class, the recombination of the rho$^-$ genomes, in rho$^+$ x rho$^-$ crosses is PIF-independent and reaches high recombination frequencies (80-90%) in both pif1 and PIF1 nuclear backgrounds. The conserved sequence is organized in palindromes. All palindromic rho$^-$ clones in crosses with rho$^+$ strains exhibit very high and similar mitochondrial DNA recombination frequencies that do not depend on the size of the conserved sequence or on the location of the genetic marker relative to the deletion end points of the conserved sequence. These factors, however, would be expected to regulate the efficiency of heteroduplex formation in the general recombination. Moreover, the palindromic rho- E41, which recombines with a frequency of more than 80% in Pif1$^+$ or Pif1$^-$ nuclear backgrounds gives rise to tandem rho- genomes that possess a very similar conserved sequence but have a recombination frequency of only 50% in the Pif1$^+$ background (3% in pif1 mutants). These results suggest that in palindromic rho$^-$ genomes, specific sequences and specific secondary structures enhance the recombination frequency. Sor and Fukuhara have shown that the junctions between inverted repeats contain an asymetric sequence of

variable length which is bordered by a pair of inverted oligonucleotide sequences". This sequence is also present in the wild-type mitochondrial DNA. These junctions which have a structure similar to that of transposable elements may play a role in the recombination between rho+ and rho- genomes.

The third category of rho$^-$ genomes is represented by the tandemly-arrayed rho$^-$ genome E1 located inside the 21S rRNA gene. The recombination frequency is similar and rather low in Pif1$^+$ and Pif1$^-$ backgrounds. Therefore, this rho$^-$ mutant is also PIF-independent. However, the conserved sequence of E1 is included in the tandem rho- genome E41 which is PIF-dependent. We do not know why these recombination abilities differ.

The pif1 mutations elicit a dramatic increase in the suppressiveness of homozygous pif1 rho$^+$ x pif1 rho$^-$ crosses with PIF-dependent and PIF-independent genomes. Suppressiveness in the pif1 background is increased whenever the rho$^-$ mutants are suppressive or not suppressive in the PIF1 background. Since the genomes of the rho$^-$ diploids are identical to that of the haploid rho$^-$ parents, the pif1 rho$^-$ genomes may have a replicative advantage in the zygote. It cannot be excluded that high suppressiveness is a secondary effect of the pif1 mutation.

In conclusion, our results emphasize that the recombination between rho$^+$ and rho$^-$ genomes in yeast is under the control of several systems depending on the specific organization of the rho$^-$ genome.

2) Characterization of a recombinogenic signal

The high level of recombination observed in certain crosses between rho$^+$ and rho$^-$ strains cannot be explained by the rules governing the frequency of genetic exchanges between rho$^+$ strains. A signal in the mitochondrial DNA of tandem rho$^-$ clones enhances the frequency of transmission of rho$^-$ alleles to the rho$^+$ diploid progeny in rho$^+$ x rho$^-$ crosses. The stimulation of recombination is dependent on the PIF1 gene.

The characterization of a large number of overlapping tandem rho$^-$ clones located either in the 21S rRNA gene or in the oli1 region has allowed us to classify them into two categories. In the PIF-independent class, the recombination frequency is low and does not depend on PIF1 gene. It is likely that the shortness of rho$^-$ repeating-units (<300 bp) severely limits heteroduplex formation between rho$^+$ and rho$^-$ DNA. In the PIF-dependent class, the recombination frequency is 10-50 times higher than in the first class. However, it is drastically decreased in pif1 mutants.

These results suggest the existence of a recombinogenic signal which is recognized by the PIF1 product or by a protein which is under the control of the PIF1 product. All the PIF-dependent genomes analyzed in the 21S rRNA gene terminate on the 3' side of their repeating-unit by the same 41-bp A+T sequence. This sequence which exhibits over 26bp a perfect dyad symmetry must contain an essential element of the recombinogenic signal. The latter, however, is not sequence-specific, since in the oli1 region, no unique sequence

can distinguish PIF-dependent and PIF-independent rho- genomes. However, a palindromic structure and a rich A+T content might be essential properties of the signal.

Our results suggest that the enhancement of recombination frequency observed in crosses with rho- clones is related with the topology of the rho- mitochondrial DNA. However, it is not the result of the organization of the mitochondrial DNA molecule, since pif1 rho- mutants, like PIF1 rho- strains, exhibit a high reiteration of the repeating-unit. An attractive hypothesis would be that the difference in the recombination efficiency observed in crosses with PIF-dependent and PIF-independent rho- clones is related to negative supercoiling of the rho- DNA molecule. Requirement for negative supercoiling has been shown in the recombination process catalyzed by the Rec1 protein in Ustilago and in Lambda bacteriophage integrative recombination. The torsional stress imposed by negative supercoiling favors local denaturation of the double helix and transition of inverted repeats to cruciforms. Local melting and cruciform extrusion are highly dependent on the base composition of the DNA molecule and favored by a rich A+T environment.

We suggest that PIF-dependent clones have the ability to form and to stabilize local DNA strand separation, possibly as cruciforms. The local denaturation in the double helix of the mitochondrial DNA of PIF-dependent clones would be preferred sites for initiation of the recombination. What might be the role of PIF1 gene product? (i) It might contribute to the destabilization of the double helix, by increasing local supercoil density. In this case, the PIF1 gene product might be related to topoisomerases (gyrases) or to DNA-unwinding proteins. (ii) It might contribute to the stabilization of the denatured region in the double helix of the PIF-dependent genomes. In this case, it might be a DNA-binding protein. (iii) Finally, the PIF1 product might recognize specific structures of the mitochondrial DNA and directly participate to the initiation of the recombination between rho+ and rho- DNA molecules.

Whatever the answer is, the present work points out the important role of DNA topology in DNA recombination and raises several questions about the role of supercoiling and cruciform structures in the initiation of mitochondrial DNA recombination which seems to occur at preferred sites.

3) Repair deficiencies in pif1 mutants

In pif1 mutants, the spontaneous production of rho- mutants vary highly from one nuclear background to another, but is always higher than in the parental strains (less than 10% in the mutants 394 and 562, 50% in the mutant 682).

After UV light irradiation, cell killing was identical in pif1 mutants and parental strain. However, after a one-minute irradiation, the induction of rho- mutants reached 75% in pif1 mutants and only 7% in the parental strain. Similarly, the induction rho- mutants by ethidium bromide was much more efficient in pif1 mutants, since with 0.25 µg/ml ethidium bromide, 90% rho- mutants were induced, while in the parent, there were only 25% rho- mutants.

Mn^{2+} petite induction was also more efficient in the pif1 mutants. It can be concluded, therefore, that in pif1 mutants, there is a defect in the recovery of intact DNA molecules after mutagenic treatments. In other words, pif1 mutants are deficient in the repair of mitochondrial DNA. Since the PIF1 gene product is involved in the recombination of the mitochondrial DNA, the simplest explanation is that it controls repair through recombination.

When the cells of a rho^+ null pif1 mutant were shifted to 36°C, they were rapidly converted to rho^- mutants. After several generations at 36°C, the probability that a cell maintains a rho^+ genome is less than 10^{-4}. The cells loose their mitochondrial DNA and become rho^0, as shown by the following evidences. i) Out of about 250 rho^- clones, none had retained the cox3 or cob1 mitochondrial markers which are, however, among the most commonly retained mitochondrial markers. ii)When the DNA from randomly-selected rho^- clones induced at 36°C was extracted, digested with restriction enzymes and subject to agarose gel electrophoresis, no mitochondrial DNA band was observed, in contrast with the rho^- clones issued from cultures at 30°C. In the same temperature and culture conditions, the wild-type strain produced only a few rho^- mutants. In the null pif1 mutant, the induction of rho^- mutants by temperature shift was immediate, since after a 5 h-incubation at 36°C , about 70% rho^- mutants were already induced in growth conditions. The induction was less pronounced in resting-cells, since for the same time of incubation only 50% rho^- mutants were induced.

Therefore, the PIF1 gene is essential for the maintenance of the mitochondrial DNA at elevated temperature.

The loss of mitochondrial DNA in pif1 mutants at elevated temperature can be the result of a defective DNA replication and/or an increased DNA degradation. First, it must be stressed that arrest in mitochondrial DNA replication does not necessarily elicit degradation of pre-existing mitochondrial DNA. For instance, in a mip1 mutant exhibiting thermosensitive mitochondrial DNA replication and DNA polymerase activity, the mitochondrial DNA is stable for several hours after a shift to 36°C, although all replication mechanisms have ceased The following experiment was performed. A rho^+ pif1 null mutant was grown at 30°C and shifted to 36°C. After a 90-min incubation, ^{32}Pi was added to the medium and the incubation at 36°C was prolonged for 4 h. The purified mitochondrial DNA was subject to restriction analysis, agarose gel electrophoresis and autoradiography. The mitochondrial DNA was still synthesized at the non-permissive temperature, although at a lower rate than at 30°C. A smear in the band pattern suggested degradation of the mitochondrial DNA. Therefore, in a second series of experiments, the fate of mitochondrial DNA after a shift to 36°C was followed. After an overnight culture at 30°C, the cells of a rho^+ null pif1 mutant were shifted to 36°C and incubated in growth and non-growth conditions. Total DNA was hybridized to the mitochondrial DNA of the rho^- clone DS400/A4, as the probe. Degradation of the corresponding DNA fragment of the mitochondrial genome of the pif1 disrupted rho^+ strain was

already observed after a 5-h incubation at 36°C. The DNA degradation was more pronounced in growth conditions, and was clearly correlated with the production of rho^- mutants. These results indicate that the loss of mitochondrial DNA at 36°C is the consequence of an increased nucleolytic attack. These results do not exclude that the degradation is the consequence of an erroneous DNA replication of the mitochondrial DNA at 36°C.

In conclusion, the PIF1 protein does not play an essential mitochondrial function in physiological conditions. However, it becomes essential for the maintenance of the mitochondrial DNA at elevated temperature. As the mitochondrial DNA of *Saccharomyces cerevisiae* is 80% A+T rich, the conformation of the mitochondrial DNA-protein complex must be highly temperature-dependent and destabilized at 36°C in the absence of DNA-stabilizing proteins. The PIF1 protein appears to play an essential stabilizing function.

5) Cloning and sequencing of the PIF1 gene

The product of the gene PIF1 is a protein of Mr=97,500, characterized by both very basic and acidic-residue stretches along the sequence. The hydropathic profile suggests that PIF1 is a soluble protein, except possibly in a glycine-rich span covering residues 280-312 which might define a transmembrane domain. This theoretical analysis agrees with recent experimental data showing that the overproduced PIF1 protein must be solubilized by detergents plus salts. However, the glycine-rich composition of the hydrophobic stretch is a typical feature of many DNA-binding proteins. The requirement of detergents for the solubilization of the PIF1 protein might result from its overexpression in the mitochondria, eliciting an aggregated state produced by multiple interactions between the negative and positive charges of the overexpressed protein. In conclusion, the hydrophobic nature of the PIF1 protein remains an open question.

The PIF1 protein shares many features with proteins involved in nucleic acid metabolism. These proteins often appear to be multifunctional, involved in recombination, repair and replication. As the vast majority of the proteins which exhibit sequence similarities with PIF1 are DNA-helicases, the simplest and more plausible hypothesis is that PIF1 is also a DNA helicase or is associated with a DNA helicase complex.

UvrD and Rep proteins are single-stranded DNA-dependent ATPases and possess a DNA helicase activity. UvrD protein (helicase II), in addition to its role in nucleotide-excision repair, is also involved in replicative unwinding of closed DNA circles, gene conversion, precise excision of transposons and mutagenesis. Rep protein would have similar functions but is in low concentration in the cell. Their exact role in DNA replication is unknown.

Perhaps more interesting is the homology found with the three components RecB, RecC and RecD of ExoV in E. coli . The RecBCD complex has a DNA unwinding activity and a nuclease activity. It is essential in DNA recombination/repair and for cell viability. The RecB polypeptide (130 kDa)

possesses the DNA unwinding activity. The nuclease activity requires correct interactions between RecC (125 kDa) and RecD (67 kDa), which binds ATP only in the presence of the other subunits. RecB and RecD belong to the helicase family described by Hodgman. RecC is completely different. The RecBCD complex plays an important role in the initiation of recombination catalyzed by RecA protein. PIF1 protein resembles the RecBCD complex. It is required in a recombination/repair process involving the recognition of a recombinogenic signal (although, in contrast to the Chi site, this recombinogenic signal is not sequence-specific, but most likely related to the topology of the DNA) and it has striking sequence similarities with the three polypeptides of the complex RecBCD.

It would not be really surprising that PIF1 protein plays a role in DNA replication, like many DNA helicases, either in association with the primase, or during DNA chain elongation, ahead of the replication fork. For example, the UL5 protein of Herpes simplex virus which is a member of the superfamily defined by Hodgman is an essential DNA helicase of the primase complex. However, there is no experimental data that indicates that PIF1 protein plays a role in mitochondrial DNA replication.

The mitochondrial DNA polymerase gene

We have isolated a conditional mutant (mip1) which is respiratory-competent at 30°C but gives rise to a population of cells completely devoid of mtDNA (rho^0) at 36°C. The replication of the mitochondrial DNA is thermosensitive in vivo. Kinetics and heat inactivation studies have demonstrated that in vitro the mitochondrial DNA polymerase of the mutant is also highly thermosensitive. These results suggest that the MIP1 gene codes for the catalytic subunit of the mtDNA polymerase. We have cloned the gene and sequenced it. It encodes a protein of 143.5 KDa which exhibits a few similarities with other eukaryotic polymerases and possesses the three typical motifs involved in the catalytic activity of the proof-reading 3'-5' exonuclease of E. coli DNA polymerase I.

No mitochondrial DNA polymerase has, thus far, been sequenced. A series of data show that the MIP1 gene encodes the catalytic subunit of the mitochondrial replicative DNA polymerase of *Saccharomyces cerevisiae*. 1/ A previous report demonstrated that the product of the MIP1 gene was absolutely required for the in vivo replication of the mitochondrial DNA. Indeed, in the thermosensitive mutant ts71 (mip1-1 SUP), the replication was thermosensitive and in a mip1-1 mutant, there was no replication of the mitochondrial DNA. 2/ The solubilized mitochondrial DNA polymerase activity of the mutant ts71 is highly thermosensitive, as shown by heat inactivation curves achieved with three different DNA or synthetic templates. 3/ The MIP1 gene encodes a polypeptide of theoretical Mr=143,500, which is in the size range of many nuclear eukaryotic/viral DNA polymerases . By comparison, the molecular weight of the purified mitochondrial DNA polymerase from *Drosophila* embryos was estimated to be125,000. Amino acid sequence analysis revealed

that the MIP1 protein shares with eukaryotic/viral DNA polymerases and reverse transcriptases several typical conserved motifs. 4/ Chromosomal disruption of the MIP1 gene does not affect cell viability. However, it kills mitochondria, eliciting complete loss of both mitochondrial DNA and mitochondrial DNA polymerase activity. 5/ In a transformant harboring the MIP1 gene on a multiple-copy plasmid under strong selective pressure for well-developed mitochondria, the mitochondrial DNA polymerase activity is 30-40 times higher than in the untransformed parental strain.

The MIP1 gene-encoded DNA polymerase offers resemblance with DNA polymerases of alpha type and reverse transcriptases, and in the same time, is highly divergent from both types of polymerases. The common motif (motif I) shared by RNA-directed RNA polymerases, reverse transcriptases and eukaryotic/ viral nuclear DNA polymerases is present in the MIP1 protein. In nuclear DNA polymerases, there are five other conserved sequences (motifs II to VI). In the yeast mitochondrial DNA polymerase, the motif I is flanked upstream by the motif II in the same linear distribution as for nuclear DNA polymerases. The cysteine/histidine rich sequences typical of DNA-binding domains in eukaryotes is present in both nuclear DNA polymerases and in the yeast mitochondrial DNA polymerase. However, major divergences are observed. First, motif I is more closely related to that of reverse transcriptases or RNA-directed RNA polymerases, since the typical threonyl residue of nuclear DNA polymerases is absent. Second, in the mitochondrial DNA polymerase sequence, the motif II is cleaved in two parts by a five-residue sequence which is absent from the nuclear DNA polymerases. Third, in the mitochondrial DNA polymerase sequence, motifs I and II are very close one to each other so that motifs III, IV and V are not detected. The motif VI is also present, slightly downstream of motif I, in contrast with the nuclear DNA polymerases. Finally, these conserved motifs are located close to the N-terminus of the sequence in the yeast mitochondrial DNA polymerase, while in the nuclear DNA polymerases,they are usually located in the C-terminus. In this respect, the question may be raised whether the MIP1 gene might result from a duplication of an ancestor gene. For instance, the motif YPTL (one letter code) and flanking sequences are present both in the N-terminus and the C-terminus of the MIP1 protein and can be found in the N-terminus of some reverse transcriptases and in the C-terminus of nuclear DNA polymerases.

The divergences and similarities observed between the sequence of the MIP1 protein and the sequences of nuclear eukaryotic DNA polymerases or reverse transcriptases might be correlated with the original properties of the yeast mitochondrial DNA polymerase activity in vitro. 1/ The yeast mitochondrial DNA replicase is a DNA polymerase - indeed, its favorite substrate is activated DNA and, in contrast with reverse transcriptases, the enzyme cannot synthesize DNA from a natural RNA template. 2/ However, the yeast mitochondrial DNA polymerase was shown in this report to share with reverse transcriptases the

capacity of copying adenylate ribohomopolymer templates. It must be noted that the enzyme has no homology with the mammalian nuclear DNA polymerase ß which has the same template requirements. 3/ Like prokaryotic DNA polymerases, and in contrast with nuclear eukaryotic DNA polymerases, the mitochondrial DNA polymerase is resistant to aphidicolin and sensitive to dideoxynucleotide triphosphates. However, except in a C-terminus track of the dnaE gene encoded subunit alpha of DNA polymerase III in *E. coli*, there is no sequence similarity with the prokaryotic DNA polymerases. This is in contrast with the yeast mitochondrial RNA polymerase which has been shown to be related to the bacteriophage T7 RNA polymerase.Therefore, in conclusion, the phylogenic relationship of the catalytic subunit of the yeast mitochondrial DNA polymerase to the other polymerases remains an open question.

Conclusions

We have shown that the mitochondrial DNA of *Saccharomyces cerevisiae* is subject to efficient repair after UV light, ethidium bromide or ionizing radiations treatments, mainly through a recombinational pathway. We have characterized an essential member of this repair pathway. The PIF1 gene which is required for the efficient recognition of a recombinogenic signal encodes a mitochondrial DNA helicase which is related to a family of multifunctional DNA helicases of *E. coli* We also have characterized the gene MIP1 that encodes the mitochondrial DNA polymerase. This enzyme must play also an important role in repair functions and we have shown that it contributes to the accuracy of the transmission of the mitochondrial genetic information through the generations.

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

V. Publications:

J. Backer and F. Foury
Repair properties in yeast mitochondrial mutators. Curr. Gen. **10**, 7-13 (1985).

F. Foury and E. Van Dyck
A PIF-dependent recombinogenic signal in the mitochondrial DNA of yeast. Embo J. **4**, 3525-3530 (1985).

A.M. Genga and F. Foury
A nuclear mutant of Saccharomyces cerevisiae deficient in mitochondrial DNA replication and polymerase activity. J. Biol. Chem. **261**, 9328-9332 (1986).

F. Foury and A. Lahaye
Cloning and sequencing of the PIF1 gene involved in repair and recombination of yeast mitochondrial DNA. Embo J. **6**, 1441-1449 (1987).

F. Foury
Cloning and sequencing of the nuclear gene encoding the catalytic subunit of the yeast mitochondrial DNA polymerase. J. Biol. Chem. **264**, 20552-20560 (1989).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-E-162-IRL

University College
IRL-Galway

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

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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.: B16-162-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, Ms. C. Gillespie, Ms. P. Mc Carthy.

I. Objectives of the project:

The objective of this project was to investigate directly the effects of radiation on the induction of chromosome abnormality in human spermatozoa. For this, it was necessary to develop the technique of human sperm chromosome analysis based on the in vitro fertilization of zona-free hamster ova to a level of simplicity and reliability so that it was applicable to the large-scale screening of ejaculated sperm samples from irradiated donors and the analysis of induced cytogenetic aberration. A secondary objective was the investigation of the effects of radiation physical, physiological and biochemical aspects of sperm function and production.

II. Objectives for the reporting period.

The objective of this reporting period was to further refine the technique of human sperm chromosome analysis based on the in vitro fertilization of zona-free hamster eggs by the use of electropermeabilization for the induction of a functional acrosome reaction in the spermatozoa and the use of a totally defined medium for gamete culture. The application of this adapted technique to the investigation of the effects of radiation on the induction of cytogenetic aberration was investigated.

III. Progress achieved:

In this project it was intended to study the effects of radiation on the chromosomes of human spermatozoa. In order to achieve this, it was necessary to develop a modified, effective technique for the visualization of the chromosomes of human sperm even from men who were severely oligospermic as a result of exposure to radiation. This technique was based on the in vitro penetration of zona pellucida-free eggs from superovulated hamsters by capacitated sperm.

Studies were caried out on men attending Radiotherapy Units in Ireland. Whenever possible, semen samples were collected before, during and following radiation treatment. Successful sperm chromosome analysis is dependent on efficient fusion of donor sperm and zona-free hamster ova. However, sperm from males who have received radiotherapy up to 5 y previously were severely disturbed, reducing the possibility of obtaining data by the standard sperm chromosome technique. A combination of chemotherapy and radiotherapy hindered the recovery of spermatogenesis more than radiotherapy alone. Unilateral scrotal shielding limited the deleterious effects of treatment and assured a more substantial stem cell base for recovery. Recovery to oligospermic status could occur in less than 27 months if the total tumour dose was 40 Gy and shielding was present. With doses of 50 Gy, even when shielded, the patient was typically azoospermic at 48 months post-treatment. Therefore, a distinct relationship existed between dose received and sperm recovery. However, there did not appear to be an association between the the results of pre-treatment analysis and post-treatment recovery. Oligoasthenozoospermic products of treatment exhibited poor sperm survival in culture and a significantly reduced possibility of undergoing a normal acrosome reaction: a prerequisite for fertilization and for sperm chromosome analysis. It was not possible to distinguish betwen the recovery potential of teratomas and seminomas. Overall, it was found that the success rate for producing analyzable sperm metaphases using the standard procedure ranged from 2-60% for normal samples and 0-3% for radiotherapy samples. In order to undertake large-scale studies on the effects of radiation on the induction of cytogenetic aberration in spermatozoa, it was necessary to develop modified procedures for sperm chromosome analysis.

Cryopreservation of semen: Samples of semen from normal, fertile and from radiation-exposed donors were frozen in GEYC diluant and the more recently developed TEST-yolk buffer. Comparative post-thaw viability was assessed in terms of motility and the penetration of zona-free hamster ova. It was found that there was no improvement in post-thaw motility or egg penetration after storage in liquid nitrogen in TEST-yolk buffer for normal, fertile samples and there was a reduction in motility and penetration of oligospermic samples compared to storage in GEYC diluant. The latter procedure was, therefore, adopted for all subsequent analyses.

Seminal Analysis: Routinely, this consisted of sperm counts, morphology, live/dead estimates and videomicrographic analysis of sperm motility. Superoxide dismutase activity (SOD), zinc, total protein, albumin, fructose and peroxidases were measured in aliquots of seminal plasma. In general terms, it was found that in the samples from radiation-exposed patients, abnormal sperm morphology was increased though not necessarily to a significant extent and sperm counts and motility were inhibited. A plot of seminal plasma SOD activity against post-irradiation therapy time has provided tentative evidence that this enzyme might be induced in the testicular compartments by clinical levels of ionizing radiation.

There was no correlation between SOD and sperm motility or performance in the zona-free hamster egg penetration assay, but it was positively correlated with seminal plasma zinc. The possible involvement of SOD in radioprotection was further investigated. UV irradiation of normal sperm in vitro in polymer-free defined media gave a charcteristic survival curve in terms of motility, with an initial 20% motility declining to zero about 3 h after irradiation. Inclusion of SOD in the medium during and after treatment led to an approximate 30% increase in motile survival, while the further presence of catalase led to an average increase of 65% over 5 h. When sperm SOD was selectively inhibited with DDC there was no survival following UV irradiation and the addition of radical scavangers did not improve the performance. Exposure to DDC in protein-free, polymer-containing medium resulted in the elimination of motility within 2 h. Decreased motility was maintained in the presence of protein but surface adhesion occurred within 3 h. The decline in motility was not associated with a significant rise in lipid peroxidation or loss in viability.

A study was undertaken of seminal plasma zinc and SOD in 43 radiation-exposed patients and controls. Mean activity of SOD following radiotherapy 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, in turn, correlated sperm concentration and plasma volume.

It is believed that these preliminary studies on the role of SOD merit further investigation and, in particular, of the effects of SOD on the survival and motility of sperm from men exposed to ionizing radiation in vivo.

Gamete Preparation: In the standard technique for sperm chromosome analysis, it is not usually necessary to select for sperm motility. However, the use of oligospermic samples from radiation-treated men necessitated the adoption of rapid, motile sperm selection procedures. Sperm were isolated by a two-step wash coupled to swim-up on iodinated ethiodized oil cushions. This sperm isolation procedure achieved >90% motile sperm preparations. When possible, sperm were incubated at a concentration of 10^7 ml^{-1} and capacitated and acrosome reacted. Oil covered drops of sperm suspension were prepared just prior to mixing with zona pellucida-free eggs from superovulated hamsters. Following incubation for 3 h and a fertilization check, eggs were transferred to droplets containing the mitotic spindle inhibitor colcemid to block nuclear division at the metaphase stage. The eggs were then fixed and dried on slides. Following banding and staining the sperm chromosomes could then be analysed.

Development of Defined Media for Gamete Interaction: 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 seveley limited the application of the standard technique to radiation protection studies. One cause of the poor penetration response was the variable nature of some of the components of the media used for sperm preparation and gamete interaction. The procedure for sperm penetration is normally conducted in modified Tyrodes medium in the presence of human serum albumin (HSA). However, albumin appears to be a major source of variation and it has been found that different batches of HSA need to be tested to select those exhibiting consistent behaviour. In this project, several undialysed defined polymers were studied as HSA replacements. High molecular weight PVA alone was found to be superior to low M.W. PVA or PVPs for maintaining sperm motility while none of the polymers alone was particularly good at achieving high penetration after < 5 h capacitation. Polymer combinations,

PVA/dextran, PVA/PVP, etc. all exhibited synergistic penetration responses to varying degrees and pronounced polyspermy occurred on prolonged exposure. Up to about 6 h incubation, sperm in defined medium demonstrated a significantly higher velocity profile than those in HSA media. Within specific concentration limits, the addition of SOD/catalase, SOD/catalase/taurine and catalase or taurine alone but not SOD alone could all increase velocity distribution. After about 6 h, taurine tended only to be effective in the presence of catalase or SOD/catalase and not all samples showed velocity advantages in defined medium. However, a significantly larger number of samples incubated in defined medium for 24 h exhibited a higher % motility than those split aliquots in HSA media, though the motility in defined medium tended to terminate rapidly on the slides as the cells stick, indicative of surface changes. Sperm in defined media were depleted of hyaluronidase more rapidly and to a greater extent than those in HSA media, this suggested that incubation in defined media resulted in improved acrosome reactions. While more subtle reorganizations in membrane structure in both gametes after sperm-egg collision in defined media could not be ruled out, it seemed likely that the enhanced penetration in the presence of these polymers was due to an increase in the rate and incidence of acrosome reactions after capacitative membrane changes. An initial EM study and biochemical investigations of sperm ATPases supported this view. The presence of SOD and catalase in the medium afforded culture protection from superoxide anions in the absence of serum rather than limiting free radical chromatin damage generated by the ionizing radiation itself.

The development of the fully defined media significantly improved the penetration ability and chromosome yield of control and sub-fertile semen samples (Tomkins, Carroll and Houghton, 1988). However, it produced little improvement with the oligospermic and asthenzoospermic samples regularly produced by radiation-exposed men. This can be illustrated by a study of 12 radiation-exposed men who were available for repeat sperm chromosome studies. Seven were still azoospermic 1-5 y after radiotherapy. Of the remaining five with demonstrable sperm counts, one (patient 1) was accidentally irradiated and the other four had received radiotherapy. A summary of the results using standard techniques and using the defined synthetic media is presented 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 was in agreement with the findings of Jendeny and Rohrborn (1987) on a smaller number of metaphases but disagreed 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 chromosomes 1,9 and 16 appeared to be elevated in radiation-exposed samples.

In spite of the improvements in sperm chromosome analysis afforded to samples from normal men by the use of defined media, it was necessary to to study other procedures for the analysis of sperm from radiation-exposed men. The main approach involved the investigation of alternative techniques for the induction of the acrosome reaction of spermatozoa from normal and radiation-exposed men prior to penetration of zona-free hamster eggs and the visualization of the sperm chromosome.

TABLE 1	Control Group (5 normal men)	Patient				
		1	2	3	4	5
Dose (Gys)	0	0.1	35	35	35	35
% 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	8	--	--	0	9

Induction of the Acrosome Reaction of Spermatozoa: On ejaculation, sperm are not immediately capable of fertilizing an egg. In order to achieve fertilization, it is essential that ejaculated sperm undergo capacitation and the acrosome reaction . The membrane alterations of capacitation normally precipitate the acrosome reaction and exposure of the fusiogenic region of the sperm head. This process normally occurs in the female reproductive tract. Failure of sperm to effectively undergo capacitation and the acrosome reaction renders them infertile. It is necessary to artificially induce the acrosome reaction in vitro if the sample of sperm is to be used for the sperm penetration assay or for sperm chromosome analysis. A variety of techniques have been developed for the induction of the acrosome reaction. However, these have all proved to be inefficient and, until recently, there was no single, rapid, completely reliable method available.

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 egg penetration scores and metaphase yields for semen samples from normal controls. However, it did not improve the performance of oligospermic samples from irradiated men an alternative procedures were investigated.

A new and innovative technique was developed during this project for the rapid induction of the acrosome reaction of human sperm using electropermeabilization. High yields of fusiogenically functional sperm were rapidly and efficiently prepared by exposing sperm suspensions to an electrical pulse of 750-1500 V cm^{-1} for 2.5 ms. This process was Ca^{++} dependent. Sperm development was not hindered and, using sperm from normal men, yields of sperm metaphases approaching 100% were achievable in the sperm chromosome analysis technique. Furthermore, this technique was applied to sperm samples from subfertile men attending fertility clinics. Preliminary studies revealed that in some cases, whilst other sperm parameters appeared to be normal, the sperm were unable to undergo the acrosome reaction using standard capacitation regimes or following electropermeabilization. However, in some of the samples from subfertile men, sperm that would not normally undergo the acrosome reaction could be induced to react by electropermeabilization and could then penetrate a zona-free hamster egg. Having penetrated an egg, the sperm could subsequently form pronuclei, the sperm chromatin decondensed and the sperm chromosomes analysed. More importantly, it was found that this technique could be used for oligospermic and asthenospermic samples from infertile and radiation-exposed men (Table 2).

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

The use of electropermeabilization for the induction of the acrosome reaction has led to a significant increase in the success of sperm chromosome analysis and has enabled the study of fundamental aspects of sperm physiology and biochemistry related to radiation damage. Furthermore, it has become apparent that modification of the pulse medium will benefit analysis of subfertile sperm from radiotherapy patients. Electropermeabilization of sperm was initially demonstrated using covalent pulse media. However, such media are incompatible with fertilization and the sperm must be transferred to culture media prior to gamete interaction, with consequential sperm loss.

The advantage of an ionic medium would be that it would permit simple post-pulse dilution without washing. A study was, therefore, carried out on several ionic media formulations, all of which contained a polymer and Ca^{++}. Within this group, the most successful was HEPES buffered KCl-inositol. For normal sperm samples it gave within 80% of the fusion response of pulsing in sucrose and for oligospermic samples from radiation-exposed men it proved superior.

For exocytotic sytems, it has been proposed that phospholipid breakdown may be the inital event that follows receptor activation. This breakdown liberates DAG and the inositol phosphates IP2 and IP3, which may function as intracellular messangers and mobilize the release of intracellular calcium stores and mediate Ca^{++} uptake. It was found that sperm pulsed in a partially ionic medium in the presence of IP3 exhibited significantly enhanced sensitivity to extracellular Ca^{++}. In the presence of <=0.75 mM Ca^{++}, the addition of IP3 induced an 85% increase in the penetration response and in the presence of 10 mM Ca^{++} it yielded a 26% increase in the level of polyspermy. Whether these results were due to increased utilization of pulsed Ca^{++}, increased uptake or release of intracellular Ca^{++} was not clear, but this was the first report of an IP3 induced effect on sperm exocytosis.

Many of the calcium-induced responses in sperm are mediated by calcium binding proteins. It has been suggested that calmodulin might be the intracellular receptor for Ca^{++} in the acrosome reaction. To study this, sperm were treated with antipsychotic phenothiazine calmodulin-binding drugs. Their effect could be overridden by the pulse procedure, but following pre-incubation methodology, there was a small but significant increase in the penetration score following incubation with >=10 μM of the drug while inhibition occurred at <=1μM. The differential response of two of the drugs (TFP and PMZ) was tentative evidence for the involvement of calmodulin or synexin-like proteins.

Studies on the application of electropermeabilization to the analysis of sperm chromosomes from radiation-exposed men is continuing and it is felt that this procedure in conjunction with the defined synthetic media will make a significant contribution to large scale studies on the effects of radiation on the chromosomes of human gametes.

Sperm Chromosome Banding Techniques: In order to study the induction of small chromosome rearrangements in sperm following radiation exposure, it was necessary to develop suitable banding techniques. Improved Q-banding of the sperm chromosomes was possible following stabilization of atebrin FS fluorescence with buffered sucrose. R-banding was achieved with chromomycin A3 and methyl green counterstain and the method was modified for the banding of sperm chromosomes from radiation-exposed men.

Standard G-banding procedures did not produce results amenable to detailed cytogenetic analysis of sperm chromosomes. Relatively effective G-banding was achieved by: (i) actinomycin D, added to the fertilized eggs cultured during G2 resulted in extended G-bands in the majority of sperm chromosomes at fixation; (ii) exposure to lower doses of AMD throughout S, G2 and M phases did not induce native chromomere banding but did ensure a controlled, reproducible response to post-fixation banding by trypsin and dilute stains.

A new silver staining method was developed for use with R- and G- banded sperm chromosomes and was useful for identifying nucleolus organizer regions, satellites and fragile sites in sperm from radiation-treated men.

Alternative Procedure for Sperm Chromosome Analysis: The technique for sperm chromosome analysis using electropermeabilization and defined media should have wide application to the study of the chromosomes of human sperm. Nevertheless, the technique is technically difficult and requires the recovery of large numbers of zona pellucida-free eggs from superovulated hamsters. During this project, attempts were made to develop an alternative technique for sperm chromosome analysis applicable to radiation studies. Following penetration of a zona-free hamster egg by a capacitated sperm, the sperm head swells and the tightly condensed sperm chromatin decondenses. A variety of methods for the in vitro decondensation of sperm was examined. A large-scale study of polymers revealed that heparin and dextran-SO_4 were the most efficient in vitro decondensers of human sperm. However, these were not sufficiently effective to permit sperm chromosome analysis. Therefore, at the present time, interspecific in vitro fertilization of zona-free hamster eggs remains the only effective method for the large-scale investigation of the effects of radiation on the induction of cytogenetic aberration in the chromosomes of human spermatozoa.

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

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V. Publications:

Tomkins, P.T., Carroll, C. and Houghton, J.A. (1985). Analysis of human sperm chromosomes and factors involved in their formation in a heterospecific in vitro environment. Journal of Embryology and Experimental Morphology 82, 202.

Tomkins, P.T., Carroll, C. and Houghton, J.A. (1986). The cytogenetic preparation of oocytes and pre-implantation embryos: a modification of the method of Tarkowski. Laboratory Practice, 35, 81-83.

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Gesellschaft für
Schwerionenforschung
Planckstrasse 1
D-6100 Darmstadt

Contract no.: BI6-E-197-D

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

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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 an energy range between 1 and 100 MeV/u are used to study the mechanisms of the action of heavy charged particles on mammalian cells. Induction of chromosome aberrations in V79 cells as a function of atomic number, LET and particle energy has been studied at various time intervals after exposure and for fractionated exposure. Changes in cell cycle progression and radiosensitivity of synchronous V79 cells are measured for various ions and energies.

II. Objectives for the reporting period:

III. Progress achieved:

A great interest in the mechanisms of radiobiological action of heavy ion beams was generated as a consequence of the increasing use of heavy charged particles in medicine and the growth in space research. In addition, the biologically effective action of neutrons consists of the recoil and the reaction components of the neutron collisions i.e. in the action of produced heavy charged particles.

Due to the different mechanism of energy deposition by heavy ions, the pattern of biologically relevant ionisation and electrons is different from X and γ rays which are typical representatives of sparsely ionizing radiation. Therefore, differences in the biological efficiency have been observed [1]. For the purpose of radiation protection, the relative biological efficiency (RBE) as a quality factor has been introduced, where the quality of the radiation is mostly given as a function of linear energy transfer (LET) [2].

Besides the problems which arise from a characterisation of the radiation quality by the LET, there is a basic problem in using RBE values measured by inactivation experiments for the other biological effects as, for instance, genetic mutations. This would imply not only that the basic mechanism for the production of the lesion is the same for all different endpoints but also that no difference in the probability of repair or non or misrepair exist between low and high LET radiation. Assuming that above the maximum of biological efficiency the cells are killed by one or a few traversals of heavy particles through a cell nucleus, the chance of surviving cells carrying a genetic mutation should differ from the situation at low LET where cells have a high chance of undergoing repair or misrepair. Therefore, the corresponding RBE between cell killing and mutation could be different. In the extreme case, when a single cell is killed by one traversal of a heavy particle through the nucleus, no mutation should occur at all. In radiation protection, for high LET radiation a high biological efficiency is assumed for all biological endpoints including inactivation, transformation, and mutation [2]. This is experimentally not justified.

In order to gain more insight into the mechanism of cellular damage after heavy ion exposure the induction of chromosome aberration has been studied over a large range of particle energies and atomic numbers. In chromosome experiments it is possible to monitor the induced damage immediately after irradiation and to follow the development of this damage over many cell cycles. In the literature, particle induced aberrations are only measured for lighter ions using V79 chinese hamster cells. L. Skarsgard et al. [3] who

measured chromosomal aberrations for He, Li, B, C, and O-beams but used one harvesting time of 12 hours only. For H-, D-, and ^{3}He-beams, Ch Geard [4] measured the chromosomal aberrations in asynchronous and synchronous populations of V79 cells. Chromosome aberration in human lymphocytes have been also studied at Dubna using various relativistic heavy ion beams [5]. The complexity of heavy ion induced chromosomal aberrations has been studied experimentally using different banding techniques by L. Sabatier et al. [6] for high energetic neon and carbon beams. It was the aim of our measurements to extend the energy- range and the particle range to that of the presently available heavy ions as well as to increase a time intervals from short time intervals directly after exposure up to 48 hours or longer.

During the measurements with very heavy ions, however, it turned out to be difficult to assess the chromosomal damage after exposure because cell proliferation strongly depends also on the LET and particle energy and on the particle fluence. From measurements using sparsely ionizing radiation a dose dependent block of asynchronous cell populations, mostly in G2- and M-phase up to 9 hours has been reported [7]. However, after this block the cells progress through cell cycle relatively homogeneously to division and into one further generation at least. Heavy ion irradiation introduces a completely different pattern of delay in the cell cycle. Cells can be blocked in all phases and the duration of these cell cycle blocks can be much longer than that known for sparsely ionizing radiation. In order to understand the changes in cell cycle progression, cells have been synchronized and the shifts in cell cycle progressions have been measured via cytofluorometric determination of the DNA content.

Using synchronous cells, the variation in radiosensitivity of cells in different cell cycle stages was also measured in comparison to the effects of sparsely ionizing radiation. However, the main emphasis of this investigation was concerned with the study of chromosomal aberrations.

Materials and methods

V79 chinese hamster cells were cultured under standard conditions as described in ref. 8. For the exposure of asynchronous cells, 24 hours prior to irradiation, cells were subcultured on 3.5 cm petri dishes and centerplated in an area of approximately 1 cm in diameter. For the experiments with synchronous cell populations, different methods of chemical synchronization have been tested including treatment with colcemid, aphidicolin, and hydroxyurea. However, treatment with these drugs interferes with radiation sensitivity and yields only partially synchronized populations. Therefore, mechan-

ical synchronization using the centrifugal elutriation method [9] has been used. With this method, a high purity in G1-cells of 90% or better can be reached while the purity of cells in the beginning and late S-phase decreases to some 70%. Therefore, normaly cells have been synchronized in G1-phase and plated immediately after synchronization. These cells then proceed through the cell cycle after attachement and reached G2-phase within the normal progression time of 8-9 hours. These cells have been used at different time intervals after synchronization when cells in different cell cycle phases have been required. The extent of synchronization was controlled by analysis of cell size using in a coulter counter and by analysis of the DNA-content in a flow cytometer.

For the synchronization measurement and the cell cycle progression experiments the DNA was stained with a fluorescent dye (Hoechst 33258) and measured in a flow cytometer (ICP 22). For the preparation of chromosomes, cells were harvested up to 24 hours after irradiation proceeded by a 2 hour colcemid treatment to accumulate the cells in mitosis. Hypotonic treatment was carried out and cells were fixed. Chromosomes were prepared according to standard procedures [10] and scored to the following categories: breaks, isobreaks, deletion, isodeletion, fragments, isofragments, exchanges, dicentrics, and disintegrations.

Track segment exposure [11] has been performed at Darmstadt and Caen using the Unilac in the energy range of 1-20 MeV/u for carbon to uranium ions and at the Ganil for carbon, oxygen, neon, calcium, and argon ions in the energy range between 30 and 95 MeV/u. Particle fluences were monitored by the use of secondary electron detectors and ionization chambers which were calibrated using nuclear track detectors. LET values have been calculated according to the tables of F. Hubert et al.[12].

Results and discussion

Cell cycle progression measurements

Radiation induced delays in cell cycle progression have been measured for α-particles and heavy ion beams using asynchronous cell populations [7,13]. These measurements using cytofluorometric methods show partially contradictory results and are difficult to correlate with measurements of cell cycle delay using the mitotic index [14] or PLM-methods [15]. An interpretation of these experiments is difficult because they are performed with asynchronous cell populations. In this case, the measured DNA spectra are only sensitive to relative changes within the population of the cell cycle phases. A simultaneous slowing down of all cells in all phases does not change the relative portion

of cells in different phases and therefore cannot be detected using this method. With synchronized cells, delays in the exposed cells can easily be monitored for each phase separately. In fig. 1, the cell cycle progression for synchronous V79 cells irradiated in G1-phase is compared for heavy ion exposure (10 MeV/u Pb ions with a fluence of 2×10^6 particles/cm^2) and of 600 rad X-ray exposure which both yield a survival level of approx. 10%.

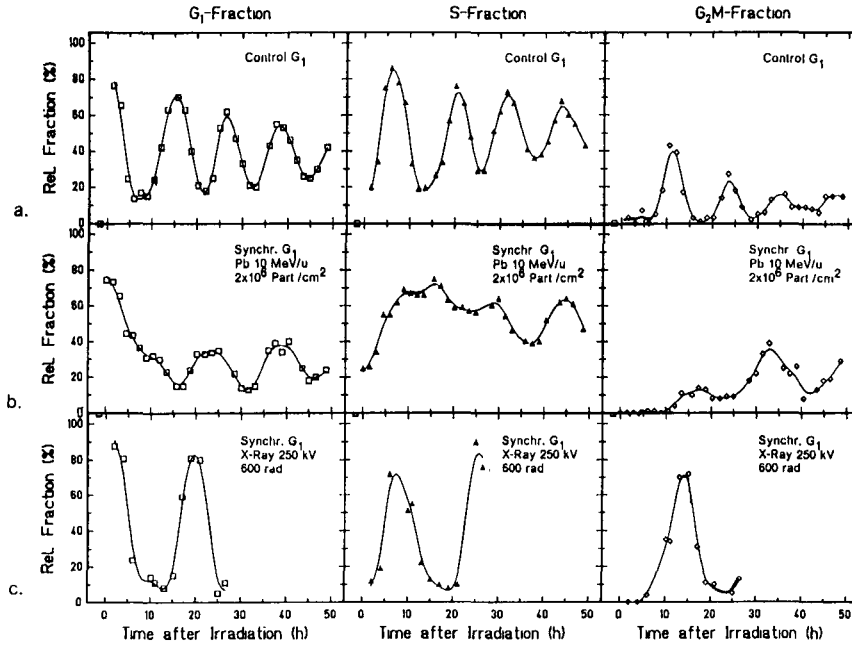

Fig. 1: *Cell cycle progression of synchronous V79 Chin. hamster cells after exposure to 10 MeV/u Pb ion with a fluence of 2×10^6 part/cm^2 and 600 rad X-rays.*

In the X-irradiation, the exit out of the G1-phase is nearly not delayed. But the maximum of the G2/M-phase is shifted and is much more pronounced than in the control, indicating an accumulation of cells in G2/M-phase. After this delay cells then cycle with a normal cycle time.

Exposure to Pb ions yields a very different pattern: The exit out of G1 is drastically slower and the first minimum of the G1 population is reached 16 hours after exposure compared to 8 hours of the control. After that time most of the cells exhibit a higher DNA content than that of G1-phase. But it is not clear whether this stage can be attributed to normal DNA-synthesis, or not.

An overhelming portion of the exposed cells does not leave this stage up to 48 hours and only a few percent proceed to G2 phase. This small fraction of cycling cells might not have been hit by a particle, because a fluence of $2 \times 10^6/cm^2$ is equivalent to an average number of 2 hits per cell nucleus. Assuming a Poisson distribution, 15% of the cell nuclei will not be hit which fits to the amount of cycling cells observed. According to this finding, many cells which are hit by at least one particle are blocked in S- and G2-phase and do not reach the next mitosis within the next 48 hours. Similar block in progression has been shown for other cell ages when exposed to very heavy ions at low energies. This is a very different finding when compared to x-rays where even at high exposures all cells proceed to the next mitosis. A summary of our measurements for different ion beams including Ne-, Ar-, Ca-, Kr-, Xe-, Pb-, and U-ions is in preparation.

Variation of radiosensitivity with cell age

Experiments with high energetic lighter ions (He, C, Ne) have shown that the variation in radiosensitivity with cell age is drastically decreased compared to the variation produced by X- or γ-rays [15]. However, for these ions the maxima and minima of radiosensitivity are still located at the same stage of the cell cycle. In the case of V79 chinese hamster cells, a maximum of radiosesistivity is found at the end of the S-phase while G2-phase and G1-phase are less radioresistant. Experiments with low energy heavy particles of high LET yield an opposite cell age response. In several experiments, the cell cycle dependent radiosensitivity of V79 cells was measured (fig.2). Cells were synchronized in G1-phase allowed to proliferate and irradiated at different time intervals after synchronization. As shown in fig. 2 for heavy ion exposure, the radioresistance is highest in G1-phase. Radioresistance decreases for the later phases of the cell cycle and reaches a minimum in late S-phase and at the G2 border. For G2/M-cells, radioresistance increases again. Up to now, all experiments performed with heavy ions showed the similar variation of radioresistance within the cell cycle.

This variations correlates strictly with the geometrical cross section of the cell nucleus. Nuclei in G1-phase have a smaller likelyhood of being hit because of their small geometric cross sections. With increasing size of the cell nucleus, the cross section increases and the chance to be hit by heavy particles increases proportionally. Therefore, cells in G2-phase have the highest probability of being killed by heavy ion exposure. In M-phase the cells round up again and have a smaller geometrical cross section. Therefore, radioresistance increases again.

A summary of our measurements together with the more detailed analysis will be published elsewhere.

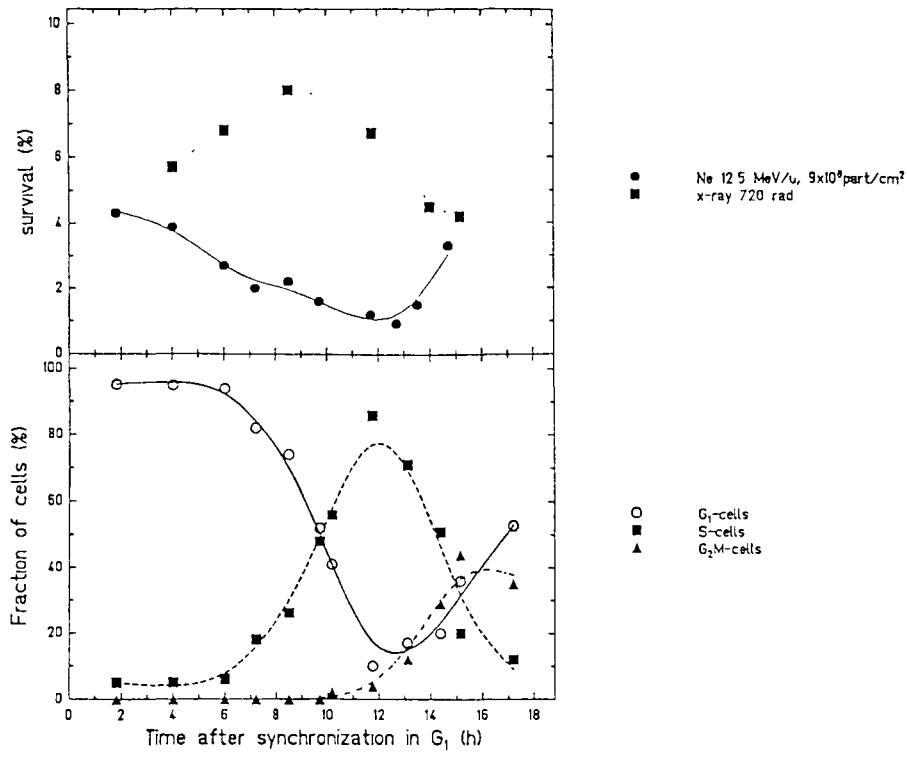

Fig. 2: *Radiosensitivity of V79 Chinese hamster cells as function of cell age is compared for X-rays and Ne-ions. In the lower panel, the proportion of cells in the different cell cycle stages at the irradiation time is given*

Chromosome aberration measurements

Chromosome aberrations induced by densely ionizing radiation differ clearly from X-ray induced aberrations. Firstly, the appearance of aberrant cells after exposure is delayed compared to X-rays. Secondly, the distribution of the aberration types is different. In fig. 3, the percentage of cells reaching the first mitosis is given as a function of time after exposure for 12.6 MeV argon ions. A minimum in the mitotic index is observed 4-8 hours after exposure and is below 2% for the higher particle fluences. At a particle fluence of $0.5 \times 10^6/cm^2$ more than 50% of cell nuclei are not hit. These cells will proceed to mitosis

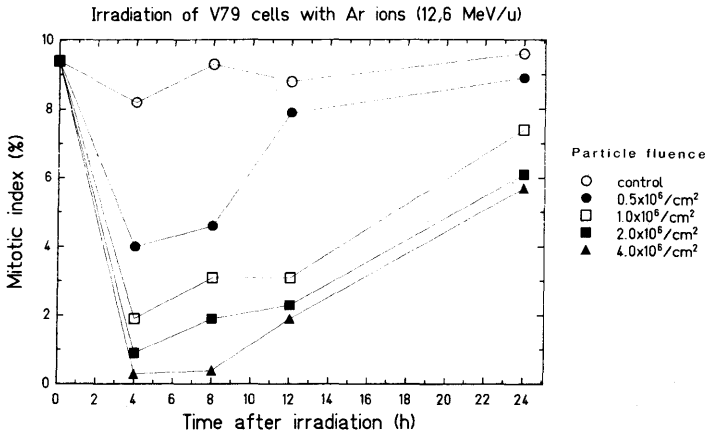

Fig. 3: *Percentage of cells reaching mitosis as a function of time after heavy ion exposure.*

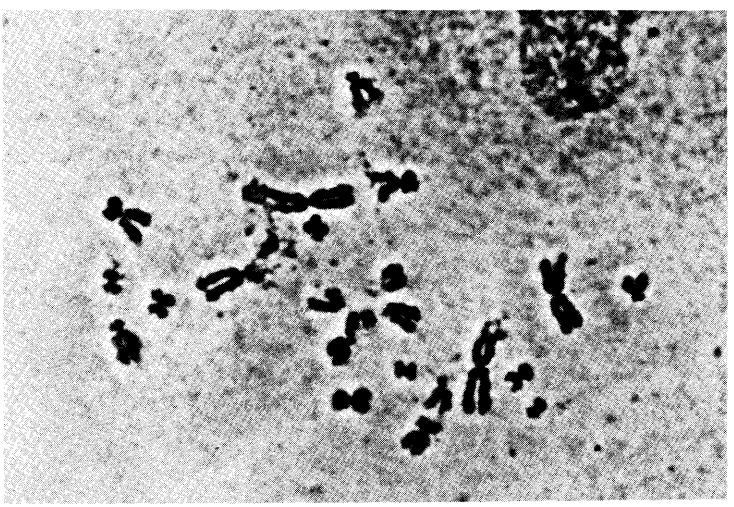

Fig. 4: *Aberrant metaphase of a V79 cell exposed to 12.6 MeV/u Ar-ion with* 4×10^6 *part/cm*^{2}*. The cells have been exposed in mitosis and exhibit a large amount of chromosomal disintegration.*

in the normal time. However, only nuclei which have been hit, have a chance to develope an aberration, but are also significantly delayed (see also the cell progression measurement). It is not clear at the moment whether all cells which carry a chromosome damage finally reach the first mitosis. In the cell progression measurements reported above a large fraction of cells appear not to reach mitosis. In contrast, cells having complete destruction of whole chromosomes or major parts of the chromosomes have been observed frequently in the first hours after heavy ion exposure (fig 4), indicating, that extreme chromosome damage does not prevent cells from progression through the cell cycle. However, the question whether the fraction of cells which have been hit will stop in proliferating or not, will studied be studied later.

From the delay in cell progression it is evident that the maximum in aberrations will be observed with a dose dependend delay after exposure. But even if the fraction of aberrant cell is normalized to the number of cells which reach mitosis, an increase in the probability of aberration is observed after 8 to 12 hours. In fig. 5, the percentage of abnormal metaphases is given as a function of particle fluence and the time after exposure. The higest probability for chromosomal damage is found always between 8 to 12 hours after exposure while at 24 hours a smaller percentage of aberrations is observed. Concerning the absolute number of aberrations normalized to the total number of cells, a maximum in aberrant cell is found much later which is due to the increase of the mitotic index.

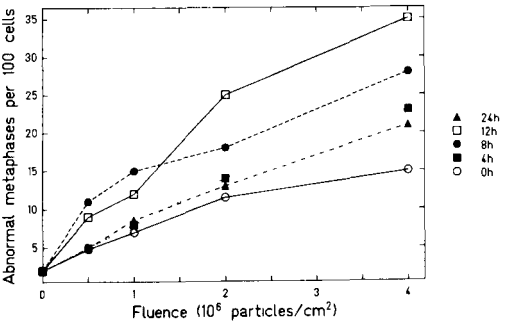

Fig. 5: *Induction of abnormal metaphases as a function of particle fluence and time after irradiation.*

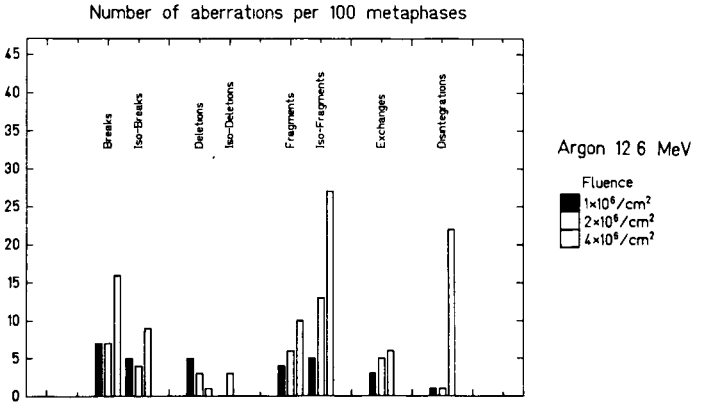

Fig 6: *Typical distribution of the various types of structural chromosome aberrations 8 hours after heavy ion exposure.*

Finally, the heavy ion induced aberrations exhibit a different distribution of the aberration type when compared to X-ray exposure. In fig. 6, a typical distribution of the various types of structural chromosome changes observed 8 hours after heavy ion irradiation is shown. The distribution is clearly dominated by the formation of breaks, deletion, and fragments. Whereas exchanges (including rings and dicentric chromosomes) are found to a lower extent. Previously, the same tendency has been found using various heavy ions and different energies [1]. Furthermore, the analysis of chromosome damage reveals that with increasing particle fluence the number of aberrations per abnormal metaphasis increases and aberrations of higher complexity are formed. This result is quite similar to that obtained from human lymphocytes exposed to the neon beam [7]. More detailed description of our chromosome experiments using asynchronous cell population and a large variety of heavy ions including 70 and 90 MeV/u Carbon-, 84 MeV/u Oxygen-, 12 and 45 MeV/u Neon-, 12, 15, 60 and 75 MeV/u Argon-, 45 MeV/u Calcium-, 6.2 MeV/u Titanium-, 15 MeV/u Krypton-, 12 MeV/u Xenon-, and 6, 13, and 15 MeV/u Uranium-ions will be presented in a forthcoming paper which is in preparation.

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

Dr. L. Sabatier, Dr. B. Dutrillaux (Fontenay Aux Roses)

V. Publications:

G. Kraft. Radiobiological effects of very heavy ions: Inactivation chromosomal aberration and strand breaks. Nuclear Science Application, Vol. 3, 1-28 (1987)
G. Kraft, W. Kraft-Weyrather, S. Ritter, M. Scholz, J.A. Stanton. Cellular and subcellular effects of heavy ions: A comparison of the induction of strand breaks and chromosomal aberrations with the incidence of inactivation and mutation. Adv. Space Res., 9, No. 10, 59-72 (1989)
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G. Kraft. On the interpretation of radiobiological experiments performed with heavy charged particles. IAEA-TAEDOC 506, Vienna, 117-127 (1989)

RADIATION PROTECTION PROGRAMME
Final Report

Contractor.

State University of Leiden
Stationsweg 46
NL-2300 RA Leiden

Contract no.: B16-E-166-NL

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

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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, Dr. M.Z. Zdzienicka.

Scientific staff:

I. Objectives of the project:

This project aims to isolate and characterize repair deficient mutants from mammalian cell cultures. Rodent cells (V79 and CHO Chinese hamster cells) are mutagenized in order to induce mutations in genes which are involved in DNA-repair. Via replica plating clones are identified which are sensitive to DNA-damaging agents.
Isolated repair deficient mutants will be characterized in terms of survival after treatment with a variety of DNA-damaging agents. Via complementation analysis it will be ascertained whether they belong to different complementation groups and whether they complement repair deficient mutants isolated by other laboratories. Also, the complementing ability with known human repair deficient syndromes will be investigated.
Furthermore the feasibility of the isolation of human genes which are involved in DNA-repair, using these repair deficient mutants, will be tested.

II. Objectives for the reporting period:

1. Isolation of repair deficient mutants.
2. Determination of cross-sensitivity to other DNA-damaging agents.
3. Genetic complementation analysis.
4. Biochemical analysis (see project 2).
5. Determination of mutability (see project 3).
6. Comparison with known human disorders.
7. Determination of stability.
8. Determination of transfectability.

III. Progress achieved:

Methodology

a. Isolation of repair deficient cell lines.
Mutagenized cells were prepared by treating large numbers of cells with 4-5 mM of ENU and growing these treated cells for four days for fixation and expression of induced mutations. After this cultivation period the cells were frozen in ampules and stored in liquid nitrogen. To isolate repair deficient cells an ampule is thawed and cells are seeded for the formation of clones. Individual clones are isolated and seeded in microwells. From these microwells six identical replicas are prepared. Five of them are treated with DNA-damaging agents: X-irradiation, ultraviolet light, MMS, EMS and MMC. The applied doses and concentrations do not visibly harm repair proficient cells but do harm repair deficient cells. Mutagen sensitive cells are propagated and their properties confirmed by the determination of a complete survival curve.

b. Genetic complementation analysis.
Cells from two mutagen sensitive mutants are co-cultivated in 1:1 ratio of each type. These cells are exposed to a medium containing PEG (poly-ethylene glycol) to induce cell hybridization. After cell hybridization the cells are seeded in a medium in which only hybrids between the two parental cell lines can survive. The mutagen sensitivity of these hybrid cells is determined. If the hybrid cells are not sensitive to the mutagen for which the parental cells were sensitive it is indicated that the two mutants complement each other and thus belong to different complementation groups.

c. Determination of stability.
For each type of mutagen sensitivity it is determined which treatment regime allows wild type cells to survive while mutagen sensitive cells die. This treatment is applied to about 10^7 viable cells. Revertants, if present, are retested for mutagen sensitivity and the reversion frequency is calculated.

d. Determination of transfectability.
Different methods of DNA transfection have been used: 1. calcium phosphate precipitate with differet buffers, 2. poly ethylene glycol and 3. lipofectin mediated DNA transfer.

Results

a. Isolation of repair deficient mutants.
Altogether thirteen repair deficient mutants have been isolated on the basis of mutagen sensitivity. These are :

UV-sensitive mutants	: V-H1, V-B11, C-A6.
x-ray sensitive mutants	: V-C4, V-E5, V-G8, V-15B.
MMC-sensitive mutants	: V-C8, V-B7, V-H11, V-H4.
MMS-sensitive mutants	: V-24B, C-G11.

b. Determination of cross-sensitivities of the repair deficient mutants.
The sensitivities of the mutants to a series of DNA-damaging agents is given in table 1. Cross sensitivity is widely found, but sofar no clear patterns are visible. MMC is most often involved in cross sensitivity and x-rays the least.

Table 1.
Cross sensitivities of repair deficient mutants. Sensitivity is expressed as the ratio of the $D_{10's}$ (dose required to reduce survival to 10%) of wild type and mutant cells. (- = not tested; ss = slightly sensitive; ns = not sensitive).

Class of mutants	mutant	UV	4NQO	MMC	x-ray	ADR	BLM	MMS	EMS
UV	V-H1	10	5	2	ss	-	-	-	-
	V-B11	3	ns	2	ss	-	-	-	-
	C-A6	3	4	2	ns	-	-	-	-
x-rays	V-C4	2	2	2	3	2	5	2	-
	V-E5	ss	ss	4	3	5	2	2	-
	V-G8	ss	ns	ns	2	2	2	2	-
	V-15B	ns	3	ns	8	-	3	2	2
MMC	V-C8	2	3	110	2	5	-	8	5
	V-C7	2	ss	8	2	-	-	ss	ss
	V-H11	ss	3	8	ss	-	-	-	-
	V-H4	ss	3	30	2	2	-	ns	ns
MMS	V-24B	ss	-	30	ns	5	-	4	5
	C-G11	ns	2	2	ss	ns	-	6	8

c. Genetic complementation analysis.
An overview of the complementation studies is given in table 2.
The three UV-sensitive mutants V-H1, V-B11 and C-A6 have been crossed to a panel of six UV-sensitive CHO repair deficient mutants, each representing a different complementation group. V-H1 and C-A6 were found to belong to complementation group 2 while V-B11 complemented all known complementation groups and thus has to be allocated to a new seventh complementation group of UV-sensitive mutants.
The four x-ray sensitive mutants fall in two complementation groups. V-15B belongs to the same complementation group as the xrs (1 to 6) mutants of x-ray sensitive mutants isolated by Jeggo while V-C4, V-E5 and V-G8 have to be placed in a new complementation group.
The complementation analysis of the MMC-sensitive mutants is not yet complete. Sofar three complementation groups have been identified and no homology with published rodent MMC-sensitive mutants has been established. An important finding was that V-H4 proved to be homologous with Fanconi anemia complementation group A. V-B7 could not be analysed as it does not behave as a recessive in crosses with repair proficient cells.

Table 2.
Complementation analysis of repair deficient mutants. (* = international classification is not yet available).

Class of mutants	mutant	complementation group	complementation with
UV	V-H1	compl. gr. 2	
	V-B11	compl. gr. 7	
	C-A6	compl. gr. 2	
x-rays	V-15B	compl. gr. 1*	
	V-C4	compl. gr. 2*	
	V-E5	compl. gr. 2*	
	V-G8	compl. gr. 2*	
MMC	V-C8	compl. gr. 1*	V-H4, V-H11, UV20, UV41 irs1SF
	V-H4	compl. gr. FA-A	V-C8, V-H11, MMC-1,2,3, irs1 irs1SF, UV20, UV41
	V-H11	compl. gr. 2*	V-C8, MMC-1,2,3, UV20, UV41
	V-B7		
MMS	V-24B		
	C-G11	compl. gr. 1*	

The complementation analysis of MMS-sensitive mutants indicates that C-G11 belongs to the same complementation group as the EM9 mutant of Thompson.

d. Biochemical analysis (see project 2).
e. Determination of mutability (see project 3)
f. Comparison with known human disorders.
The complementation analysis indicated that V-H4 is homologous with Fanconi anemia complementation group A. This proved to be in agreement with the biochemical and mutational analysis of V-H4 which corresponds with the characteristics of cells derived from patients with Fanconi anemia (see projects 2 and 3).
g. Determination of stability.
Three mutants have been tested for reversion: V-H1, V-15B and V-H4. V-H1 proved to be stable with a reversion frequency of $3.5X10^{-7}$. These revertants still are about 2-fold more sensitive to UV than wild type cells. V-15B is unstable with a reversion frequency of about 10^{-3}. V-H4 proved to be very stable; no revertants have been observed which means that the reversion frequency must be smaller than $2.5X10^{-8}$.
h. Determination of transfectability.
Good results have been obtained with the first transfection method. About 200 to 1000 transfectants were obtained per petri dish with 10^5 cells.

Discussion

During the course of this project a large number of repair deficient mutants have been obtained from Chinese hamster cells. The V79 cell line

appears to be very suitable for the isolation of repair deficient mutants.
The complementation analysis has been completed for 9 of the 13 repair deficient mutants and two new complementation groups have been identified, one for UV and one for X-rays. Especially rewarding was the finding that V-H4 is homologous with Fanconi anemia complementation group A. As presently conditions have been found which permit an efficient transfection of DNA into V79 Chinese hamster cells, this opens the exciting possibility of isolating the human gene which is defective in Fanconi anemia.
The isolation of revertants from V-H1, which are still phenotypically different from wild type, will be important for the study of the function of the ERCC-2 gene of complementation group 2 which has been recently isolated by Thompson.

IV. 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)
Department of Biochemistry, Leiden (Prof. Dr. P. van de Putte)
Department of Molecular Carcinogenesis, Leiden (prof. Dr. A. van de Eb)
Medical Biological Laboratory, Rijswijk (dr. G.P. van der Schans.
Anthropogenetisch Instituut, GU Amsterdam (Dr. F. Arwert)
Lawrence Livermore National Laboratory, California (Dr. L.H. Thompson).
Department of Genetics, California (Dr. J. Boyd).
School of Biological Sciences, Swansea U.K. (Dr. R. Waters).

V. Publications:

Zdzienicka, M.Z. and J.W.I.M. Simons (1986) 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, 59-69.

Zdzienicka, M.Z. and J.W.I.M. Simons (1987) DNA-repair deficient mutants are induced with a high frequency in V79 Chinese hamster cells, Mutation Res., 178, 235-244.

Zdzienicka, M.Z., G.P. van der Schans, A. Westerveld, A.A. van Zeeland and J.W.I.M. Simons (1988) Phenotypic heterogeneity within the first complementation group of UV sensitive mutants of Chinese hamster cell lines, Mutation Res., 193, 31-41.

Zdzienicka, M.Z., G.P. van der Schans and J.W.I.M. Simons (1988) Identification of a new seventh complementation group of UV-sensitive mutants in Chinese hamster cells, Mutation Res., 194, 165-170.

Zdzienicka, M.Z., J.W.I.M. Simons and P.H.M. Lohman (1990) Chinese hamster cell lines defective in DNA repair. In: DNA repair mechanisms and their biological implications in mammalian cells. (M.W. Lambert, ed.) Plenum Press. N.Y. (in press).

Zdzienicka, M.Z., N.G.J. Jaspers, G.P. van der Schans, A.T. Natarajan and J.W.I.M. Simons (1989) Ataxia-Telangiectasia-like Chinese hamster V79 cell mutants with radioresistant DNA synthesis, Chromosomal instability and normal DNA strand break repair. Cancer Res., 49, 1481-1485.

Title of the project no.: 2

Biochemical analysis of DNA repair

Head(s) of project: Dr.Ir. A.A. van Zeeland
Dr. L.H.F. Mullenders

Scientific staff: Dr.Ir. A.A. van Zeeland
Dr. L.H.F. Mullenders
Dr. M.Z. Zdzienicka
Drs. J. Venema

I. Objectives of the project:

In this project we will study the biochemical aspects of DNA repair induced by radiation, in normal cells as well as radiosensitive mutants, in relation to biological endpoints such as cell killing, induction of gene mutations, and chromosomal aberrations. Radiosensitive cell lines in which the radiosensitivity is complemented by the introduction of a cloned repair gene will also be investigated. Emphasis will be put on: (a) the role of chromatin structure in the distribution and repair of damage induced by radiation, (b) the nuclear localization of the various steps of the repair process, and (c) the structure of DNA repair patches using inhibitors of DNA synthesis.

II. Objectives for the reporting period:

III. Progress achieved:

1. Methodology

1.1 The distribution of repaired sites in higher-order chromatin loops has been analyzed in UV-irradiated stationary human fibroblasts. Repair synthesis was detected by incorporation of ^{3}H-TdR in the presence of hydroxyurea which is a specific inhibitor of normal replicative synthesis. The distribution of repaired sites in chromatin loops was investigated by digestion of DNA-nuclear matrix complexes with DNAsel or nuclease S_1. The structure of repair patches was analyzed by nuclease S_1 or Bal31 digestion of DNA isolated from UV-irradiated human fibroblasts.

1.2 Removal of UV-induced pyrimidine dimers (PD) from defined DNA fragments was studied in human fibroblasts using the dimer specific enzyme T4 endonuclease V (T4-endo). DNA samples treated with or without T4-endo were subjected to alkaline electrophoresis, Southern blotting and hybridization. The PD content of defined fragments of (in)active genes was determined by quantification of intensities of full size restriction bands on DNA samples treated or untreated with T4-endo.

1.3 A permeable cell system aimed to study UV-induced repair in vitro, was established using the procedures described by Dresler et al. (Biochemistry 21, 2557, 1982).

1.4 Induction and repair of DNA breaks was determined by alkaline elution. Repair of PD from the genome overall was quantified by T4-endo incubation of permeabilized cells and alkaline sucrose gradient centrifugation. DNA repair-replication was performed by using cesiumchloride density gradients. Removal of 6-4 photoproducts (6-4 PP) was measured via a radioimmunoassay by Dr. D. Mitchell, Univ. of California, USA.

2. Results

2.1 DNA repair and chromatin structure.

The eukaryotic DNA is organized in supercoiled loops by anchorage to a protein structure termed nuclear matrix (interphase) or scaffold (mitosis). Topoisomerase II has been identified as an integral component of both structures and this finding has led to the suggestion that the nuclear matrix somehow poise chromatin domains for replication and transcription by introducing or removing torsional stress in defined chromatin regions. Furthermore specific sequences have been found at the base of loops associated with the nuclear matrix. Such an organization is thought to bring about functional compartimentalization within the nucleus and facilitates DNA replication and transcription. These processes are intimately associated with the nuclear matrix. A model in which attachment of DNA to the nuclear matrix is a necessary precondition to create proper substrates for enzymatic processes can be readily extended to DNA repair. Repair can occur when DNA lesions become attached to repair enzymes located at the nuclear matrix.

The initial experiments aimed to investigate the intranuclear localization of DNA repair, were performed with confluent human fibroblasts exposed to 30 J/m² (Mullenders et al., Biochem. Biophys. Acta, 740, 328 [1983]; Carcinogenesis, 7, 995 [1986]). Repaired sites were marked by pulselabelling (5-10 min) of the cells at different time periods following UV-exposure. DNA-nuclear

matrix complexes were isolated by extraction with high concentrations of NaCl, and the relative positions of repaired sites to the nuclear matrix were determined by DNAsel digestion. No indications were found for a preferential localization of repair synthesis at the nuclear matrix. Enzymatic analysis showed that the majority of repair patches was not ligated during a 10 min pulse ruling out the possibility that the repair process is too fast to be trapped at the nuclear matrix (Mullenders et al., J. Cell. Sci., 6, 243 [1987]). However, incubation with nuclease S_1 which converts the single stranded DNA regions at sites of repair into double strand DNA breaks, revealed that repair events were not randomly distributed along the DNA loops, i.e. sites of repair appeared to be clustered within DNA loops. These clusters can reside at any position within DNA loops (Mullenders et al., Biochim. Biophys. Acta, 826, 38 [1985]). Further analysis revealed that the distribution of repair in chromatin loops was markedly different at low UV-exposure (5 J/m²). In confluent normal fibroblasts exposed to 5 J/m², repair synthesis was preferentially found in nuclear matrix associated DNA, when cells were pulselabelled immediately following UV-irradiation. At later time periods (2 hours after UV-treatment) repair approached a random distibution. The non-random distribution of repaired sites at early times after irradiation, represented the preferential repair of DNA permanently bound to the nuclear matrix, as deduced from pulse-chase experiments (Mullenders et al., Nucleic Acids Res., 16, 10607 [1988]). These results do not favour prior attachment of damaged sites to the nuclear matrix in order to be repaired. Instead it became obvious that some chromatin domains located at the base of loops, are preferentially repaired initially after irradiation. The observation that preferential repair of nuclear matrix associated DNA was only restricted to a short period after UV-treatment, fits in with the concept that in human fibroblasts exposed to a low UV-dose, repair of functionally important domains in the genome including transcriptionally active DNA, occurs quickly during a short period after treatment.

The biochemical approaches require the use of confluent cells and the presence of inhibitors. To study repair in exponentially growing cells in the absence of inhibitors we employed the fluorescence DNA-halo technique, which allows to examine repair at single cell level. The results obtained with this methodology (Mullenders et al., Carcinogenesis 7, 995 [1986]) also showed evidence for a dose dependent preferential repair of nuclear matrix associated DNA.

When DNA repair was labelled during the first two hours following irradiation, pronounced differences were found in distribution pattern of repaired sites in DNA loops among normal and UV-sensitive cell lines. In both normal and xeroderma pigmentosum group D (XP-D) fibroblasts exposed to 5 J/m², repair was about 2.0 fold more efficient in nuclear matrix associated DNA than in loop DNA. In xeroderma pigmentosum group C (XP-C) fibroblasts repair was highly specific for nuclear matrix associated DNA independent of the time period following irradiation, whereas in Cockayne's syndrome (CS) fibroblasts repair was found to be 2-fold less efficient than in loop DNA (Mullenders et al., Carcinogenesis 7, 995, 1986; Nucleic Acids Res. 16, 10607, 1988). This was the first demonstration of a repair defect in CS cells. Since transcriptionally active genes are located proximal to the nuclear matrix our

results suggest that the residual repair capacity of XP-C cells is specific for active DNA, whereas CS cells possess a defect in excision of UV-damage from transcriptionally active DNA. Detailed analysis of XP-C cells revealed that not all loops were repaired at their base, and that large domains were excluded from the repair process. Treatment of XP-C cells with sodiumbutyrate (to make the chromatin more accessible) did not change the non-random distribution indicating that the selective repair of UV-damage in XP-C cells is not simply due to the inability to render bulk chromatin accessible to repair enzymes.

To demonstrate preferential repair of UV-induced damage in transcriptionally active DNA in a more direct way we study the removal of pyrimidine dimers (PD) in defined fragments from (in)active genes using the methodology described by Bohr et al. (Cell, 40, 359, 1985). The adenosin deaminase (ADA) and dihydrofolate reductase (DHFR) housekeeping genes and the X-chromosomal 754 gene were chosen as active and inactive loci respectively. In confluent normal human fibroblasts exposed to 10 J/m² PD were 2-3 fold faster removed from the active ADA gene than from the inactive 754 gene. The kinetics of repair of the 754 gene was comparable to the PD removal for the genome overall. Confluent XP-C cells with a residual repair capacity of 15%, were able to perform efficient repair of the active ADA and DHFR genes, but were not able to repair the 754 locus. Fine structure analysis revealed that the 3' end of the ADA gene was repaired as good as in normal cells, whereas DNA fragments located at the 5' end of the ADA and DHFR genes were repaired to 60% of the level seen in normal cells (J. Venema et al., Nucleic Acids Res., in press). Further analysis with strand specific DNA probes showed that XP-C cells were only capable to remove PD from the transcribed strands of active genes. The occurrence of transcripts on both strands at the 3' end of the ADA gene explains the very efficient repair in this part of the gene. In CS cells exhibiting a normal repair level, the preferential repair of active DNA was found to be absent (J. Venema et al., submitted for publication). Instead, PD in active genes were processed with similar kinetics and to similar extent as in inactive genes.

So far, the heterogeneity in distribution of repaired sites in chromatin loops correlates well with preferential removal of PD in normal and XP-C cells, and its absence in CS cells. Analysis of DNA fragments still associated with the nuclear matrix following restriction of DNA-nuclear matrix complexes, revealed that the 5' end of the ADA gene upstream of the putative promotor region was bound to the nuclear matrix.

In UV-irradiated hamster V79 cells we found a rapid and efficient removal of PD from the HPRT gene, in contrast to the slow repair of the genome overall. The UV-sensitive derivative VH-1 was completely deficient in preferential repair of PD from the HPRT gene. These differences in repair correlate well with the 7-fold increase of UV-induced HPRT mutations in VH-1 compared to V79 wild type cells.

2.2 Characterization of a permeable cell system for in vitro repair studies

In vitro UV-induced repair synthesis was performed by αdCTP labelling of permeabilized cells. Only low levels of repair synthesis could be detected in stationary human fibroblasts permeabilized prior to UV-irradiation. However, repair synthesis was strongly enhanced when stationary cells were UV-irradiated prior to perm-

eabilization or when the pyrimidine dimer specific enzyme T4 endonuclease V was introduced into the permeable cells. Excision repair deficient xeroderma pigmentosum group A cells were not capable to perform repair synthesis, unless T4 endonuclease V was added to the cells. In preliminary experiments we were not able to correct the repair deficient phenotype XP-A cells by adding cellular extract of normal human fibroblasts, possibly due to inappropriate concentration of the extract.

2.3 Biochemical characterization of repair deficient mutants

The biochemical analysis of UV-sensitive repair deficient mutants is given in table 1 and of X-ray sensitive mutants in table 2.
Table 1 shows that different mutants which belong to the same complementation group differ in phenotype. This heterogeneity indicates 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. Despite that V-H1 is at least as sensitive and as mutable as UV-5 there is still 60% removal of (6-4) photoproducts although there is no repair of dimers. Interestingly revertants of V-H1 which are only slightly sensitive to UV and have a normal mutability after UV still are not able to remove dimers while the removal of (6-4) photoproducts is 100%. This could indicate that the (6-4) photoproducts are the main cytotoxic and mutagenic lesions. Therefore, the mutants of this complementation group and their revertants will be of great value to study this question.
Table 2 shows that V-B15 has the characteristics which have been published for mutants which belong to the same complementation group. The mutants V-E5, V-C4 and V-G8, which belong to a new complementation group demonstrate a normal number of induced single and double strand breaks, normal kinetics of their rejoining and a normal final level of unrejoined single and double strand breaks. These mutants all have a diminished inhibition of DNA synthesis compared to wild type cells. After exposure to 20 Gy the residual rate of DNA synthesis was about 40% for wild type cells while it was about 80% for the X-ray sensitive mutants. This biochemical characterization of the new complementation group indicates a similarity to the phenotype of cells derived from patients with Ataxia telangiectasia (see also project 3).

Table 1.
Biochemical characterization of UV-sensitive repair deficient mutants (- = not tested).

mutant and complementation group	removal of dimers	removal of (6-4) photoproducts	capacity of incision	repair replication
V-H1 (c.gr. 2)	0%	60%	50%	50%
revertant 1	0%	100%	-	-
revertant 2	-	100%	-	-
UV-c (c.gr. 2)	0%	0%	0%	0%
V-B11 (c.gr. 7)	reduced	normal	30%	-
C-A6	-	-	-	-

Table 2.
Biochemical characterization of X-ray sensitive repair deficient mutants (- = not tested).

Mutant and complementation group	repair of SSB	repair of DSB	inhibition of DNA synthesis
V-15B (c.gr. 1)	100%	50%	normal
V-C4 (c.gr. 2)	100%	100%	reduced
V-E5 (c.gr. 2)	100%	100%	reduced
V-G8 (c.gr. 2)	100%	100%	reduced

3. Discussion

Our results suggest that UV-induced repair synthesis is not confined to the nuclear matrix, as has been shown for replication and transcription. The efficiency of repair may be based on a sliding mechanism as has been proposed for incision of bulky damage by the UVR ABC enzymes in E. coli. The occurrence of clustered repaired sites within DNA loops is in favour of repair enzymes processively operating along DNA molecules. In fact a substantial fraction of incisions was observed at interdimer distance which is consistent with a processively acting repair process. In the presence of hydroxyurea ligation or polymerization following the incision step was delayed leading to single strand breaks within DNA molecules.

It is clear that in human fibroblasts certain domains within the chromatin are more rapidly repaired than the bulk of chromatin. These domains are located proximal to the nuclear matrix end comprise transcriptionally active DNA. We have shown that the heterogeneity in distribution of repaired sites in chromatin loops correlates with the heterogeneity in removal of pyrimidine dimers from the genome. Yet it is more likely that the preferential repair of nuclear matrix associated DNA observed in normal cells exposed to low UV-dose, reflects preferential repair of another important type of photoadduct namely the 6-4 photoproduct (6-4 PP) since repair incorporation initially after irradiation can be almost exclusively attributed to repair of 6-4 PD. Our data suggest that repair of 6-4 PP is subject to the same regime as removal of PD i.e. preferentially directed towards repair of transcriptionally active DNA. The absence of preferential repair at high UV-dose may be due to a sufficient local disruption of chromatin structure to allow efficient interaction of repair enzymes with bulk chromatin. Our findings suggest the existence of two independently operating pathways directed towards repair of PD in either active or inactive chromatin. XP-C cells have lost the capacity to repair inactive chromatin, but are still able to repair active chromatin. The reverse situation may exist in cells from CS patients, which appear to be unable to perform efficient repair of active genes. Although direct evidence is lacking repair of PD in active chromatin may be mediated by the transcription process itself. The experiments with XP-C cells indicate that the preferential repair of PD from active genes confers considerable UV-resistance to confluent cells in the absence of efficient repair of 6-4 PP. In growing cells UV-resistance seems to be provided both by preferential repair of PD from active genes and efficient removal of 604 PP from the genome overall, pointing to a strong cytotoxic potency of 6-4 PP.

The absence of preferential repair of PD in the HPRT gene concomitant with a high frequency of mutations in this locus in UV-sensitive hamster V-H1 cells points to a dominant role of PD in mutagenesis. However recent data obtained with revertants of V-H1 cells showed the absence of PD repair in the HPRT gene, but mutation frequencies similar to wild type V79. This phenomenon is not well understood at the moment, but indicates a role of 6-4 PD in mutagenesis as well.

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

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Dr. G.P. van der Schans, Medical Biological Laboratory, The Netherlands

V. Publications.

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Venema, J., L.H.F. Mullenders, A.T. Natarajan, A.A. van Zeeland and L.V. Mayne (1990) The genetic defect in Cockayne's syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA, submitted for publication.

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 0 UV-sensitive mutants of Chinese hamster cell lines, Mutation Res., 193, 31-41.

Zdzienicka, M.Z., Q. Tran, G.P. van der Schans and J.W.I.M. Simons (1988) Characterization of an X-ray-hypersensitive mutant of V79 Chinese hamster cells, Mutation Res., 194, 239-249.

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Title of the project no.: 3

DNA repair and mutagenesis.

Head(s) of project: Prof. Dr. A.T. Natarajan.
Dr. J.W.I.M. Simons.

Scientific staff: Dr. M.Z. Zdzienicka
Dr. F. Darroudi
Dr. H. Vrieling

I. Objectives of the project:

To study the mechanisms involved in the induction of gene mutations and chromosomal abberations 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 and (radiomimetic) chemicals.

II. Objectives for the reporting period:

1. Characterization of repair deficient mutants.
2. Determination of the fidelity of DNA replication after treatment with DNA-damaging agents.
3. Cytogenetical characterization of repair deficient radiosensitive mutants of Chinese hamster ovary cells

III. Progress achieved.

Characterization of repair deficient mutants.

Methodology.

Dose response relationships for the induction of mutations at the HPRT and Na/K-ATPase loci have been determined. For each dose about $5X10^6$ cells were used. After treatment the cells were propagated for expression of the induced mutations and subsequently seeded for selection of mutants. After eight days of selection mutant colonies are fixed, stained and counted.

Results and discussion.

a. Consequences of the presence of the human ERCC-1 gene in repair deficient 43-3B cells.

The human gene restores the sensitivities to DNA damaging agents of the repair deficient rodent cells to normal levels. Also the frequency of induced mutations after treatment with UV returns to the level of rodent wild type cells.

The data suggest that the ERCC-1 gene largely corrects all impaired functions in the defective 43-3B cells and that therefore the ERCC-1 gene is probably homologous with respect to function to the defective gene in 43-3B cells.

b. Characterization of the UV-sensitive mutant VH-1.

The dose repose 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.

V-H1 cells were also compared with V79 wild type cells and with two UV-sensitive CHO mutants of the same complementation group with respect to induction of UDS (unscheduled DNA synthesis) by UV. The level of UDS in V-H1 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. This is in agreement with the data on repair replication and incision (see project 2). These data show that a phenotypic heterogeneity exists within the second complementation group of UV-sensitive mutants. It is hypothesized that the repair gene of this complementation group has more than one functionally important domain or that the gene is involved in preferential repair of active genes.

c. Charaterization of the x-ray sensitive mutants V-15B, V-C8, V-C4 and V-E5.

Despite that V-15B cells are approximately 8-fold more sensitive to x-rays than wild type cells the mutation induction was not significantly enhanced. The mutation induction at the HPRT locus was $0.9X10^{-5}$ per Gy for control cells whereas it was $1.7X10^{-5}$ for V-15B.

Mutants from the same complementation group have been shown to depend on epigenetic alterations as they can be induced to revert by 5-azacytidine. We found that reversion of V-15B cannot be induced by 5-azacytidine.

Despite the fact that V-G8, V-C4 and V-E5 belong to the same complementation group, different levels of spontaneous chromosomal aberrations were observed. For V-G8 these frequencies were similar to that observed in wild type cells whereas an increase of about 2- and 6-fold was found for V-E5 and V-C4 respectively. In all three mutants the frequencies of x-ray

induced aberrations were higher in comparison to wild type V79 cells in both G1 and G2 cells. The type of abberations induced in G1 cells were mainly of the chromosome type in the normal cells, whereas in all three x-ray sensitive mutant both chromosome and chromatid exchanges were found and occurred within the same cell. This level and pattern of chromosomal aberrations are similar to those observed in cells derived from patients with Ataxia telangiectasia. As the biochemical characteristics of these cells (see project 2) also agree with those observed for Ataxia telangiectasia our mutants from the new complementation group are the first rodent repair deficient mutants which phenotypically resemble Ataxia telangiectasia cells.

d. Characterization of the MMC-sensitive mutant V-H4.
The level of spontaneous chromosome aberrations is almost 2-fold increased in V-H4 and the level of chromosome abnormalities induced by treatment with MMC or cis-DDP is 2-3 fold higher in this mutant than in the wild type cells. Preliminary results indicate that V-H4 is rather hypomutable after treatment with 8-MOP plus UVA, 4NQO or EMS.

Determination of the fidelity of DNA replication ater treatment with DNA-damaging agents.

Methodology.
GRSL mouse lymphoma cells are treated with a mutagen and seeded in subpopulations of about 100 viable cells each. Each subpopulation is grown to $1.5X10^6$ cells and the mutant frequency per subpopulation is determined. This procedure allows to discriminate between directly induced mutations and delayed mutations. The directly induced mutations lead to large numbers of mutant clones in a few of the subpopulations while delayed mutations, if present, will lead to smaller numbers of mutant colonies per subpopulation. The mutation spectrum has been analysed by molecular analysis of the mutants. To this end mutant RNA is used for the production of cDNA and the HPRT-cDNA is amplified by PCR. The HPRT cDNA is ligated in a M13 vector and sequenced.

Results and discussion.
Treatment with ENU leads to significant numbers of delayed mutations, which contribute to more than 10% in the total mutational response. Sequence analysis of ENU-induced mutations shows that many directly induced mutations are as expected transitions, which occur in both strands, while the delayed mutations are predominantly AT-transversions, which occur only in one strand and show site-specificity.
As explanation for the delayed mutational response the induction of infidelity of DNA synthesis is preferred to the presence of persistent ethylated adducts or their AP-sites which could give rise to delayed mutations if they are less mutagenic. Firstly because the kinetics of the delayed response show an increase of mutational events with time and not a decrease which would correspond with disappearance of adducts, secondly not enough ethyl adducts appear to be available for the number of observed mutations, and thirdly the delayed mutational response can be significantly reduced by 3AB. The inducible error-prone response, if present, peaks before the fifth generation after treatment and has disappeared at the ninth generation after treatment. It leads to targeted mutations, probably of spontaneous cryptic lesions such as apurininc sites, presumably via insertion of adenine. It is thought to affect the fidelity of a strand- specific poly-

merase.
Also experiments with UV led to a significant induction of delayed mutations. This effect however could only be observed when all four experiments were pooled. Therefore the induction of infidelity of DNA replication after UV is much weaker than after ENU.
Five experiments have been performed with x-rays. Although there is some evidence for the induction of infidelity of DNA replication the difference is not significant.

Cytogenetical characterization of repair deficient radiosensitive mutants of Chinese hamster ovary cells

Methodology
Chinese hamster cells were grown in Ham's F10 medium supplemented with 15% new born calf serum and antibiotics. When necessary, cells were synchronized by mitotic shake off technique. Cells growing as monolayers were irradiated using an ENRAF apparatus (150 kV, 6 mA). For chromosome analysis, cells were incubated in the presence of colcemid for 2 h. prior to fixation. For determining the frequencies of SCEs, cells were grown for 2 cycles in the presence of 5 uM 5 bromo-deoxyuridine. Air dried preparations were made and stained with aqueous Giemsa solution (for chromosomal aberrations) or fluorochrome plus Giemsa staining (for sister chromatid exchanges). X-ray sensitive mutants were kindly provided by Dr. P. Jeggo.
For visualising interphase chromosomes, nondividing cells were fused with mitotic CHO cells in the presence of polyethyleneglycol to generate premature condensation (PCCs) of the interphase chromosomes.
For detection of HPRT- mutants Cells were grown in selection medium containing 5 ug/ml. 6-thioguanine and for detection of Ouabine resistance, the cells were grown in medium containing 1 mM oubaine.

Results and discussion
Several mutants deficient in DNA double strand break (DSB) repair have been isolated in CHO cells by Jeggo (Jeggo and Kemp, Mutation Res. 112, 313-327, 1983). Two of these mutants, namely xrs 5 and xrs 6 have been studied in great detail by us, with the aim of correlating the degree in deficiency in rejoining of DSBs to the frequencies of chromosomal aberrations induced by X-rays. Though both the mutants belong to the same complementation group they were found to exhibit different characteristics with regard to biological response to X-rays.
When the cells were irradiated in G2 stage of the cell cycle, the frequencies of induced chromosomal aberrations correlated well with the extent of defect in rejoining of DSBs. Xrs cells which are about 90% deficient had more aberrations (576 aberrations/100 cells after 1 Gy) than xrs 6 cells (305 aberrations/100 cells after 1 Gy) which are about 60% deficient in DSB repair. Wild type cells yielded 81 aberrations/100 cells after 1 Gy (Darroudi and Natarajan, 1987a).
When synchronized cells were irradiated in G1 stage, these mutants responded with similar frequencies of aberrations at higher doses (0.7 Gy and above). However at lower dose (0.3 Gy), Xrs 5 responded with more aberrations than xrs 6. Similar frequencies of aberrations in both mutants at higher doses may be due to the proportion of heavily damaged cells reaching mitosis may vary between these mutants. The assumption proved to be correct, when the frequencies of X-ray induced aberrations were evaluated in these mutants using PCC technique (in which no cell selection operates), xrs 5 cells had more aberrations than xrs 6 cells (Darroudi and Natarajan,

1989b).
One of the characteristic feature of xrs mutant cells irradiated in G1 is the yield of both chromosome and chromatid types of aberrations in contrast to wild type cells in which only chromosome type of aberrations are encountered (Darroudi and Natarajan 1987a). This is very similar to the situation found in radiosensitive, cancer prone human disease ataxia telangiectasia (Taylor, Mutation Res. 50, 407-418,1978, Natarajan and Meyers, Human Genetics, 52, 127-132, 1978). This increase in chromatid type of aberrations in these mutants following G1 irradiation could be due to a cells with some unrepaired DSBs reaching the S phase. If this assumption is correct, one would expect the frequency of X-ray induced chromatid aberrations in G1 in xrs 5 cells should be greater than the one obtained in xrs 6 since xrs 5 has a larger defect in DSB repair than xrs 6 cells. However, the reverse was found to be true, namely xrs 6 had more chromatid aberrations than xrs 5 cells. This led us to search for alternative type of lesions responsible for the increased frequency of chromatid aberrations. Since both these mutant cells are known to be proficient in the repair of DNA single strand breaks (Kemp et al, Mutation Res.132, 189-196, 1984), X-ray induced base damages appeared to be a possible candidate. If the persisting lesion that rised to chromatid type of aberrations following G1 radiation in Xrs 6 cells is indeed DNA base damage, then one would expect an induction of sister chromatid exchanges (SCEs) by X-rays in this cell line. When synchronized xrs 6 cells were irradiated with different doses of X-rays in G1 stage, a dose dependent increase in the frequency of SCEs was obtained. (Darroudi and Natarajan, 1987b). There was a small increase in xrs 5 cells and no increase in wild type cells.
It is possible to alter the proportion of radiation induced DNA strand breaks to base damages by altering irradiation conditions. Irradiation in air leads to more strand breaks due to indirect action of radiation mediated by free radicals, in comparison to irradiation in nitrogen, while the frequency of base damage remains relatively unaltered (Paterson and Setlow, Proc. Natl Acad. Sci. US. 69, 2927-2931,1972). Ionizing radiation is known to be apoor inducer of SCEs. In earlier experiments we have demonstrated that when wild type CHO cells are irradiated under O2 and N2 conditions, the frequency of chromosomal aberrations increased under O2 conditions and the frequency of SCEs increased under N2 conditions. If one compares the relative induction of SCEs to chromosomal aberrations, the ratios vary very much , a higher ratio being obtained for irradiation under nitrogen conditions in comparison to oxygen conditions (Uggla and Natarajan, Mutation Res., 122, 193-200,1983). When similar experiments were done with xrs mutants, the increase in the frequency of SCEs was higher in cells which were irradiated under nitrogen in comparison to irradiation in air. The ratio between SCEs and aberrations was highest for xrs 6 cells in comparison to xrs 5 and wild type CHO cells (Darroudi and Natarajan, 1987b; Darroudi et al., 1990a). These results support the conclusion that xrs 6 mutant is defective in repair of both DSB and base damage.
Inhibitors of DNA replication such as cytosine arabinoside (ara C) inhibit also inhibit repair of DNA strand breaks and base damage. In view of the complexity in the repair defect of xrs 6 mutant, we investigated the influence of ara C (alone or with hydroxy urea, HU) on the frequency of UV (which induces lesions similar to base damage) induced chromosomal aberrations. There was a potentiating effect of ara C on the frequency of aberrations induced by UV in wild type cells and xrs 5 cells, but not in xrs 6 cells (Darroudi et al, 1990b). This response was not due to the defect in uptake of ara C by xrs 6 cells as similar non-potentiating effect was observed with aphidicolin (another inhibitor of polymerase alpha)

(Darroudi and Natarajan, 1987b). Post-treatment with ara C and HU of UV irradiated cells leads to an accumulation of SSBs and this reflects the incision capacity of the cells under investigation. We measured the accumulation of SSBs at 20 and 120 min. in wild type CHO cells and xrs mutants following UV treatment and post treatment with ara C and HU (Darroudi et al., 1990b). While the frequency of SSBs increased in wild type and xrs 5 cells with time, the frequency remained at the same level in xrs 6 at both times of determination, around 35% of the wild type CHO cells. These results suggest that xrs 6 cells, in addition to the defect in the repair of DSBs, are defective in the incision of other types of damage (base damage, dimers) as well. This additional defect is also reflected by the increased sensitivity of xrs 6 mutants to bleomycin and monofunctional alkylating agents in comparison to xrs 5 cells (Darroudi and Natarajan, 1989a). In a series of experiments designed to evaluate the relative induction of mutations in these cell lines, xrs 5 cells responded with higher frequency of mutations (HPRT-) following X-irradiation than xrs 6 and wild type CHO cells. This can be expected on the broad nature of HPRT- mutations, namely base pair changes, small and large deletions and rearrangements involving this locus. However, xrs 6 cells responded with higher frequencies of mutations (HPRT-, Oubaine resistant) following treatment with a monofunctional alkylating agent such as methyl methanesulfonate (Darroudi and Natarajan, 1989a).

These results point out that DNA DSB appears to be the most important radiation induced lesion leading to chromosomal aberrations in repair competent cells. However, in repair defective cells, radiation induced lesions other than DSBs can also lead to chromosomal aberrations.

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V. Publications:

Darroudi, F., and A.T. Natarajan (1987a) 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 (1987b) 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 α-polymerase, Mutation Res., 177, 149-160.

Darroudi, F., and A.T. Natarajan (1989a) Cytogenetical characterization of Chinese hamster ovary X-ray-sensitive mutant cells xrs 5 and xrs 6. III. Induction of cell killing, chromosomal aberrations and sister-chromatid exchanges by bleomycin, mono- and bifunctional alkylating agents, Mutation Res., 212, 123-135

Darroudi, F., and A.T. Natarajan (1989b) Cytogenetical characterization of Chinese hamster ovary X-ray-sensitive mutant cells xrs 5 and xrs 6. IV. Study of chromosomal aberrations and sister-chromatid exchanges by restriction endonucleases and inhibitors of DNA topoisomerase II, Mutation Res., 137-148.

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Darroudi, F., A.T. Natarajan and P.H.M. Lohman (1989) Cytogenetical characterization of UV sensitive repair deficient cell line 43-3B. II. Induction of cell killing, chromosomal aberrations and sister-chromatid exchanges by 4NQO, mono- and bifunctional alkylating agents, Mutation Res., 212, 103-112.

Darroudi, F., A.T. Natarajan, G.P. van der Schans and A.A.W.M. van Loon (1990a) Biochemical and cytogenetical characterization of X-ray-sensitive Chinese hamster ovary mutant cells xrs 5 and xrs 6. V. The correlation between DNA strand breaks and base damage to chromosomal aberrations and sister-chromatid exchanges induced by X-irradiation, Mutation Res., (in press).

Darroudi, F., A.T. Natarajan and G.P. van der Schans (1990b) Biochemical and cytogenetical characterization of Chinese hamster oary X-ray-sensitive mutant cells xrs 5 and xrs 6. VI. Correlation between UV-induced DNA lesions and induction of chromosomal aberrations, and their

modulations with inhibitors of DNA repair synthesis, Mutation Res., (in press).
Darroudi, F. A. Westerveld and A.T. Natarajan (1989b) Cytogenetical characterization of Chinese hamster 43-3B transferants with the amplified or non-amplified human DNA repair gene ERCC-1, Mutation Res., 212, 113-122.
Darroudi, F., T.S.B. Zwanenburg, A.T. Natarajan, O. Driessen and Langevelde (1989c) Reduced tumor progression in-vivo by inhibitor of poly (ADP-ribose) synthetase (3-aminobenzamide) in combination with X-rays or cytostatic drug DTIC, in: M.K. Jacobson and E.L. Jacobson (Eds.), ADP-Ribose Transfer Reactions: Mechanisms and Biological Significance, Springer-Verlag, pp. 390-395.
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Oussoren, Y., F. Stewart, A. Bergg and F. Darroudi (1987) Chromosome damage in mouse kidneys after in vivo irradiation assessed by premature chromosome condensation (PCC), in: E.M. Fielden, J.F. Fowler, J.H. Hendry and D. Scott (Eds.), Radiation Res., vol. 2, Taylor & Francis, pp. 254.
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Zdzienicka, M.Z., N.G.J. Jaspers, G.P. van der Schans, A.T. Natarajan and J.W.I.M. Simons (1989) Ataxia-telangiectasis-like Chinese hamster V798 cell mutants with radioresistant DNA synthesis, Chromosomal instability and normal DNA strand break repair, Cancer Res., 49, 1481-1485.

Title of the project no.: 4

The relationship between DNA repair processes and magnitude of genetic damage induced by X-irradiation.

Head(s) of project: Prof. A.T. Natarajan

Scientific staff:

Prof. K. Sankaranarayanan	Dr. A.D. Tates
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I. Objectives of the project

The project is aimed at gaining information on (1) the nature of radiation induced DNA lesions and their repair on the manifestation of genetical effects in mammalian cells, both under in vivo and in vitro conditions and (2) the effect of radiation on germ cells of rodents and primates as measured by induced chromosomal translocations, with the aim of using such data to estimate genetic risks due to radiation in man.

II. Objectives for the reporting period:

(1) To study the nature of the lesions induced by ionizing radiation in human peripheral blood lymphocytes which lead to chromosomal aberrations, the early repair events which influence the yield of aberrations scored both in interphase (PCCs) and mitosis and the role of adaptive response and chromatin structure on the yield of aberrations. In addition, investigations are made to study spontaneous and radiation-induced mutations in human lymphocytes.
(2) To study the induction by ionizing radiation of stable chromosomal aberrations in spermatogonial stem cells of rhesus monkeys for a quantitative extrapolation to the human situation and in mice, to gain information about the biological processes underlying such aberration production.

III Progress achieved

1.1 Role of DNA repair on the frequency and types of aberrations in lymphocytes

Methodology

Human lymphocytes, either as isolated cells or as whole blood, were grown in a medium containing the mitogen (phytohemagglutinin) and fixed at 48h. culture time,with a pre-incubation with colcemid for 3h. Chromosomal preparations were made by routine air-dry technique. For making premature chromosome condensation (PCC) preparations, non-dividing lymphocytes were fused with mitotic Chinese hamster ovary (CHO) cells in the presence of polyethyleneglycol (PEG). In the hybrid cells, the mitotic factors from CHO cells induce the interphase nuclei of the lymphocytes to condense to form chromosome like structures. In order to visualise PCCs better, CHO cells were grown in 5-bromodeoxy-uridine for several cycles, so that their chromosomes can be faded by differential staining. The slides were stained with aqueous Giemsa. To identify dicentrics in PCCs, the chromosomes were C banded. 150 kV X-rays from an ENRAF machine, at a dose rate of 1 Gy/minute were used.

Results and Discussion

(a) Repair kinetics in relation to chromosomal aberration formation: When irradiated human lymphocytes were post-treated with a repair inhibitor, such as cytosine arabinoside (ara C), the yield of aberrations (dicentrics and fragments) increased. When the ara C treatment was given for 15, 30, 60 or 120 minutes, most of the increase in the frequency of aberration was found to occur in the first 15 mins. If irradiated lymphocytes were allowed to recover for 30 min. and then challenged with ara C, there was no increase in the frequency of aberrations indicating that fast repairing lesions lead to the formation of aberrations (Natarajan et al, 1986). Parallel studies with PCCs, in which lymphocytes were irradiated and fused immediately or allowed to repair for 60 min.in the presence or absence of ara C indicated that about 50% of breaks disappear during this repair, while in the presence of ara C, there was a complete inhibition of repair of breaks. When nucleoid sedimentation technique (which measures the induction of DNA strand breaks) was used under a similar protocol, it was seen that ara C completely inhibited the repair of strand breaks (Natarajan et al., 1986). These results pointed out that fast repairing DNA strand breaks induced by X-rays lead to chromosomal aberrations.

The PCC technique and conventional metaphase chromosome analysis were used to examine the kinetics of X-ray induced primary breaks, their rejoining and formation of dicentrics in human lymphocytes. A dose dependent increase in the frequency of fragments and a linear quadratic increase in the frequency of dicentrics were observed (Natarajan et al, 1989, 1990). The frequency of fragments was much higher (factor of about 10) when determined by PCC technique immediately after irradiation in comparison to the frequencies observed in metaphases. For an assessment of repair kinetics irradiated lymphocytes were fused with mitotic CHO cells either immediately or after several recovery times after irradiation. It was found that the frequency of chromosome fragments decreased with time whereas the dicentrics were formed

very fast and their frequency remained the same, despite the decrease in the number of fragments with increased recovery time (Vyas and Natarajan, 1990). These results confirm our earlier findings using repair inhibitor ara C, which indicated that early repairing lesions (strand breaks) lead to chromosomal aberrations, especially the exchange types (see above)

(b) Chromatin structure in relation to yield of chromosomal aberrations:

We have demonstrated earlier that when lymphocytes were incubated in a medium containing sodium butyrate, which hyperacetylates histones, thus relaxing DNA, prior to X-irradiation increased the yield of aberrations (Sankaranarayanan, et al., Mutation Res.151, 269-274, 1985). When human lymphocytes were fused to mitotic CHO cells irradiated with X-rays (2 Gy), immediately or after 30 or 60 min. the frequency of induced breaks fell dramatically with increased condensation (Vyas and Natarajan,1990). It has been suggested that treatment with hypertonic salt solution suppresses the fast component of repair of radiation induced strand breaks (Hittelman, 1987). This should increase the frequency of chromosome breaks. Hypertonic treatment is also expected to condense the chromatin which could prevent accessibility to repair enzymes, thus increasing the frequency of induced breaks. Two types of experiments were carried out to study the role of hypertonic salt solution on the yield of X-ray induced chromosomal aberrations in lymphocytes. In a fractionation regime, lymphocytes were irradiated in the medium or a hypertonic salt solution (0.3 M Na Cl), with 1 Gy and allowed to recover for 30 or 90 min. and irradiated further with 1 Gy. The lymphocytes were fused with mitotic CHO cells and the frequencies of dicentrics and fragments were evaluated in PCCs. Contrary to the expectation,the yield of dicentrics and fragments was reduced in the lymphocytes irradiated in the presence of hypotonic solution. In another experiment, lymphocytes were X-irradiated with 3 Gy alone, or they were irradiated in the medium or treated with a hypertonic salt solution before and after irradiation, or irradiated in cold isotonic solution and transferred immediately to hypertonic salt solution. After 30 min. post incubation, lymphocytes were cultured for 48 hrs. and the frequency of aberrations was evaluated. The frequency of dicentrics was very similar in all treatments except the one in which the lymphocytes were irradiated in hypertonic salt solution, which responded with lower (about 40%) number of dicentrics. These results indicate that at least in isolated lymphocytes irradiation under hypertonic conditions reduces the frequencies of aberrations probably due to induced chromatin condensation.

(c) Inducible repair in human lymphocytes:

It has been reported that human lymphocytes subjected to a small conditioning dose of radiation leads to some protection (in terms of yield of chromosomal aberrations) to a challenging higher dose of radiation, a phenomenon termed as adaptive response.

Two series of experiments were carried out with whole blood from healthy volunteers: one in which radioisotopes were used to administer the "conditioning dose", and the other in which a low dose of X-rays was used as the conditioning treatment. In the first series, 6 h after PHA stimulation in cultures, the radioisotope was added to the culture medium [^{3}H-TdR (0.01 uCi/ml);

tritiated water (5 uCi/ml); ^{14}C-TdR (0.01 uCi/ml) or ^{32}P (0.01 uCi/ml]. 50 h later, the cultures were X-irradiated with 50 rad ("challenge dose"). Colcemid was added 1 h later and the cells were fixed at 53 h. In series II, the cultures were exposed to 5 rad of X-rays 32 h after PHA stimulation, to 150 rad of challenge dose at 48 h and fixed 6 h later. A total of 9 donors were used to obtain blood samples and the frequencies of chromosomal aberrations were ascertained in at least 300 cells per group, including the appropriate controls.

Two important findings emerged from these experiments: first, in cells that received both the conditioning and the challenge doses, the frequencies of chromosomal aberrations (chromatid and isochromatid deletions) were lower than expected on the basis of additivity of the effects of individual treatments; these results thus support those published from Wolff's laboratory in showing that human lymphocytes can become "adapted" by prior exposure to low level irradiation so that they become less sensitive to the chromosome-breaking effects of X-rays delivered subsequently. Second, the magnitude of reduction in aberration frequencies in the adapted cells varied between the different donors (and also between blood samples from the same donor exposed to different radioisotopes),was generally lower than those observed in the studies from Wolff's laboratory and, with lymphocytes from some donors, no adaptive response could be demonstrated. This finding is consistent with the existence of inter-individual differences with respect to the magnitude of the adaptive response, a finding which has received support from the work of Bosi and Olivieri (Mut. Res 211, 13-17, 1989).

(d) Chromosomal aberrations induced by restriction endonucleases: Among the DNA lesions induced by ionizing radiations, DNA double strand breaks (DSBs) appear to be the most important one leading to chromosomal aberrations . We have provided both biochemical and cytological evidences for this conclusion (for a review Natarajan et al., 1989). When CHO cells are permeabilized with inactivated Sandai virus and treated with restriction endonucleases (RE) (which exclusively induce DNA DSBs), chromosomal aberrations are induced in a pattern similar to that induced by ionizing radiation, namely chromosome type in G1 and chromatid type in G2 and a mixture in the S phase (Natarajan and Obe, 1984). When CHO cells were treated with the restriction enzyme Alu I and post treated with ara C, the frequency of aberrations increased indicating the ara C can inhibit rejoining of RE induced DSBs. Ara C has a similar influence on X-ray induced breaks. This indicates that X-ray induced DSBs respond in a similar way as RE induced DSBs to ara C.(Obe and Natarajan, 1985).

Influence of incorporated BrdU on the induction of chromosomal aberrations and sister chromatid exchanges (SCEs) and cleaving of DNA by different REs differing in their recognition sequences, especially the number and position of thymidine nucleosides was investigated. CHO cells were grown in medium containing different concentrations of BrdU to give different levels of substitution in the DNA (0,30,50,70 and 100%). The cells were permeabilized and treated with REs, namely, Alu I, Cfo I,Taq I and EcoR I. Parallel to cytological studies (evaluation of induction of chromosomal aberrations and SCEs), isolated nuclei from different

groups were digested with different REs and the DNA fragments were electrophoretically separated in 0.6% neutral agarose gels in the presence of ethidium bromide in the running buffer. Samples were run overnight at 25 v and photographed under UV illumination. For induction of SCEs, it was found that the efficiency of RE did not depend on the nature of the initial cleavage, blunt and cohesive breaks were equally effective in inducing SCEs, whereas blunt ends were more effective in inducing chromosomal aberrations. It was found that mid S phase was the most sensitive phase for the induction of chromosomal alterations. Restriction pattern analysis of DNA from BrdU (100%) substituted and unsubstituted nuclei following Alu I digestion, showed some inhibition at 100% level, which may be responsible for less efficient induction of SCEs by this RE. Some increase in the frequency of induced chromosomal aberrations in BrdU substituted cells was found which may indicate that incorporated BrdU may interfere with the repair of the induced strand breaks, since BrdU substitution was not found to significantly influence the induction of strand breaks by different REs. (Stoilov et al.,1986).

1.2 HPRT$^-$ mutants in T-lymphocytes from normal donors and patients that received a low dose of gamma irradiation from Technetium-99 m for a diagnostic test of heart function

(a) Spontaneous HPRT$^-$ mutations in lymphocytes:
In order to improve our understanding of spontaneous mutants in human lymphocytes, use was made of the cloning method for genetic alterations in the hypoxanthine guanine phosphoribosyl transferase (HPRT) gene. This method is routinely used in our laboratory. Mutant frequencies (Mf) were determined in 28 non-smokers and 23 smokers. Mean Mf values and standard deviations for the two groups were $10.0 \pm 10.7 \times 10^{-6}$ and $11.1 \pm 15.7 \times 10^{-6}$ respectively. The effect of smoking was not statistically significant. For the pooled data of the two groups it could be calculated that Mf values increased significantly with age (range 19-60 yrs) with a rate of 1.8% per year.
A total of 31 mutants from 10 normal non-smoking donors was used to determine the spectrum of DNA sequence alterations in the coding region of the HPRT gene. For this purpose mRNA from expanded HPRT$^-$ mutant clones was converted into cDNA which was then used for DNA sequence analysis after PCR amplification. In 12 out of 31 mutants a single base pair substitution was observed. These were equally divided in transitions and transversions. Furthermore, 5 frame shift mutations and 2 small deletions of 9 and 12 base pairs were found. In 12 out of 31 mutants one or two exons were completely or partially deleted in the HPRT mRNA, probably because of a mutation in a splice acceptor site of the HPRT gene.

(b) HPRT$^-$ mutant frequencies in patients receiving a low dose of gamma rays:
Blood samples were collected from 13 nuclear medicine patients undergoing a ventriculographic examination of heart function with Technetium-99 m. The effective dose equivalent due to this procedure was 6.4 mSv resulting in a dose equivalent to the lymphocytes of about 14 mSv. The first sample was taken just before the treatment with Technetium and the second one 8 to 120 days thereafter. Mean Mf values plus standard deviations for pre- and

post treatment samples were 21.2 ± 19.9 x 10^{-6} and 11.1 ± 7.2 x 10^{-6} respectively. The decrease of 52% in the mutant frequency after the nuclear medicine procedure proved to be statistically significant ($P < 0.03$). This result does not confirm that of Seifert et al. (Mutation Res., 191, 1987, 57-63) who concluded that Technetium treatment of a similar type of patients resulted in a significant increase of HPRT$^-$ mutant frequencies in post-treatment samples. When, however, Seifert's data were recalculated with the same statistical test that was used for our own data, it was found that the results of Seifert did in fact not show any effect of Technetium exposure.

2 Study on germ cells of primates and rodents

Methodology

Local testicular or whole body irradiation was carried out for primates (rhesus monkey and stump-tailed macaque) and rodents respectively. ^{60}Co and ^{137}Cs gamma-rays, 250-300 kV X-rays and 2.1 MeV neutrons were used. Chronic gamma irradiation was given with dose rates of 140 mGy and 0.2 mGy/min.

In some experiments mice were pretreated with hydroxyurea (HU) as an S-phase killer to synchronize the spermatogonial stem cells or with 3-aminobenzamide (3-AB), a potent inhibitor of poly (ADP-ribose) synthetase, to sensitize these cells. Moreover in vivo pulse labelling with ^3H-thymidine was performed of meiotic stages from mice with radiation history.

For all experiments meiotic preparations were made at appropriate time intervals after treatment and analyzed for the presence of reciprocal translocations.

Results and discussion

Primates

The analysis of spermatocytes from X-irradiated stump-tailed macaques showed that the induction of reciprocal translocations in spermatogonial stem cells was significantly lower than in the rhesus monkey (0.28% vs. 0.86% at 1 Gy). From X-irradiation of prepuberal rhesus monkeys it could be concluded that the chromosomal radiosensitivity of spermatogonia is identical to that of adult monkeys.

Data from the rhesus monkey after chronic or semi-chronic gamma-ray exposures indicated that in this species the reduction in yield of genetic damage with reduced dose-rates, is the same as observed in the mouse for semi-chronic irradiation but much less than observed in the mouse for chronic irradiation. For neutron irradiation of rhesus monkeys an RBE of 2.1 (neutron vs. X-rays) was obtained, which is clearly lower than the value of 4 recorded for the mouse. Differential cell killing between semi-chronic and chronic exposures or between high and low LET irradiation with ensuing differential elimination of aberration-carrying cells is the most likely explanation for the differences in radiation response between primates and the mouse. This further stresses the point that in general mammalian species with high sensitivities for the cytotoxic effects of ionizing radiation, such as the rhesus monkey (and also man), will exhibit relatively high threshold dose rates below which no further reduction in aberration yield occurs, whereas in more resistant species such as the mouse, the threshold dose rate will be at a very low level. Similarly, resistant species will show relatively high RBE values for neutron irradiation

and sensitive species low ones.

Mouse

Through the use of combined HU and X-ray treatments we were able to demonstrate a clear correlation between spermatogonial stem cell killing and recovered genetic damage. In addition the results provided experimental evidence that in mouse spermatogonia the probability that a radiation induced basic laesion in the DNA gives rise to cell killing is about 10 times the probability that a recoverable reciprocal translocation will be formed.

The influence of 3-AB pretreatment on the dose response relationship for radiation induced reciprocal translocations was studied. The results showed that the shape of the dose effect curve was similar to that obtained without 3-AB pretreatment, i.e. a humped curve, but the initial slope was clearly higher and the position of the peak was shifted from 7 to 9 Gy. These observations could be explained by a 3-AB mediated sensitization of original radioresistant resting stem cells that are at the stage of stimulation to enter the mitotic cycle. Using in vivo pulse labelling of meiocytes from mice irradiated with relatively high doses of X-rays (6 Gy and 3-AB + 7 Gy), evidence could be obtained that translocation carrying cells tend to spend longer times at the meiotic prophase than normal cells. This forms, at least partially, a good explanation for the well established observation that the transmission of translocations to the next generation is only half that expected on base of the frequency of multivalent configurations present at diakinesis-metaphase I of meiosis.

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

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Title of the project no 5

Studies on mutations and their repair in Drosophila

Head(s) of project: Dr. J.C.J. Eeken

Scientific staff

Dr. W. Ferro,
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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.

II. Objectives for the reporting period:

1. Repair of UV-induced DNA damage in the genome overall and in individual genes of the repair proficient cell line Kc and the excision-repair deficient cell lines mei-9 and mus-201.
2. The molecular nature of X-ray mutations, recovered from irradiated spermatozoa, in repair proficient and repair deficient Drosophila.
3. The molecular nature of chemically (ENU/EMS) induced vermilion mutations in repair proficient Drosophila.
4. The genetic effect in vivo of a post-replication repair deficient mutation (mei-41) in somatic tissues.
5. Isolation of bleomycine sensitive mutants.
6. Isolation of P-insertion mutations at repair genes.
7. The characterization of a mobile element (P-element) transposition system in Drosophila.

III. Progress achieved:

Repair of UV-induced DNA damage in the genome overall and in individual genes of the repair proficient cell line Kc and the excision-repair deficient cell lines mei-9 and mus-201.

Methodology

The repair capacity of repair proficient (Kc) and excision repair deficient (mei-9, mus-201) cells for UV-and X-ray-induced strand breaks in total DNA is measured using the nucleoid sedimentation technique.

Repair of UV-induced thymidine dimers in total DNA in the repair proficient Kc cells is measured using alkaline sucrose gradients.

The removal of UV induced thymidine dimers from the individual genes GART, RpII, white and Notch is measured by blot-hybridization. Cells are irradiated with 10 or 15 J/m^2 UV. After a period of repair-incubation (0, 4, 8, 16, 24 and 48 hours) the DNA is isolated and purified. DNA is digested with restriction enzymes to obtain fragments on which an entire gene (GART, Xho - 15 kb; RpII, $BstE_2$ - 9.4 kb; white, EcoRI - 15 kb) or part of a gene (Notch, Xho - 17 kb (intron) and - 15 kb (exon)) is situated. Part of the digested DNA is incubated with T4-endonuclease. This enzyme recognises thymidine dimers and introduces specifically single strand breaks at these sites. T4-endonuclease treated and untreated DNA is fractionated on an alkaline agarose gel, blotted and hybridized with a probe of one of the four genes (GART, RpII, white Notch). If no thymidine dimers are present, a discrete band can be detected in the DNA independent whether this DNA is treated or not treated with T4-endonuclease. However, if thymidine-dimers are present in the selected fragment, than in the T4-endonuclease treated DNA, some DNA from the discrete band is cut and a smear of lower molecular weight DNA is detected. The ratio of DNA in the discrete bands of the T4-endonuclease treated and untreated DNA is a measure for the number of thymidine dimers present in this DNA-fragment.

Results and discussion

Nucleoid sedimentation experiments were performed to compare the repair capacity for strand breaks of two permanent cell lines deficient in excision-repair (mei-9, mus-201) with that in repair proficient cells (permanent cell line Kc). After UV-treatment (10 J/m^2; 256 nm.) the number of breaks detected in the mei-9 and mus-201 cells is lower than in the repair-proficient Kc 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.

To determine the repair capacity of Kc cells (proficient repair) to remove UV-induced thymidine dimers from DNA of the complete genome, several experiments were performed using alkaline sucrose gradients. Kc cells were UV-irradiated and after a period of repair-incubation (0, 4, 8, 16, 24 and 48 hours) the cells are lysed. The DNA is incubated with T4-endonuclease, and the molecular weight of the DNA is determined. The results show that the removal of dimers is very slow during the first 8 hours after irradiation. After 8 hours, the repair increases and at 24 hours after irradiation 95% of the dimers are removed.

It has been shown that in mammalian cells and also in the lower eukaryote Saccharomyces cerevisiae UV-induced DNA repair is not randomly distributed over the genome. Evidence has been presented for the preferential repair of actively transcribed genes. In addition to studies on the in vivo induction of mutations in germline and somatic cells in Drosophila repair proficient

and deficient (mei-9 and mus-201) strains, repair of UV-induced damage in four specific genes (GART, RpII, white and Notch) in cellines derived from these strains, is investigated.
GART is a 10 kb gene that is involved in the de-novo purine synthesis. The RpII (RNA polymerase II) gene (9.4 kb) encodes for the largest subunit of RNA polymerase II. The product of the white gene (6 kb) plays a role in the distribution of pigment in the fly. The Notch gene (34 kb) is expressed during embryogenesis and appears involved in the development of neurogenic tissues.
Transcripts from the GART and the RpII gene were found in poly A^+ RNA isolated from the repair proficient Kc cell line, by Northern blotting. In contrast, no expression of the white and Notch genes could be detected.
It is found that the active gene GART as well as the inactive genes white and Notch are all repaired to the same extent. 4 hours after UV-irradiation no repair is measured. After 8 hours only 20 to 30% of repair is found, 60% after 16 hours and about 80% after 24 hours up to 90% after 48 hrs. The repair-kinetics for these genes appears independent of the doses studied. The repair of the RNA polymerase II gene was found to be faster. Preliminary data, however, indicate that less pyrimidine dimers are induced in this gene as compared to the other genes investigated.
In both excision-repair deficient cell lines (mei-9 and mus-201) no removal of thymidine dimers from the GART and Notch genes was found. These results are in agreement with the results obtained with other biochemical methods; i.e. these cells cannot introduce single strand nicks at thymidine dimers (in total genomic DNA) nor do they show Unscheduled DNA Synthesis (UDS) after UV-irradiation.

The molecular nature of X-ray mutations, recovered from irradiated spermatozoa, in repair proficient and repair deficient Drosophila.

Methodology
Amherst-M56i wild type males are irradiated (15 Gy) and mated with: (1) repair proficient (mei^+), (2) excision-repair deficient (mus-201) and (3) post-replication repair deficient (mei-41) females with the genetic constitution $In(1)sc^8In(1)dl-49$, y^{31d} w^a v^{0f} f; (1) mei^+ or (2) mus-201 or (3) mei-41. The F_1 females can be scored for (induced) X-linked mutations at the y (yellow body color), w (white eye color), v (vermilion eye color) and f (forked bristle) genes. Only mutations induced in mature sperm are collected. The mutants are analysed and classified according to genetic and cytogenetic characteristics in 1. intragenic mutations and 2. gross chromosomal aberrations (multi-locus deletions, translocations, inversions). DNA from whole body white mutants from the first class (intragenic mutations) was analysed by molecular techniques including: restriction enzyme pattern, S1 protection experiments and, when neccesary, sequencing.

Results
a. In repair proficient condition, 23 yellow, 44 white (including white-Notch) and 41 vermilion among 108291 F_1 females were found (see Table 1). Of these 108 mutations 87 are whole body (wb) and 21 mosaic (mos) mutations. 29 out of 87 wb mutations are sterile as F_1 females are sterile indicating probable multilocus deletions. Of the remaining 58 mutations, 21 proved to be male lethal in the F_2. If these are classified, in addition to the F_1 female sterile mutations, as multi-locus deletions, the total fraction of multi-locus deletions is 29+21/87= 57%. Of the remaining 37 intra-locus mutations, 12 are white mutations. Ten of these were analysed

cytologically and at the molecular level. One is a translocation, two are deletions of 700 and 400 bp, 6 have deletions smaller than 50 bp and 2 are base-pair changes (for details see Table 2).

b. In excision-repair deficient mus-201 background, 83636 F_1 female progeny was scored and 7 yellow, 69 white (including the white-Notch) and 15 vermilion females were found (Table 1). Of these 91 mutations 44 are wb and 47 mos mutations. 15 F_1 wb mutant females are sterile, 8 proved to be male lethal in the F_2 and 2 white mutations show in addition a rst/vt phenotype. The total frequency of multi-locus deletions is therefor 15+8+2/44=57%. The white mutations were analysed in more detail, using cytological and molecular techniques. The presumptive five multi-locus white deletions proved in fact not to be deficiencies but double mutations. In conclusion, the analysis shows that, in contrast to the recovered white mutations in repair proficient females, the calculated frequency of multi-locus deletions recovered in a mus-201 background is over-estimated. The molecular analysis of the remaining 11 intra-locus white mutations from mus-201 females showed that one is an inversion, one has a larger deletion of 400 bp, 6 have small deletions (4-20 bp) and 3 are base changes (for details see Table 3). In conclusion it appears that the spectrum of X-ray mutations recovered in a mus-201 background may differ from that recovered in proficient repair condition in that the fraction of multi-locus deletions appears lower. Furthermore, it is obvious that the fraction of 'mosaic' mutations in the mus-201 background is 2-3 times higher.

c. In a post replication deficient background (mei-41), 124782 F_1 females were scored and a total of 9 yellow, 44 white and 44 vermilion females found. Of these 97 mutations 73 are wb mutations. 39 of the F_1 wb mutant females are sterile and classified as multi-locus deletions. Of the remaining 34 mutations, 16 proved to be male lethal in the F_2. These were in addition to the F_1 female sterile mutations classified as multi-locus deletions. The total frequency of multi-locus deletions is therefor 39+16/73=75%. Most of the mutations were analysed cytologically. The two F_2 male lethal yellow mutations are a deletion and an inversion. Of the 8 F_2 male lethal white's 7 were analysed; 6 are deletions and one appeared normal. Of the 6 F_2 male lethal vermilion mutations, 5 carry a detectable deletion and one an inversion with a break at 10A1/2 (v). Of the 18 intra-locus F_2 male viable mutations, 9 are white mutations. Seven of these were analysed at the molecular level. One is a translocation, one is a deletions of more than 100 bp. Of the remaining 5, one has a small deletion in the 3' end of the gene. The remaining 4 are being tested for the middle and the 5' end of the gene. The two remaining male viable white mutations are in fact pigmented and may represent base changes. One of the analysed F_2 male lethal white mutations is also pigmented. The cytological examination showed a deletion from around 3C2 to 3E. This in fact may represent again two, closely linked, mutations; one a base change in the white gene, the other a multi-locus deletion.
The fraction of exceptional (mutant) F_1 females that are sterile is in mei^+ females 33% (29/87), in mus-201 females 34% (15/44) and in mei-41 females 53% (39/73). The fraction of F_2 male lethal mutations is in the three cases respectively, 36% (mei^+:21/58), 27% (mus-201:8/28) and 47% (mei-41:16/34). A comparison of all the available cytological data show that, among the mutations recovered in the mus-201 background 81% appears normal, 8% carries a multi-locus deletion and in 11% a rearrangement was observed. In the mei-41 background, only 50% appears normal, 38% carries a multi-locus deletion and in 12 % a rearrangement was observed.

Table 1.
CLASSIFICATION OF X-RAY-INDUCED MUTATIONS IN EXCISION REPAIR DEFICIENT (mus-201) CONDITION, WITH RESULTS OF FERTILITY AND TRANSMISSIBILITY

	yellow		white		white-Notch		vermilion
	wb	mos	wb	mos	wb	mos	wb
15 Gy, mus$^+$, 108,291 F_1							
Exceptional females:	17	6	17	12	12	3	41
Fertile and transmitted:	16		16		7		20
F_2 mutant male lethal:	5		4		7		6
male viable:	11		12		0		14
15 Gy, mus-201, 83,636 F_1							
Exceptional females:	3	4	20	41	5	2	15
Fertile and transmitted:	3		16		3		6
F_2 mutant male lethal:	1		3		3		1
male viable:	2		13		0		5
15 Gy, mei-41, 124782 F_1							
Exceptional females:	7	2	21	17	5	1	40
Fertile and transmitted:	6		15		2		11
F_2 mutant male lethal:	2		6		2		6
male viable:	4		9		0		5

Table 2.
THE MOLECULAR ANALYSIS OF 10* X-RAY INDUCED INTRA-LOCUS white MUTATIONS IN REPAIR PROFICIENT CONDITION.

mutant	description
83B	Tr(1;3)3C;64BC, with a break between coordinates -3.0 and -0.7
25B2	deletion of 400 bp between the Sal I sites at 12727 and 14242
35A2	deletion of 14 bp in exon 4 (12035 - 12048)
73A1	deletion of 29 bp in exon 6 (13105 - 13133)
80B2	deletion/insertion smaller than 5 bp or base-pair change**
8801-10A	deletion of 700 bp between the Sal I site at 11862 and the Bam H1 site at 9815
8801-17A	small (5 bp) deletion between the Cla I site at 12670 and the Sal I site at 14242
8801-35A	deletion/insertion smaller than 5 bp or base-pair change**
8802-44A1	small (5 bp) deletion between the Cla I site at 12670 and the Sal I site at 14242
8803-19A	small (5 bp) deletion between the Xba I site at 10765 and the Sal I site at 11867

Table 3.
THE CYTOLOGICAL AND MOLECULAR ANALYSIS OF 16 X-RAY INDUCED white MUTATIONS IN EXCISION REPAIR DEFICIENT (mus-201) CONDITION.

mutant	description cytology	
8705-44A (male lethal)	not deter.	not tested
8706-68B (male lethal)	Df(1)3A4-3B4	not tested
8631- 1A (semi lethal)	In(1)w;18C	normal restriction enzyme map
8624- 4A ($w^{-}rst^{-}vt^{-}$)	normal X	normal restriction enzyme map
8633- 6B ($w^{-}rst^{-}$)	normal X	normal restriction enzyme map
8631-22A (plain white)	In(1)w;14C;20	breakpoint between the Hind III site at 8027 and the Xba I site at 10765
8623-12 (" ")	normal X	deletion of 400 bp between the Xba I site at 10765 and the Sma I site at 12085
8622-14 (" ")	normal X	deletion of 9 bp in exon 3 (11552 - 11560)
8625- 7A (" ")	normal X	GC --> AT at nucleotide 11371 (Gln into stop)
8632-15B (" ")	normal X	deletion of 4 bp in exon 6 (13076 - 13079)
8701-35A (pigmented)	normal X	deletion/insertion smaller than 5 bp or base-pair change
8705-14A (")	normal X	deletion of 7 bp + 4 bp insertion in exon 5 (12586 - 12592)
8706-12A (")	normal X	deletion of few base-pairs in exon 1
8706-55A (")	normal X	deletion/insertion smaller than 5 bp or base-pair change
8706-63B (plain white)	normal X	deletion of 5 bp in exon 3 (11654 - 11658)
8707-39A (male sterile)	normal X	deletion of 20 bp + 2 bp insertion in exon 3 (11772 - 11791)

Discussion
A comparison of all the data indicate, that due to the defect in the excision repair of mus-201 females, among the recovered X-ray induced mutations relatively more intragenic and less multi-locus deletions are present than in repair proficient condition. The reverse appears true after recovery in post-replication repair deficient mei-41 females.

The molecular analysis of chemically (ENU/EMS) induced vermilion mutations.

Methodology
Vermilion mutants were induced by ENU or EMS treatment. Mutant alleles were cloned in phage lambda using the recombinational screening method or cloned directly in M13 vectors after amplification in-vitro (PCR). Single-stranded plasmid or M13 DNA was then used for the sequence determination using a set of oligo-nucleotide primers.

Results
In total we have analysed 25 (16 F1 and 9 F2) ENU-induced and 28 (17 F1 and 11 F2) EMS-induced mutants. Three ENU mutants and one EMS mutant represent double mutations. Transition mutations are the most prominent sequence change after ENU treatment: GC-AT (61%) and AT-GC (18%). The ENU spectrum also includes a significant fraction of tranversions, namely 21% (3 AT-TA, 1 AT-CG, 1 GC-CG and 1 GC-TA). No deletions were observed. In case of EMS the percentage of GC-AT is even higher, namely 79%. In addition, 3 AT-TA and 1 GC-TA base-pair changes and 2 deletions were seen.

Discussion
Both GC-AT and AT-GC transition mutations can be explained by mispairing of the O6-ethyl-guanine and O4-ethyl-thymine adducts with thymine and guanine, respectively. The AT-TA transversions in case of ENU can be explained by the O2-ethyl-thymine adduct. However, AT-TA transversions are also induced by EMS although EMS treatment does not result in O2-ethyl-thymine adducts. Most likely the transversion mutations result from alkylation of the base-nitrogen atoms. Comparison of the alterations in F1 and F2 mutants did not show significant differences. The strong contribution of O6-ethyl-guanine adduct to the mutagenicity of both ENU and EMS possibly explains the absence of distinct differences between the type of mutations observed in F1 and F2 mutants.

The genetic effect *in vivo* of a post-replication repair deficient mutation (*mei-41*) in somatic tissues.

Drosophila repair deficient mutants were characterized biochemically in primary cell cultures, i.e. somatic cells. In contrast, most of the genetic effects of these mutants have been studied in cells of the germ line. To improve the correlation between these two levels it was decided to investigate the effects of repair-deficient mutants on the mutation induction in somatic cells.

Methodology
Female larvae of repair deficient mutant strains, heterozygous for an X-linked recessive *white eye* mutation (w/w^+) were X-irradiated. The emerging adult flies were scored for (induced) clones of *white* spots (ommatidia) in the eyes. These spots arise as the result of (1) mutations (point mutation, deletion), (2) complete/partial chromosome loss and (3) recombination events. The number and size of the clones was used to estimate mutation rates.

Results
The frequency of spontaneously occuring small spots is significantly higher in *mei-41* females (compared to *mei*$^+$). This increase is not observed among the larger spots. After X-irradiation, especially the induction of the larger spots is enhanced in *mei-41* females. This effect appears stronger after irradiation in anoxic than oxic condition. These results differ from those obtained in excision-repair deficient (*mei-9*) females; in these females the spontaneous and induced frequency of both types of spots is increased. In both cases (*mei-41* and *mei-9*) the induced frequency of spots is much higher in these w/w^+ heterozygous females than in w^+/Y hemizygous males.

Discussion
The last result indicates that especially recombination events are increased after X-irradiation in repair deficient condition. The fact that this increase in mei-41 females is stronger in anoxic condition may indicate that in these flies recombinational events result from the misrepair of X-ray induced base damage.

Induction of bleomycine sensitive mutants.

A series of mutants of Drosophila melanogaster have been reported that are deficient in repair of UV induced DNA damage. Although the mutants alter in general the frequency of X-ray induced genetic damage, no repair defects could be demonstrated yet for X-ray induced DNA damage. Also the detected increases and decreases in the frequencies of X-ray induced genetic damage were relatively small. The direct selection of X-ray hypersensitive mutants was considered but was supposed to be complicated as X-rays are known to induce a wide range of DNA lesions. Instead bleomycin was chosen as the selection agent as it produces primarily DNA strand breaks which are also an important type of lesion induced by X-irradiation.

Methodology
Male flies were mutagenized with ENU and mated to attached-X females. The F_1 males were crossed individually to attached-X females and the F_2 cultures were treated with bleomycin. The absence or decreased frequency of males in the F_2 indicates a potential bleomycin sensitive mutant.

Results
In total 13807 mutagenized X-chromosomes were screened. Based on the dose of ENU used for the induction and the number of X-linked genes, it can be calculated that (assuming a random induction of mutation) each gene was mutated 4 times. Five mutants have been isolated that shows a slight hypersensitivity to bleomycin.

Discussion
The frequency of recovered bleomycin hypersensitive mutants is low compared to that of MMS hypersensitive mutants. As bleomycin induces far less different types of DNA damage than MMS, it is to be expected that mutations at fewer loci will confer bleomycin hypersensitivity. Presumably only a small number of loci are involved in the repair of DNA strand breaks.

Induction of P-insertion mutations at repair genes.

The mobile element P can be used to clone specific genes. Mutations due to the insertion of a P-element in a gene, will provide a 'tag' which can be used to isolate the DNA-sequences of the mutated gene. To clone DNA repair genes, mutations have to be made in these genes with the P-element.

Methodology
Dysgenic flies are generated in which the mobile element P is transposing at high frequency in germ line cells. Dysgenic males carrying a Pm balancer chromosome are crossed to C(1)DX females. The F_2 Pm males are mated individually to mus-201 (excision-repair deficient, MMS hypersensitive) females for 2 days after which the parental flies are transferred to new culture-vials. MMS is added to the first vial. The vials are scored for the

presence or absence of Pm flies in the F_3. If a P-induced mus-201 is present on the Pm chromosome, only Pm^+ flies will survive the MMS treatment.

Results
A total of 27033 second chromosomes and no P-induced mus-201 mutation has been detected.

The characterization of a mobile element (P-element) transposition system in Drosophila.

In Drosophila it is evident that many spontaneous mutations and chromosome aberrations are the result of transposition of mobile elements. Since spontaneous genetic endpoints form a convenient frame of reference for physically and chemically induced DNA-damage, it is important to study the effect of mutagenic agents on mobile elements and the process of transposition. One of the systems to study such interactions in Drosophila is the transposition of P-elements. However, since there exists considerable heterogeneity among the strains carrying P-elements, these strains have to be characterized in detail.

Methodology
We studied a number of particular P-strains, MR-strains, that are characterized by the fact that the P-activity in principle is limited to one isolated chromosome only. Functional P-elements are 2.9 kb long, including an intact 2.4 kb Acc I fragment. We determined the number of copies of P-elements carrying an intact 2.4 kb Acc I fragment in the MR-strains MR-h12/Cy, MR-n1, MR-GB39/Cy, MR-T007/Cy and MRF-31.1/CyL^4. This analysis was combined with genetic studies determining the activity of these MR-strains to induce sex-linked lethal and visible mutations and to revert P-insertion mutations.

Results
The results show that the strains MRF-31.1/CyL^4 and MR-T007/Cy carry respectively more than 16 and 7-8 intact P-elements. The MR-strains n1 and GB39/Cy appear very similar, and possibly contain the same functional P-elements (5-6). The MRh12/Cy on the other hand carries two intact P-elements.
A detailed analysis of the MR-strains revealed the presence of part of the P-elements with an intact 2.4 kb Acc I fragment on other chromosomes than the MR-chromosome, whereas the genetic activity is restricted to this chromosome. The P-elements containing the complete 2.4 kb Acc I fragment of two strains (MR-h12/Cy and MR-T007/Cy) were cloned and analysed. From respectively MR-h12/Cy and MR-T007/Cy, two and five different elements were isolated that are identical to the complete and functional P-element pπ25.1 (they do not have larger deletions/insertions than 4-5 bp, as indicated by S1 protection experiments). The ends of all elements were sequenced and their inverted repeats and internal P-transposase binding sequences are intact. It is shown that in the MR-h12/Cy strain one complete element resides at 38A (HA41) on the MR-chromosome, whereas the other element (HA51) is present at 2B on the X-chromosome. This last element was completely sequenced and is identical to pπ25.1, however, it has no genetic activity.

Discussion
The results show that (1) the genetic effects in MR-h12/Cy are correlated

with one complete P-element only, (2) complete P-elements exist without any genetic activity and (3) since the activity of complete P-elements in the same P-strain can vary in activity, the differences in genetic activity of the different MR-strains cannot directly be related to the number of complete elements.

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V. Publications:

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Title of the project no.: 6

The production of chromosome aberrations in human lymphocytes by low doses of X-rays

Head(s) of project Prof. A. T. Natarajan

Scientific staff: Prof. K. Sankaranaryanan
Dr. A. T. Natarajan

I. Objectives of the project:

To irradiate blood in vitro to low doses of X-rays and to examine the lymphocytes in metaphases 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.

II. Objectives for the reporting period:

Results and Discussion:

This is a multi-laboratory project. For a common report on the project, see the report of Contract no. B.16-E-225-UK (NRBP).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: State University of Leiden
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Contract no.: BI6-E-226-NL

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Title of the research contract:

Studies on spontaneously-arising genetic and partially genetic disorders in man within the framework of the evaluation of genetic radiation hazards.

List of projects.

1. Studies on spontaneously-arising genetic and partially genetic disorders in man within the framework of the evaluation of genetic radiation hazards.

Title of the project no 1

Studies on spontaneously-arising genetic and partially genetic disorders in man within the framework of the evaluation of genetic radiation hazards

Head(s) of project. Prof. Dr. K. Sankaranarayanan

Scientific staff: Prof. Dr. K. Sankaranarayanan

I. Objectives of the project:

1. To make a detailed analysis of the population prevalence of naturally-occurring multifactorial diseases, of mendelian and chromosomal diseases (in that order) to examine the validity of the estimates of prevalence currently used in the context of the evaluation of genetic radiation hazards in man;
2. To make use of these data and those that bear on the severity of these diseases and arrive at estimates of detriment; and
3. To make an in-depth analysis of the mutation component of diseases of multifactorial diseases which can be used in the context of genetic risk assessment.

II. Objectives for the reporting period:

The main objectives of the work carried out during the period 1986-89 have been (i) to make a systematic analysis of the prevalence of naturally-occurring multifactorial diseases in the population of Hungary, compare these estimates with those published in the literature and summarize their epidemiological, clinical and genetic features and (ii) on the basis of these data and information on mortality and other aspects, arrive at estimates of detriment at the individual and population levels.

III. Progress achieved:

1. Methodology.

In the first part the project, carried out in 1987-88, 26 common multifactorial diseases (CMDs) were selected for consideration and in the second part carried out in 1989, attention was focused on mental retardation (MR). In the selection of CMDs, three principal criteria were used: (i) they should be common-defining common as potentially present in more than 1 per 10^4 individuals at stage in life; (ii) they should be multifactorial and (iii) the estimates of narrow heritability of liability (h^2) should be at least 0.30. Based on clinical severity, these diseases were arbitrarily divided into three groups, namely, group I, clinically very severe (schizophrenia, multiple sclerosis, epilepsy, acute myocardial infarction and systemic lupus erythematosus), group II, moderately severe and/or episodal or seasonal (Graves' disease, diabetes mellitus, gout, affective psychoses, glaucoma, essential hypertension, asthma, peptic ulcers, idiopathic proctocolitis, coeliac disease, calculus of the kidney, psoriasis, rheumatoid arthritis and ankylosing spondylitis) and group III, less severe than those in groups I and II (varicose veins, allergic rhinitis, atopic dermatitis, Scheurmann disease and adolescent idiopathic scoliosis).

For the CMDs, baseline figures on population sizes, mortality and its causes were extracted from the Hungarian Demographic Year Books published by the Central Statistical Office (CSO), Budapest, while data on age-standardized prevalences, mean ages at onset, and heritability estimates were from different epidemiologivcal studies carried out in Hungary. Data published in the literature were used to make comparisons with the Hungarian data. The indicators used to assess detriment were: years of lost life (LL), potentially impaired life (PIL) and actually impaired life (AIL). While the first two could be estimated from the epidemiological data, for the third, use was made of data on premature retirement provided by the Office of Hungarian Medical Specialists.

For the MR part of the study, the starting point was the Budpaest survey of MR and its aetiology among 1,276 school-age (7-14 years) children in Budapest (Czeizel et al Am. J. Ment. Def 85, 120 (1980), which was supplemented with additional data on aetiology and other data obtained from CSO. The indicators used to assess detriment for the different aetiological groups of MR are: years of life lost and impaired.

2. Results and discussion

A. Common multifactorial diseases (CMDs).

a) Prevalences and epidemiological features.

The total prevalence estimate for the 26 diseases included in our analysis is about $6500/10^4$ individuals in the population (group I, $512/10^4$; group II, $3218/10^4$ and group III, $2811/10^4$) and this figure is in agreement with that (i.e., the total of median estimates for the individual conditions) for other countries. Apart from a few diseases (or disease sub-entities) such as insulin-dependent-diabetes-mellitus, epilepsy and coeliac disase, most of the diseases included in our compilation have mean onset ages in middle life. There are no sex differences (i.e., sex ratio of 1:1) in the prevalence of diabetes mellitus, schizophrenia, epilepsy, essential hypertension etc. Diseases such as Graves', varicose veins, systemic lupus erythematosus have female preponderance whereas those such as gout, acute myocardial

infarction and ankylosing spondylitis have male preponderance. Heritability estimates range from about 0.30 for diabetes mellitus to 0.90 for systemic lupus erythematosus.

b) Mortality profiles.
With the exception of epilepsy (mostly childhood epilepsy cases), these CMDs are not associated with mortality between ages 0 and 19, but are among the leading causes of death between ages 20 and 69 and thereafter. A sizeable proportion of those with essential hypertension, diabetes mellitus, rheumatoid arthritis etc survive to 70 years and beyond, as do those with gout, glaucoma, allergic rhinitis, psoriasis etc. The average annual mortality rates are $13.5/10^4$ individuals for group I diseases, $6.68/10^4$ for group II diseases and $0.03/10^4$ for group III diseases. Overall about 16% of deaths that occur in Hungary every year (all age groups) can be attributed to these diseases.

c) Years of life lost (YLL).
The mean number of YLL is substantial only for five of the diseases (epilepsy, 30 y; affective psychoses, multiple sclerosis and systemic lupus erythematosus, 18-20 y; schizophrenia, 13 y). At the population level, the total YLL is about $2700/10^4$ individuals; those with acute and sub-acute myocardial infarction accounts for about one-half of this total.

d) Years of potentially and actually impaired life (PIL and AIL).
The mean PIL was calculated by subtracting the mean age at onset from the mean age at death. This covers a wide range (about 20-40 y, 12-70 y and 40-60 y for groups I, II and III, respectively), the overall mean being about 24 y. However, the nature and degree of impairment and the impact on life quality of those afflicted differ for the different diseases. The mean AIL was estimated as the difference between the mean age at death and the mean age at premature retirement. This encompasses, again, a wide range from 16 to 45 y, but the overall averages for the diseases included in each of the three groups are roughly the same, being about 20 y. At the population level, the diseases considered herein cause about 96,000 y of PIL and about 5,800 y of AIL per 10^4 individuals in the population.

e) Comparison of estimates of detriment for multifactorial diseases with those for mendelian diseases.
For this purpose, we used estimates of detriment arrived at by Carter (Prog. Mut. Res 3, 1-8, 1982) for mendelian and chromosomal diseases and by Czeizel and Sankaranarayanan (Mut. Res 128, 73-103, 1984) for congenital abnormalities. Our analyses show that relative to mendelian diseases as a whole, multifactorial diseases (congenital abnormalities + other CMDs discussed thus-far) are associated with much greater detriment (Life loss: 1.4x; potentially impaired life years: 30x and actually impaired life years: 3.9x).

B. Mental retardation

a) Prevalence of MR among school-age children.
The data made available to us by the CSO, Budapest show that about 30 per 10^3 school-age children (7-14 y; about 35,000

children) are mentally retarded (mild + severe; IQ of 70 or less) and this figure is essentially the same over the school-years 1974/1975 to 1986/1987. One-tenth of these children have severe MR (IQ of less than 50) about half of whom are institutionalized in Hungary.

b) Aetiology.

The prevalences of the different aetiological categories of MR, based 1,276 school-age children (from special schools and residential institutions) included in the analysis are as follows: (i) mendelian, $2.7/10^3$; (ii) chromosomal, $1.7/10^3$; (iii) prenatal causes, $2.8/10^3$; (iv) perinatal causes, $6.4/10^3$; (v) post-natal causes, $2.1/10^3$; (vi) familial (multifactorial) causes, $12.1/10^3$ and (vii) unknown causes, $1.5/10^3$. Grand total, $29.3/10^3$.

c) Estimates of detriment.

The estimates of mean number of years of lost life range from 42 to 68 (depending on the aetiological category) with an overall mean of 58 years. The total number of years of lost life is about 36,000 per 10^4 livebirths of which over 70% is due to pre-, peri-, and post-natal causes, 18% due to "familial" causes and the remainder due to mendelian and chromosomal diseases. The total number of years of impaired life is about 7,300 per 10^4 livebirths 50% of which is due to "familial" causes. While these figures are admittedly approximate, they do suggest that detriment associated with MR-related causes is not inconsiderable. Additionally, they provide some indication of causes of MR which are minimizable.

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

Dr. Andrew Czeizel, Director, Department of Human Genetics and Teratology, National Institute of Hygiene, Gyali UT 2-6, Budapest.

V. Publications:

Sankaranarayanan. (1988) Invited review: Prevalence of genetic and partially genetic diseases in man and the estimation of genetic risks of exposure to ionizing radiation, Am. J. Hum. Genet., 42, 651-661.

Sankaranarayanan, K. (1988) Mobile genetic elements, spontaneous mutations and the assessment of genetic radiation hazards in man, In: Banbury Report 30, Eukaryotic transposable elements as mutagenic agents (M. E. Lambert et al., eds.) Cold Spring Harbor Laboratory, N. Y, pp 319-336.

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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

Contract no.: BI6-E-190-UK

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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

Effect of dose-rate on mutation frequency in V79 Chinese hamster cells.

Head(s) of project:

Dr A Morgan

Scientific staff:

Dr PD Holt
Dr GR Morgan
Mr CJ Roberts

I. Objectives of the project:

To determine whether an inverse dose-rate effect exists for mutation of the HGPRT locus of V79 Chinese hamster cells.

II. Objectives for the reporting period:

To establish whether α-particle induced mutation is independent of dose-rate.

III. Progress achieved:

INTRODUCTION

The biological effect of ionizing radiation can depend on dose-rate. However, the consequences of changing dose-rate depend on radiation quality. Decreasing the dose-rate generally reduces the effectiveness of low linear-energy-transfer (LET) radiation, while the effect of high LET radiation is acknowledged to be independent of dose-rate. Thus, lowering the dose-rate should, if anything, produce a sparing effect (UNSCEAR, 1986). Consequently, when predicting the effect of a dose of radiation, any error introduced by ignoring a reduction in dose-rate will be conservative, i.e. the predicted effect will be an overestimate. However, it has been suggested that this is not correct for certain end-points. Results from various transformation assays (Hall and Miller, 1981; Hill, Han and Elkind, 1984; Jones and Elkind, 1987) and carcinogenesis studies (Ullrich, 1984) demonstrate that lowering the dose-rate of high LET radiation can increase its biological effect, a phenomenon designated the reverse, or inverse, dose-rate effect (IDRE). Though initial attempts to reproduce this result using different radiation qualities have failed (Hieber *et al.*, 1987; Roberts *et al.*, 1987), its implications for radiological protection mean that an explanation must still be found for the original observations.

It is increasingly obvious that transformation *in vitro* is not a simple phenomenon. The transformed phenotype can be regulated by epigenetic factors, whose influence is ill-defined (Kennedy and Little, 1978; Terzaghi and Little, 1976; Terasima, Yasukawa and Kimura, 1981). To avoid these complications and establish whether the IDRE applies to end-points other than transformation, the effect of dose-rate on the mutation frequency at the HGPRT locus of V79 Chinese hamster cells was measured. In this system the conditions required for reliable measurement of the mutation frequency are well characterized. Log-phase cultures of V79 cells were irradiated with either 2.9 MeV neutrons, produced by the $^{9}Be(d,n)^{10}Be$ reaction on a Van der Graaf accelerator, or by 3.2 MeV α-particles from a ^{238}Pu source. In addition, the contribution of cell-cycle traverse and cell division to the IDRE were investigated by comparing mutation yields from log-phase and plateau-phase cultures.

Plateau-phase cultures contain synchronised, growth-arrested cells. Thus their radiosensitivity should be homogeneous and all cells receive the same dose during a protracted exposure. This permits a true, direct comparison of high and low dose-rates without needing to compensate for variations in cell sensitivity during the irradiation period.

METHODOLOGY

V79 AH1 Chinese hamster cells were used for all experiments. The cells were propagated in Eagle's minimum essential medium, supplemented with 10% fetal calf serum, 2mM glutamine, 50 μg ml^{-1} streptomycin and 50 iu ml^{-1} penicillin (complete medium and all cell culture materials obtained from GIBCO). Stock cultures were maintained in exponential growth. Log-phase cultures for neutron irradiation were prepared by seeding $3x10^5$ cells onto 25 cm^2 culture flasks containing 10 ml complete medium 40-48 hrs before irradiation. Plateau-phase cultures were prepared by seeding $5x10^5$ cells 96 hr prior to irradiation. Log-phase cultures for α-irradiation were prepared by seeding $1.5x10^5$ cells onto specially prepared 30 mm diameter culture dishes made of titanium with a base (growth surface) of Hostaphan 2.5 μm thick. The dishes had airtight lids with entry and exit ports for a throughflow of 5% CO_2:air to maintain pH. As for the neutron irradiations, cultures were seeded 40-48 hrs before irradiation and incubated at 37°C in a 5% CO_2:air atmosphere. A 5-MV Van de Graaf accelerator was used to provide a 3-MeV deuteron beam incident on a thick beryllium target. The cultures were irradiated with neutrons emanating at 90° to the direction of the deuteron beam. The neutron beam contained a wide range of energies up to 6 MeV, with a mean energy of 2.9 MeV when weighted by kerma. The cultures to be irradiated were placed in a chamber aligned parallel to the horizontal axis of the target assembly. The chamber was flushed with air at 37°C during the irradiation and the flasks were sealed to maintain their internal atmosphere. The dose in each exposure was checked with a sulphur disc placed on top of the flask, the ^{32}P β-activity induced being checked later with an end-window GM counter. High dose-rate irradiations were carried out at approximately 10 cGy min^{-1} and low dose-rate irradiations at <0.5 cGy min^{-1}. Cultures irradiated at a high dose-rate were kept in the irradiation chamber for the same length of time as the low dose-rate samples and irradiated at the end of the

period. After irradiation, each culture was returned to the laboratory and processed alongside its control which had been taken to the irradiation site and then kept in a hot room at 37°C during the irradiation. The cells were recovered by trypsinization, counted and diluted to yield 200 survivors per 90 mm dish. The remaining cells were inoculated into 900 cm^2 roller bottles containing 100 ml complete medium and allowed a period of growth before the mutation frequency was measured. The α-particle source used for these experiments has been described in detail elsewhere (Roberts and Goodhead, 1987). Briefly, the α-particles traverse a path of 65 mm of helium at atmospheric pressure, a containment chamber window of 0.35 mg cm^{-2} Hostaphan (polyethylene terephthalate; Hoechst) and 3 mm air before entering the base of the culture dish. The dishes are contained in a wheel which rotates over the chamber window. The two edges of the irradiation field are sharply defined by a sector plate. Dose-rate can be adjusted by using sector plates with different apertures combined with low-scatter apertures immediately above the ^{238}Pu disc. The whole assembly was kept at 37°C during the irradiation and the dishes were gassed from a 5% CO_2:air supply to maintain pH.

In these experiments the α-dose was restricted to 0.72 Gy given at either 0.016 Gy h^{-1} or 25.2 Gy h^{-1}. Low dose-rate, high dose-rate and controls were located on the same wheel (3 dishes for each), the high dose-rate and controls being screened by an aluminium disc. When the low dose-rate irradiations were completed the dishes were removed from the wheel and the blanking discs removed from three of the remaining six dishes. The wheel was then returned to the chamber, the appropriate sector plates inserted and the same dose given at high dose-rate. All nine dishes were then returned to the laboratory. The cells were recovered by trypsinisation from a 28 mm dia. circle of Hostaphan cut from the bottom of each dish. Replicate dishes were combined, the cells counted and prepared for survival and mutation measurements as described for the neutron irradiations.

Measurements of the mutation frequency were conducted according to the methods of Thacker and colleagues (Thacker, Stretch and Stevens, 1977; Thacker and Stretch, 1983). Each of twenty 90 mm tissue culture dishes (NUNC) containing 10 ml complete medium, supplemented with 6-thioguanine at 0.5 μg ml^{-1}, were seeded with 10^5 cells. At the same time, sufficient

cells to yield 200 survivors were seeded into each of five 90-mm dishes containing 10 ml complete medium to determine the surviving fraction. The survival dishes were incubated for seven days and the mutation dishes for ten days before being stained with methylene blue. Colonies were counted by eye. On the mutation dishes, only colonies >2 mm diameter were scored. In some experiments the identity of the colonies was checked autoradiographically (Thacker, Stretch and Brown, 1982) to ensure that the size discrimination gave correct results. Average control mutation frequencies were in the range $((0.4\text{-}1.5)\pm0.4)\times10^{-5}$ mutants per surviving cell.

RESULTS

Analysis of plateau-phase cultures

A typical growth curve of the V79 cultures shows an initial exponential increase in cell numbers, lasting three to four days. The number of cells per culture reaches a maximum after which there is no further change (Fig. 1). This 'plateau-phase' condition can be maintained for several days without detriment to the cells, as judged by their plating efficiency.

Further investigation of the dynamics of logarithmic and plateau-phase cultures using a fluorescence-activated cell sorter (FACS) revealed an accumulation of cells in the G_1 stage of the cell cycle on entry into plateau phase (Fig. 2). During logarithmic growth approximately 65% of the cells were in S phase, synthesizing DNA, while 25% were in G_1. Entry into plateau phase was a gradual rather than an abrupt transition. However, 96 h after inoculation, approximately 80% of the cells were in G_1 with a concomitant fall in the number of S phase cells. Thereafter, the composition of the culture changed little until the cells began to deteriorate.

Measurement of survival and mutation - neutron irradiations

The effect of dose-rate was established using plateau-phase cultures to exclude cyclic variations in radiosensitivity and repopulation leading to non-uniformity of dose. At high dose-rates the survival curve for 2.9 MeV neutrons was linear over the range 0 to 3 Gy (Fig. 3). Lowering the dose-rate led to a significant increase in survival, with the dose response remaining linear but a small shoulder appearing. Survival in

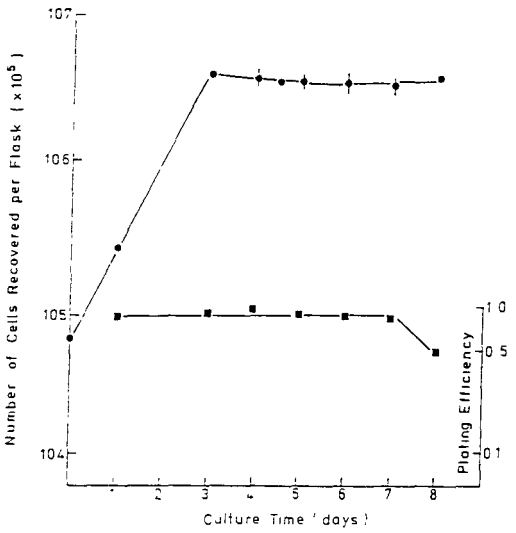

FIG.1: THE GROWTH KINETICS AND PLATING EFFICIENCY (PE) OF V79 CHINESE HAMSTER CELLS.

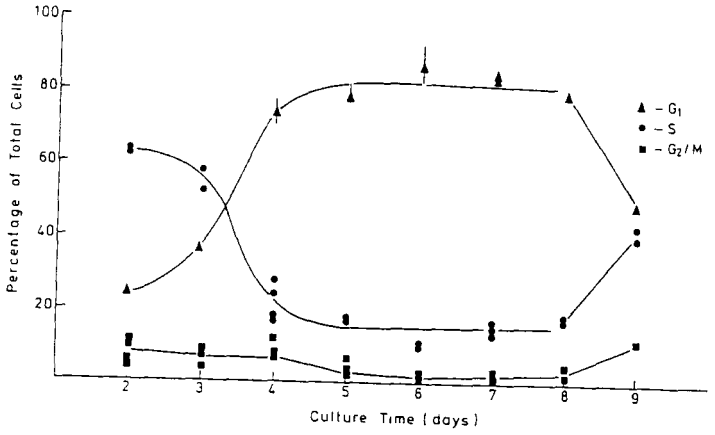

FIG.2: ANALYSIS OF COMPOSITION IN TERMS OF CELL-CYCLE PHASE OF V79 CULTURES ON SUCCESSIVE DAYS AFTER PLATING AS DETERMINED BY FACS ANALYSIS.

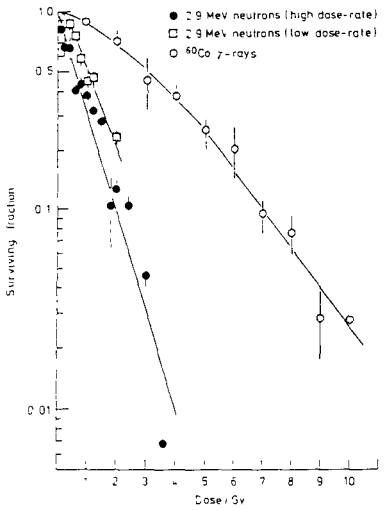

FIG.3: SURVIVAL OF PLATEAU-PHASE V79 CELL EXPOSED TO 2.9MEV NEUTRONS OR TO 60CO -RAYS.

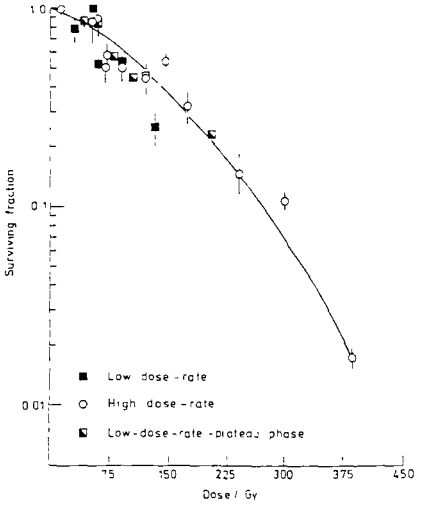

FIG.4: SURVIVAL OF LOG-PHASE V79 CELLS EXPOSED TO 2.9 MEV NEUTRONS AT. THE SURVIVAL OF PLATEAU-PHASE CELLS IRRADIATED AT LOW DOSE-RATE IS ALSO SHOWN FOR COMPARISON.

log-phase cultures was independent of dose-rate and was coincident with the plateau-phase low dose-rate survival curve (Fig. 4). As shown in Table 1, the RBE of the neutrons was greatest for the plateau-phase cells irradiated at high dose-rate (4.7). Lowering the dose-rate or changing to log-phase cultures reduced the RBE to approximately 3.3.

TABLE 1

Parameters of the best-fit lines obtained by weighted regression according to the relationship $S = \exp[-(\alpha D+\beta D^2)]$ for ^{60}Co γ-rays and $S = \exp(-\alpha D)$ for 2.9 MeV neutrons.

	α (cGy^{-1})	β (cGy^{-2})	RBE
^{60}Co γ-rays (16 Gy min^{-1})	0.23351±0.00260	0.01278±0.00330	-
2.9 MeV neutrons (10 cGy min^{-1})	0.01182±0.0010	-	4.72±0.2
2.9 MeV neutrons (0.4 cGy min^{-1})	0.00815±0.0014	-	3.28±0.3

The effect of dose-rate and culture kinetics on mutation.

The effect of dose-rate on mutation frequency was measured by its influence on a) expression time and b) on the dose-response relationship. To ensure that the interval before mutants appeared in the population did not depend on dose-rate, the mutation frequency was monitored at various times after irradiation. The relationship between expression time and mutation frequency for a dose of 40 cGy (Fig. 5a) delivered to plateau-phase cells showed that a post-irradiation incubation period in excess of 96 h was needed to ensure maximum recovery of mutants. Thereafter some variability was observed between successive assays but there was no consistent significant difference between high and low dose-rates. Log-phase cultures followed a similar time-course, again independent of dose-rate (data not shown). Measurements made at three or more successive expression times between four and ten days, were averaged to obtain the yield of mutants for each dose of radiation. The level of mutation in control cultures was calculated in the same way and subtracted to obtain the yield of radiation-induced mutants. In plateau-phase cultures the dose-response relationship for mutants induced by 2.9 MeV

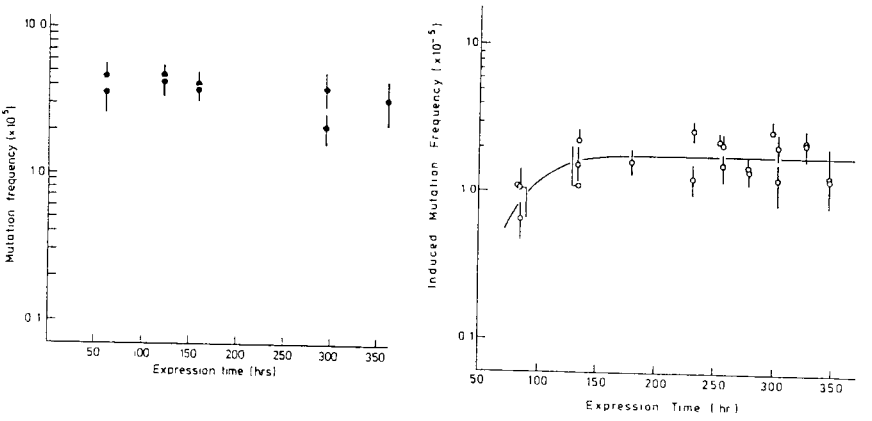

FIG. 5: MUTATION AS A FUNCTION OF EXPRESSION TIME FOR V79CELLS IRRADIATED WITH A) 2.9 MEV NEUTRONS AND B) 3.2MEV α-PARTICLES AT HIGH OR LOW DOSE-RATE. NO CONSISTENT DIFFERENCES WERE OBSERVED BETWEEN HIGH AND LOW DOSE-RATE; THEY HAVE NOT BEEN DISTINGUISHED IN THIS FIGURE.

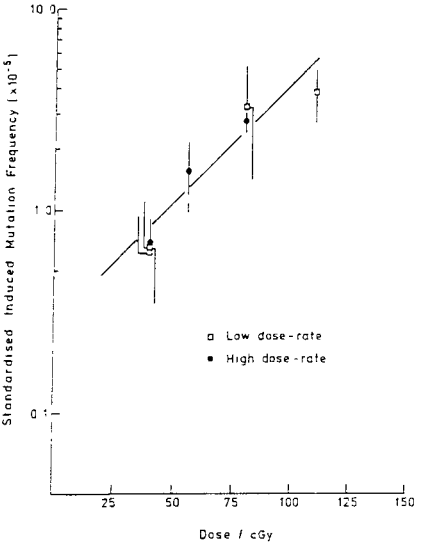

FIG. 6: DOSE-RESPONSE RELATIONSHIP FOR MUTATION INDUCED IN PLATEAU-PHASE CELLS BY 2.9 MEV NEUTRONS.

neutrons was linear (Figure 6) and no significant effect of dose-rate was detected. In log-phase cultures, irradiation at high dose-rate induced mutation yields similar to those obtained in plateau-phase cells. However there was evidence for a small increase in mutation frequency when combining a low dose with a low dose-rate. Expressing the mutation frequencies at high and low dose-rates as a ratio (Table 2) revealed a trend towards enhanced mutation frequencies at the lower doses.

Measurement of survival and mutation - alpha particle irradiation

Irradiation of the log-phase V79 cells with 0.72 Gy α-particles gave a mean survival of (44.9±10.3)% at high dose-rate and (43.7±5.6)% at low dose-rate. The same amount of cell killing resulted from a dose of approximately 1.2 Gy of 3.2 MeV neutrons, making the α-particles more effective. The relationship between expression time and mutation frequency is shown in Fig. 5b. Although the mutant yield fluctuated between experiments there was no consistent difference between the high and low dose-rate results. This is borne out by the average mutation frequencies which were (3.8±1.1) and (3.6±0.6) at high and low dose-rates respectively, a yield comparable to that from an equitoxic dose of neutrons, giving a low dose-rate/high dose-rate ratio of 0.95.

DISCUSSION

These results show no evidence of an IDRE for mutation at the HGPRT locus when non-dividing cells were irradiated with either 2.9 MeV neutrons or 3.2 MeV α-particles. However, there was a small, but consistent, trend toward an enhanced mutation frequency when cultures of dividing cells were irradiated with neutrons using a combination of low dose and low dose-rate. Unfortunately the sensitivity of the mutation assay is relatively poor and it was not possible to measure the mutation frequency at doses lower than 0.5 Gy.

These observations suggest that the IDRE effect seen in these experiments could be attributable to variations in radiosensitivity during the cell-cycle.

TABLE 2
The mutation frequency in log-phase cultures exposed to 29 MeV neutrons at high and low dose-rates

Dose (Gy)	mutation frequency at low dose-rate / mutation frequency at high dose-rate
0.48	1.60 ± 0.45
0.72	1.14 ± 0.12
0.90	0.72 ± 0.10
1.04	1.01 ± 0.17
1.40	0.61 ± 0.10

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Dr D.T. Goodhead, MRC Radiobiology Unit, Chilton, Oxfordshire.

V. Publications:

Morgan, G.R., Pepperall, D., Roberts, C.J. and Holt, P.D. The effect of dose-rate on mutation frequency induced by high LET radiation in V79 Chinese hamster cells. In: Low Dose Radiation, Biological basis of risk assessment, Ed. Baverstock, K.F. and Stather, J.W. Pub. Taylor and Francis 1989, 560-570.

Morgan, G.R., Goberdahn, N.J., Roberts, C.J. and Holt, P.D. The effect of dose-rate on mutation frequency in V79 Chinese hamster cells. (Abstr) International Journal of Radiation Biology, in press.

Morgan, G.R., Roberts, C.J. and Holt, P.D. The influence of dose-rate on the biological effect of low doses of radiation. In: Radiation Research, Proc. 8th Int. Cong. for Radiat. Res. Edinburgh 1987. Pub. Taylor and Francis 1987, p. 129 (Abst).

Title of project No. 2

Cell Transformation in C3H 10T½ mouse cells by alpha particles

Dr A Morgan

Mr C J Roberts
Miss S Futter
Dr GR Morgan
Dr PD Holt

To establish whether an inverse dose-rate effect exists for ^{238}Pu α-particles.

To evaluate the influence of environmental factors on the plating efficiency and transformation frequency of C3H 10T½ cells.

III. Progress achieved:

INTRODUCTION

While decreasing the dose-rate generally leads to a sparing effect for low-LET radiation, the effect of high-LET irradiation is usually independent of dose-rate. However there is now considerable evidence that lowering the dose-rate can increase the effect of high LET radiation. This phenomenon, the inverse dose rate effect (IDRE), has been observed in a variety of biological systems from cell-cultures to man. In many cases the effect is only seen at relatively high doses of relatively little concern to general radiological protection. Of much greater concern are those studies which demonstrate that a combination of low dose and low dose-rate can enhance the carcinogenic or transforming effect of a low dose of high LET radiation. This has been observed in epidemiological studies on humans (Darby, 1989), in vivo experiments (Ullrich, 1986), and in cell culture experiments. In particular, experiments on the C3H 10T½ mouse cell-line demonstrated a 9-fold increase in transformation at doses below 1 Gy (Hill et al, 1982). However, attempts to reproduce these experiments failed to show any IDRE (Balcer-Kubiczek et al, 1988). Several other laboratories, while demonstrating an IDRE, have found much smaller enhancements at low dose-rate (Miller et al, 1988). This lack of consistency has made it difficult to develop a model to explain the IDRE and it is not clear whether, or to what extent, different types of radiation can elicit an IDRE, what factors govern the size of the effect, or whether it is determined by environmental modulation of transformation at a cellular level. Against this background experiments have been carried out to determine whether an IDRE exists for α-particles, as these are of great importance for general radiological protection. Also, factors affecting the expression of transformation in C3H 10T½ cultures, particularly cell density, have been examined.

Methodology

C3H 10T½ cells were maintained and propagated in Eagles Basal Medium supplemented with 10% FCS, 2 mM glutamine, 50 $\mu g\ ml^{-1}$ streptomycin and 50 $i.u.\ ml^{-1}$ penicillin (complete medium-CM). The stock cultures were incubated at 37°C in a 5% CO_2: air atmosphere. In some experiments 10 % Nu-serum (Universal Biologicals) replaced FCS (CMNS).

Plating efficiency

For plating efficiency (PE) measurements, the cells were recovered from stock cultures by trypsinisation, counted and diluted appropriately in complete medium. Various numbers of cells were seeded onto either 90 mm dia.(63.6 cm^2) and 60 mm dia.(28.3 cm^2) culture dishes or 25 cm^2 tissue culture flasks, to yield cell densities ranging from 0.16 to 16 cells cm^{-2}. The cultures were incubated for 2 weeks before being stained with methylene blue and colonies containing more than 50 cells counted by eye.

α-particle irradiations

A description of the source and experimental protocol has been given elsewhere (Roberts and Goodhead, 1987). Briefly, two days before irradiation, 10^5 cells from cultures in CM or CMNS, between passage 11 and 14, were seeded into 30 mm glass culture vessels having bases of 2.5 μm thick Hostaphan to give a non-overlapping monolayer of cells in exponential growth at the time of irradiation. For irradiation, the cells were located in a wheel which traversed the aperture of the beam tube three times a minute, the dose-rate being adjusted by a combination of a sector plate immediately beneath the wheel and low scatter apertures immediately above the ^{238}Pu disc. The apparatus provided essentially monoenergetic α-particles of 3.2 MeV. High dose-rate irradiations were done at room temperature at 0.4-1.7 Gy min^{-1}, and low dose-rate irradiations at 37°C at $4.8x10^{-3}$ Gy min^{-1}. After irradiation the dishes were returned to the laboratory and the cells recovered by trypsinisation. A glass cloning ring (26 mm i.d.) was inserted to exclude cells near the periphery of the dish.

γ-ray irradiation

γ-ray irradiations were carried out at room temperature in a ^{60}Co Hotspot Mark 4B irradiator at a dose-rate of 30 Gy min^{-1}. The source had been calibrated using LiF thermoluminescent dosimeters in the form of Teflon discs with a 6% phosphor loading (Teledyne Isotopes Type SD-LiF-7-0.4). For the γ-ray experiments, cells grown in CM or CMNS were irradiated either in suspension or as a monolayer on Hostaphan dishes prepared as described above. After trypsinisation of the monolayer cultures, the cells were diluted and seeded into 90 mm culture dishes to give about 100 surviving cells per dish. The dishes were incubated for 14 days being

refed every 4 days with CM or CMNS as appropriate. They were then stained and colonies scored as described above.

RESULTS

Survival and RBE measurement for 3.2 MeV α-particle irradiation

The dose-response relationship for survival of C3H 10T½ cells irradiated with γ-rays is shown in Fig. 1. The use of CM or CMNS did not affect the response significantly and both sets of data were pooled for curve fitting. The curve shown is the best fit obtained by the linearised least-squares method according to the equation $\ln S=-(\alpha D+\beta D^2)$, giving $\alpha=0.185$ Gy^{-1} and $\beta=0.0215$ Gy^{-2}. Survival of the 10T½ cells irradiated with α-particles was independent of dose-rate (Fig. 2); consequently the data were again pooled for extra precision in curve fitting. The α-particle dose-response relationship was exponential with no evidence of a shoulder within the range of survival down to 1%. The survival curve shown is the best fit obtained from the equation $\ln S=-\alpha D$, giving the value of α as 1.65 Gy^{-1}. The dotted line in Fig. 2 represents the γ-ray survival curve. Comparison of the two radiation qualities gave a RBE of 6.2 for the α-particles at 37% survival.

Effect of dose-rate on transformation by ^{238}Pu α-particles

Preliminary experiments using a combination of high dose and high dose-rate (2.65 Gy, 0.4 Gy min^{-1}) gave a transformation frequency of 4.5×10^{-2} transformants per surviving cell, a value in good agreement with that obtained by Lloyd *et al*. (1979) for 10T½ cells irradiated with 5.6 MeV α-particles from an accelerator. Subsequent experiments compared the yield of mutants following a single dose of 0.34 Gy delivered at 1.0 or 0.05 Gy min. For these experiments, the cells were grown in Nu-serum which resulted in much higher transformation yields in both control and irradiated cultures. The results showed that, under the conditions of the experiment, the transformation frequency was independent of dose-rate, within the statistical uncertainties of the method (Table 1) although the absolute frequency was clearly very dependent on the plating density.

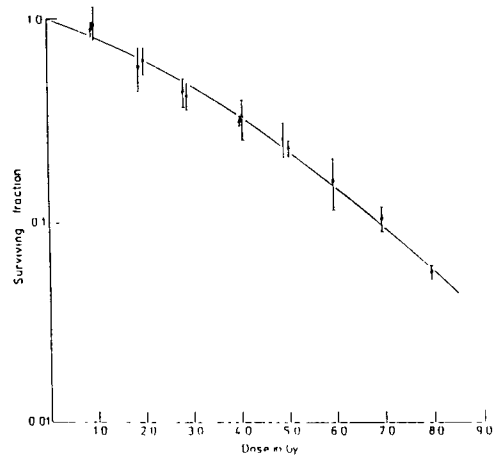

FIG. 1: SURVIVAL OF 10T½ CELLS EXPOSED TO -RAYS.

●, CULTURES GROWN IN NU-SERUM .
○, CULTURES GROWN IN FCS.

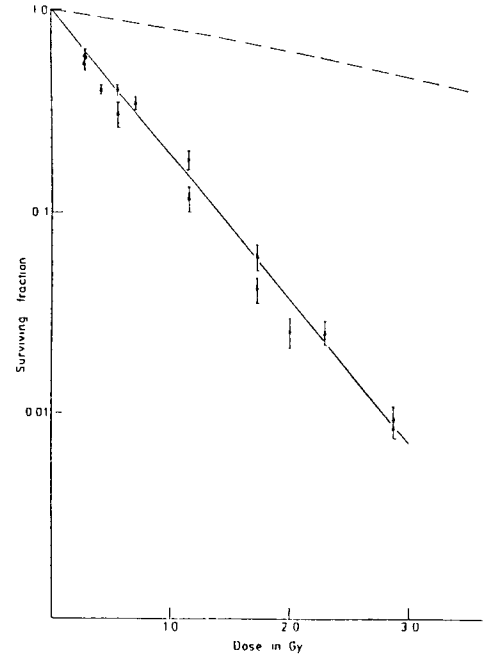

FIG. 2: SURVIVAL OF 10T½ CELLS EXPOSED TO 3.2 MEV 238PU α-PARTICLES.
●,▲ HIGH DOSE-RATE
△ LOW DOSE-RATE

Dose Rate Gy/min	Dose Gy	Surv. Frac.	Surv. cells/ Dish	Dishes	Foci	Dishes without Foci	Av.No. Foci/ Dish	Trans/ Surv. Cell
control	0.0	1.0	58.03	20	44	3	2.23	0.0379
0.004	0.29	0.56	32.38	49	112	6	2.29	0.0840
1.1	0.29	0.51	29.7	50	136	3	2.72	0.077
control	0.0	1.0	542.0	31	18	19	0.58	0.0011
0.004	0.29	0.36	386.99	50	89	9	1.78	0.0046
1.1	0.29	0.40	432.5	40	71	12	1.78	0.0041
control	0.0	1.0	194.4	50	55	21	1.1	0.0057
0.004	0.29	0.60	116.0	50	114	5	2.28	0.0197

Table 1. Transformation of 10T½ cells by ^{238}Pu-α-particles

Factors governing the response of 10T½ cells to radiation - the effect of cell density. The effect of cell density on the plating efficiency (PE) of 10T½ cells was examined by plating a series of dilutions into vessels of different growth surface area. 10T½ cells from two different sources were used, designated lines 1 and 2. Both cell lines showed similar results (Fig. 3).

The PE of low numbers of 10T½ cells was influenced by the choice of culture vessel. 25 cm^2 flasks proved superior to either of the culture dishes, while the smaller-sized dish offered a slight, but consistent, improvement over the larger. However, cell density profoundly influenced the PE. Increasing cell density depressed the PE on all three types of culture vessel. This effect was particularly striking on the 25 cm^2 flask where a four-fold increase in density from 4 to 16 cells cm^{-2} reduced the PE from >80% to <30%. The effect was present, but less marked, with the culture dishes. Taking the 90 mm dishes as an example, a 100-fold increase in cell density, from 0.16 to 16 cells cm^{-2}, was needed to reduce the PE by approximately 50%. In all three vessels the depression in PE appeared to be proportional to the logarithm of the cell density. On the culture dishes there was a suggestion of a greater effect at cell densities >10 cells cm^{-2}.

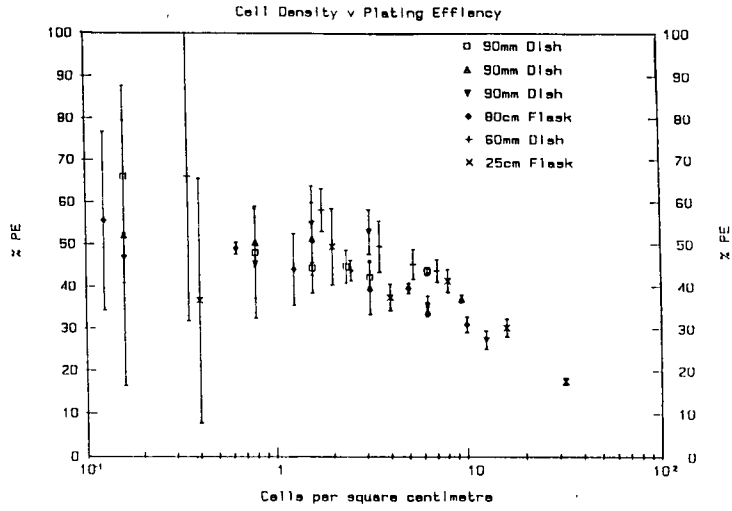

FIG. 3: RELATIONSHIP BETWEEN CELL DENSITY AND PLATING EFFICIENCY FOR 10T½ CELLS ON VARIOUS SIZES OF VESSEL.

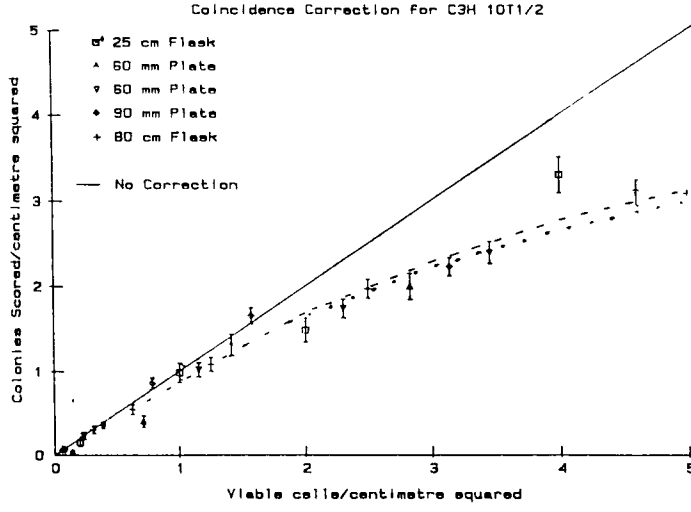

FIG. 4: THE EFFECT OF CORRECTING FOR COINCIDENCE ON THE RELATIONSHIP BETWEEN CELL DENSITY AND PLATING EFFICIENCY SOLID LINE SHOWS 1:1 RELATIONSHIP EXPECTED, BROKEN LINES INDICATE CORRECTION FOR COINCIDENCE DISTANCE OF (----) 2.4MM AND 2.5MM (---).

Effect of cell density on transformation frequency

Transformation frequencies were recorded under three different conditions; using a 10T½ strain with a high spontaneous transformation rate: a) with cells, physically separated from each other so that no cell-cell interactions could occur.

b) With an equal number of cells seeded onto 90 mm culture dishes so that the cell concentration was very low but interaction between cells was possible.

c) Under the standard conditions for a transformation experiment.

Colonies from single cells in the wells of a microtitre dish could be recognised as transformed by the same morphological criteria as applied to a conventional 10T½ transformation assay. The transformation frequencies obtained under each set of culture conditions are recorded in Table 2. The spontaneous transformation frequencies were similar under conditions (a) and (b). In both cases the level of spontaneous transformation was significantly greater than on dishes prepared according to the standard protocol (c).

Table 2 Plating efficiency and transformation in different vessels

Culture vessel	Numbers of cells seeded	PE (%)	Transformation frequency (%)
Microtitre plate	96	27	35
90 mm dish	96	51	26
90 mm dish	600	21	7.3

DISCUSSION

The D_0 value of 0.61 Gy for 3.2 MeV α-particles from a ^{238}Pu source obtained in these experiments is in good agreement with values reported for 10T½ cells by Bettega *et al.* for 4.3 MeV α-particles from a ^{244}Cm source (D_0 0.61 Gy, Bettega, pers. comm.) and by Hieber *et al.* for α-particles from a ^{241}Am source (D_0 0.61 Gy, Hieber *et al*, 1987). This suggests that the radiosensitivity of the cells is unaffected by the use of a serum substitute such as Nu-serum, an argument supported by the results from the γ-ray irradiations where the use of Nu-serum did not significantly change the dose-response relationship. However, the use of

Nu-serum clearly changed the transformation frequency of the cells. We suspect this may have been caused by high concentrations of EGF, which is known to enhance transformation frequencies to the same extent as TPA in the CREF cell line (Fisher et al, 1981). The increase in background levels of transformation may preclude the use of Nu-serum where absolute sensitivity is required, for example, when measuring the effect of very low doses of radiation. In these experiments such a change does not materially affect the conclusion (Hill et al, 1987), that transformation by α-particles is independent of dose-rate. The possibility than an IDRE may exist under other experimental conditions cannot be excluded at present. The conditions used in these experiments were based on those found to give an IDRE for fission spectrum neutrons. The conditions that give an IDRE for α-particles may well be different, although other laboratories have found the same result at other doses and other dose-rates, (Hieber et al, 1987, Bettega et al, in press). However, very recent epidemiological data on uranium miners suggests an inverse correlation between the concentration of radon/radon daughters in the mine and the incidence of lung tumours in the work force (Darby, 1989). Consequently it may be possible to assess whether the in vitro conditions used in these experiments were likely to elicit an IDRE.

One of the difficulties in quantitating transformation of 10T½ cells lies in determining the actual transformation frequency per surviving cell. Generally, far fewer cells are plated to calculate the surviving fraction than the transformation frequency. However, investigation of the relationship between cell density and plating efficiency (PE) shows that it is misleading simply to assume that the plating efficiency is the same in both cases. Increasing the cell density above 1.5 cells cm^{-2} leads to an apparent suppression of plating efficiency. This represents approximately 100 cells on a 90 mm culture dish, probably more than would be plated normally to estimate survival but fewer than would be plated to assess transformation. This suppression of PE appears to be due to coincidence of colonies. As the plating density is increased, cells are more likely to lie close to one another. When the distance between two cells is sufficiently small, their colonies will coincide and appear as one. A computer programme based on Monte-Carlo techniques was written to establish whether the observed data fitted this model and, if so, to

calculate the maximum coincidence distance. A full description of the programme will be published elsewhere. The model fitted the observed plating efficiencies very well (Fig.4) giving a maximum coincidence distance of 2.5 mm.

It is also well known that transformation frequency can depend on cell density. The exact reason for this is not clear but is thought to reflect the size of the potentially transformed population at confluency and/or whether a potentially transformed cell has gone through sufficient divisions for the transformation to be expressed. These parameters in turn reflect the density of the initial inoculum. Clearly, when single cells were isolated in the wells of a microtitre dish the transformation frequency was much higher than when cells from the same population were seeded at high density in a culture dish. This did not reflect actual physical separation of the cells as the same result was obtained when the cells were seeded into the culture dish at low density, hence some kind of diffusible factor is probably not required for suppression. Similar high transformation frequencies at low cell densities have been reported previously (Kennedy et al, 1980; Bettega et al, in press). It is not clear at present whether the simple coincidence model involving contact between transformed and untransformed cells could explain this suppression of transformation. Preliminary work has shown an approximate fit to transformation data (kindly provided by Dr Bettega of Milan University) with a coincidence distance of 2.2 mm (data not shown).

In conclusion, the transformation frequency by 3.2 MeV α-particles was independent of dose-rate, suggesting that the IDRE may not be a general feature of high LET radiation. A computer model has been developed, based on the idea of coincidence, which successfully accounts for the effect of cell density on plating efficiency. This model is currently being applied to understanding the effect of cell density on transformation.

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IV Other research group(s) collaborating actively on this project:

Dr D Goodhead, MRC Radiobiology Unit, Chilton, Oxfordshire

Dr A J Mill, Radbiology Laboratory, Berkeley Nuclear Laboratories, Berkeley, Glocs.

V Publications:

Roberts, C.J., Goodhead, D.T., Morgan, G.R. and Holt, P.D. (1987) in Low Dose Radiation Fielden, E.M. et al. (Eds), Taylor and Francis, Low Dose Radiation, p. 182. Proc. 8th Int. Cong. Rad. Res. Edinburgh 1987.

Roberts, C.J. and Goodhead D.T. Int. J. Rad. Biol. 52, 871-882, 1987.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor.

Contract no.: BI6-E-151-F

Institut Curie
Section de Biologie
Rue d'Ulm, 26
F-75321 Paris Cédex 05

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

Dr. E. Moustacchi
Section de Biologie
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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 in Fanconi's anemia

Head(s) of project: Dr. Ethel MOUSTACCHI

Scientific staff:

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D. Papadopoulo
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I. Objectives of the project:

Fanconi's anemia (FA) belongs with xeroderma pigmentosum (XP) and ataxia telangiectasia (AT) to a class of autosomal recessive diseases associated with cancer-proneness, genetic instability and hypersensitivity to more or less specific genotoxic agents. Cells from FA patients have increased frequencies of chromosomal aberrations, an hypersensitivity to DNA cross-linking agents and anomalies in the cell cycle. "FA like" mutants exist in rodent cells and may serve, in parallel with FA human cells, as recipients in view of correction of the defect as successfully performed recently with genes involved in excision-repair of UV-induced lesions. The basic biochemical abnormalities associated to the processing of DNA cross-links in FA being unclear, our project aimed at the correction of the defect in view of the identification of the relevant genes for the characterization of their expression.

II Objectives for the reporting period:

- Characterization of mouse DNA sequence which corrects defects of the FA phenotype

- Dissociation of functions in corrected FA.

III. Progress achieved:

The repair of the majority of DNA lesions requires the sequential and coordinated operation of genetically controlled enzymatic steps. Our interest in the cellular processing of DNA interstrand cross-links and monoadducts stems from a number of observations including their induction by a variety of agents present in the human environment. Indeed, several antitumoral drugs (mitomycin C, cis-platinum, nitrogen mustards), environmental pollutants (alkylating agents) and products such as furocoumarins used in photochemotherapy of certain skin diseases and in cosmetology, have been shown to produce interstrand cross-links (CL) in DNA. In all cases, described so far, the production of CL is accompanied by the induction of monoadducts (MA) in various proportions according to the agent used and the treatment conditions. Also, in some instances as for ionizing radiations, oxidative type DNA damage is concomitantly induced by cross-linking agents (mitomycin C or 3-carbethoxypsoralen, for instance). In bacteria and yeast, it has been demonstrated that the excision-repair and the recombinational repair systems are both required for the processing of CL and MA. The fate of these lesions in human cells still raises questions. Taken together with the presence of cross-linking agents in a variety of practical situations, this provides compelling reasons for attempting to understand their genotoxic potentials in human cells. In this respect, furocoumarins are of particular interest. These tricyclic aromatic compounds contain two reactive sites, the 3-4 pyrone and the 4'-5' furan double bonds. They intercalate into DNA and form covalent adducts only with pyrimidine bases after exposure to near ultraviolet light (UVA) ; when activated by UVA either of the reactive sites of the furocoumarins can react specifically with the 5,6-double bond of pyrimidine bases forming cyclobutane-type monoadducts on the furan or pyrone-side (MA_f and MA_p) between a psoralen molecule and a pyrimidine base. Upon absorption of a second photon of 365 nm, a fraction of the MA_f can react with a pyrimidine base on the opposite strand by engaging its 3,4-pyrone double bond to form an interstrand CL (for review, see Cimino et al., 1985). Psoralens photoinduced MA and CL are stable to biochemical treatments and are not chemically lost from the DNA in vivo. Their induction can be quantitatively controlled by the UVA fluence, and they do not require metabolic activation to react with DNA. The ratio of CL to MA, at a constant total number of adducts, can be controlled by a double irradiation protocol after elimination of unbound psoralen molecules. Moreover, in certain irradiation conditions (405 nm) only MA are formed by otherwise bifunctional psoralens. Since these advantageous features are not shared by many other agents, most of our studies on MA and CL processing by normal and defective human cells deal with psoralen photoaddition.

Mutants characterized by a more or less specific hypersensitivity to cross-linking agents have been isolated from yeast (Henriques and Moustacchi, 1980 ; Cassier and Moustacchi, 1981), hamster (Robson and Hickson, 1986 ; Zdzienicka and Simons, 1987) and mouse cells (Hama-Inaba et al., 1983, 1988). This hypersensitivity is shared by cells derived from patients affected by Fanconi's anemia (FA), an inherited autosomal recessive disorder in humans. Attempts to correct this defect by normal DNA sequences or by cocultivation with normal cells, the molecular cloning of the sequences involved as well as complementary studied on the fate of lesions in normal and FA cell lines are reported here.

MATERIAL AND METHODS

FA cell lines belonging to the genetic complementation groups A and B (Duckworth-Rysiecki et al., 1985) were used. Their phenotype in terms of rates of DNA semi-conservative synthesis following a cross-linking treatment differ (Moustacchi and Diatloff-Zito, 1985 ; Moustacchi et al., 1987) and was systematically defined. Both skin fibroblasts and lymphoblasts, derived in some case from the same FA patient have been used in parallel with cell lines from normal donors. Treatments of cells with DNA cross-linking agents (psoralen plus UVA, mitomycin C) or with radiations were performed as previously described. Transfection of high molecular weight human or mouse DNA in FA cells was done either by the Ca phosphate precipitation method for fibroblasts or by electroporation for lymphoblasts as detailled elsewhere (Diatloff-Zito et al., 1986 ; Diatloff-Zito et al., 1990, submitted). The selection of transfectants and characterization of correcting sequences are also described.

The fate of cross-links and monoadducts was followed either by alkaline elution (Papadopoulo et al., 1987 ; Averbeck et al., 1988) or by the electron microscopy (Rousset et al., 1990).

RESULTS AND DISCUSSION

A) Correction of the FA defect and toward the cloning of the correcting DNA sequences. Correction of the abnormal response to DNA cross-linking agents of FA fibroblasts by transfection with high molecular weight normal human DNA has been achieved (Diatloff-Zito et al., 1986). Since in our conditions exogenous correcting DNA could not be distinguished from the host DNA, the correction of sensitivity to mitomycin C (MMC) of FA cells with DNA from mouse cells has been attempted. A similar strategy using interspecies complementation by DNA transfection has been effective for identification and isolation of several human genes concerned with excision repair (Westerveld et al., 1984 ; Rubin et al., 1983).

1) The protocol which allowed correction of FA fibroblasts by transfection

with normal human DNA was re-adopted when using mouse DNA. We show that the degree of correction of MMC sensitivity of FA group B cells (FA 145) with DNA from mouse lymphoma L5178Y cells is close to 90%. The relative D_{37} (lethal dose leaving 37% survivors) of transfected FA 145 cells compared to untransfected FA 145 equals 2.75, whereas the relative D_{37} of FA 145 cells over that of normal 1 BR/3 cells equals 3.9. DNA from transfected cells was hybridized with ^{32}P labelled C_0t1 mouse probe, which detects only highly repetitive interspersed mouse sequences, and it was shown that FA 145 human transfectants contain a noticeable amount of foreign mouse DNA sequences. This is true even two months after transfection.

Secondary transfectants were also generated by transfecting FA 145 cells with high molecular weight DNA extracted from primary FA 145 transfectants. Survival curves to MMC were established, and it was shown that FA 145 secondary transfectants were only 1.5 times more resistant than the untransfected FA 145 cell line. The hybridization signal of the secondary transfectants DNA with the C_0t1 mouse probe is below the level of detection by Southern blot analysis. Secondary transformants obtained by transfer of human DNA from primary transformants into the X-ray sensitive Chinese hamster ovary cell line EM9 also displayed intermediate resistance to EMS and X-rays and weak hybridization to human Alu sequences (Spiro et al., 1986). Such partial resistance may be due to the fact that expression of more than one gene may be required, following transfection, for acquisition of full resistance. Alternatively, it is possible that the gene responsible for CL and MA processing was damaged during transfection and selection and that this gene is now only partially active. High variability (increase in mutation rates and in rearrangements of DNA fragments) has indeed been reported for transfected DNA (Calos et al., 1983).

The presence of mouse DNA in FA primary transfectants being demonstrated, a genomic library in phage lambda was constructed and screened for the presence of mouse DNA as well as for correction of FA cells. Three of such recombinant phages restaured resistance to DNA cross-linking agents of FA 145 cells following transfection and their restriction map has been established. The minimal sized correcting sequence is now being analysed. Transfection of other FA group B and A cell lines as well as of "FA like" mouse and rodent mutants has been performed in order to define the degree of specificity of the correction.

2) Mouse DNA from L5178Y cells was found to be ineffective in correcting MMC sensitivity of FA cell lines belonging to complementation group A (FA 150 and E.K.) in spite of several trials. However, when mouse DNA extracted from total

mouse embryo was used for transfection of FA group A cells, a partial correction of the MMC sensitivity was observed. It is of interest to note that, in comparison to FA group B cells, FA cells belonging to group A are : a) more sensitive in terms of clonogenic cell survival, b) appear to be more affected with respect to removal of CL (Papadopoulo et al., 1987), and c) demonstrate a lack of recovery of a normal rate of DNA semi-conservative synthesis (Moustacchi et al., 1987) following a DNA cross-linking treatment. It seems that in FA group A cells, in which the defect is more pronounced, it is more difficult to supply, by transfection with mouse DNA, the proper sequences suitably expressed. Moreover, DNA from adult and embryonic mouse cells are not in the same state of competence for expression of gene(s) necessary for correction of FA group A cells.

Correction of FA fibroblasts hypersensitivity to the effects of diepoxybutane, another DNA cross-linking agents, was achieved by transfection of Chinese hamster lung cell DNA (Shaham et al., 1987). Taken together with our results, these findings indicate that DNA sequences correcting the FA defect are present not only in human but also in rodent DNA.

3) Mitomycin C-sensitive mutants derived from mouse lymphoma L5178Y cells (Hama-Inaba et al., 1983) share with FA several features : a) they are highly sensitive to the lethal effect of a number of DNA cross-linking agents whereas they are clearly less sensitive to their monofunctional counterparts (Hama-Inaba et al., 1988), b) the mouse mutants have been classified by somatic hybridization into two genetic complementation groups I and II (Hama-Inaba et al., 1983) which, as in FA, demonstrate either a normal capacity of recovery of the rate of DNA semi-conservative synthesis after a cross-linking treatment (group II of mouse mutants equivalent to group B in FA) or an absence of such a recovery (group I of mouse mutant equivalent to group A in FA) (Moustacchi et al., 1989), c) cytogenetic analysis demonstrates high rates of chromosomal aberrations in the mouse mutants compared to the original L5178Y cell line which is clearly enhanced by treatment with MMC (Rosselli and Moustacchi, 1989). This is comparable to the well established spontaneous and induced chromosomal instability which is one of the major characteristics of FA. Such mouse mutants serve now as recipients for transfection with human DNA with a selectable marker. Optimum conditions for transfection of these cells which grow in suspension were established. Assays by classical transfection by the Ca phosphate procedure having failed, a range of electroporation conditions were tried using several parameters : cellular viability and uptake of a fluorescent dye, challenge for MMC resistance of surviving cells and expression of the $\underline{neo}^R$ gene held by the pCD vector containing the cDNA inserts of

a human DNA library (Chen and Okayama, 1987). A transfection frequency in the order of 5×10^{-5} for the neo gene was found using the optimum conditions for the mouse mutants (30 nF at 5 kV and 3 pulses with 8 seconds between pulses using an "Apelex" electropulsing unit).

The same experiments were carried out on FA lymphoblastoid cell lines and optimum conditions differ from those found for the mouse lymphoblastoid cells. The cDNA library derived from normal mouse cells could also be used in the near feature since the subsequent rescue would be similar to that for the human library.

The strategies adopted are complementary of each other and progress toward the molecular cloning of sequences correcting the hypersensitivity of FA cells to DNA cross-linking agents has been achieved.

B) Complementation of chromosomal hypersensitivity to DNA cross-linking agents by cocultivation of FA and mouse lymphoma mutants. To determine whether the mutations in FA and mouse mutant cells are the same, complementation studies using somatic hybridization have been attempted. Difficulties have been encountered in this approach and it is why complementation has been performed by cocultivating human lymphoblastoïd cells with mouse cells. The frequencies of MMC or of psoralen plus UVA induced chromosomal aberrations were taken as parameters of complementation. FA cells from the complementation groups A and B were cocultivated in suspension with normal mouse lymphoma L5178Y cells or with the derived "FA like" mutant cells MCN-151 and MCE-50 assigned to complementation groups I and II, respectively.

Table 1 : Complementation of the chromosomal hypersensitivity of FA and of mouse mutant after treatment with MMC or with 8-methoxypsoralen plus UVA (from Rosselli and Moustacchi, 1990)

cocultivation of			chromosomal effect on	
human		mouse	human	mouse
FA "A"	&	normal	+	u
FA "A"	&	mutant "I"	-	-
FA "A"	&	mutant "II"	+	-
FA "B"	&	normal	-	u
FA "B"	&	mutant "I"	-	+
FA "B"	&	mutant "II"	-	-
normal	&	mutant "I"	u	+
normal	&	mutant "II"	u	-

+ complementation as expressed by a reduction in the frequency of induced chromosomal aberrations, is observed. - complementation is not observed. u unchanged frequency of chromosomal aberrations.

The results summarized in table 1 show a partial complementation of the defect (i.e. a reduction in the frequency of chromosomal aberration) in FA (A) cells by coculture with normal or group II mouse cells, and also a partial correction of

mouse group I cells cocultived with normal or FA (B) human cells. No reciprocal effects were observed between FA (A) and mouse group I mutant cells ; the frequencies of MMC-induced chromosomal aberrations in these cells remained unchanged by cocultivation. Also, for both FA (B) and mouse group II cells, no complementation was observed after cocultivation with normal cells from either mouse or human origin. This implies that a diffusible factor released by normal human and mouse cells, as cell as by FA (B) and mouse group II mutant cells, can correct at least in part the chromosomal defect of FA (A) and mouse group I mutant cells. With normal cells, human or mouse, the frequency of chromosomal breakage after cocultivation remains the same as observed in non-cocultived cells. In other words, no detectable clastogenic factor is released by human FA or "FA like" mouse cells. The nature of the FA correcting diffusible factor is now investigated.

C) Further characteristics of the FA defect

1) Repair capacity, interaction between lesions. a) The incision of CL induced after 8-methoxypsoralen (8-MOP) plus 365 nm radiation is present in FA cells belonging to the two genetic complementation groups A and B. However, the process is slower and the final amount of CL incised is lower in FA cells than in normal cells (Papadopoulo et al., 1987 ; Rousset et al., 1990). Using a 405 nm-365 nm reirradiation protocol with trimethylpsoralen (TMP), we demonstrate that, for a constant number of total adducts (20 or 440 per 10^8 bp) and different ratios of CL over MA, the incision of CL is systematically hampered in FA compared to normal cells (Averbeck et al., 1988). In other words, in normal cells, the incision of CL is progressivelyy diminished by increasing amounts of MA_f. We show that this inhibition is even more pronounced in FA cells.

It can be asked, why, despite the fact that the total number of 365 nm photoinduced TMP adducts per 10^8 base pairs is low, the repair of CL can be inhibited by the presence of MA and even more specifically by the furan-side MA. The first explanation may be that MA_f trap the incision-excision repair complex which may then not be available to efficiently incise CL. In contradiction with this hypothesis is the fact that when the number of total adducts is enhanced by a factor of 100, the efficiency of incision changes very little (Papadopoulo et al., 1987). Alternatively, the stabilization of the helical structure of DNA due to MA_f (Shi and Hearst, 1986) may lead to conformational alterations which in turn change the way in which repair enzymes have access to DNA CL. Such stabilized regions may be located at the specific sequences defined as "strong sites" which are the preferential targets for psoralen derivatives (Boyer et al., 1988). For 254 nm, UV-induced pyrimidine dimers, cluster formation has been observed (Lam and Reynolds, 1987).

Similarly, the production of MA_f at given DNA sites is likely to occur in clusters which in turn may favor the induction of a CL at the closest vicinity compatible with a favorable sequence. These possibilities are investigated using DNA sequencing methodology combined with specific enzymatic digestion.

b) The sensitivity of normal and FA cells to MA and repair of cross-linkable furan-side MA. It is accepted that FA cells are more or less specifically hypersensitive to DNA cross-linking agents. However, as mentioned above, MA are always induced concomitantly to CL. In order to precisely define the contribution of MA and CL to the particular sensitivity of FA cells, we determined the lethal effect of different types of adducts formed, using the same bifunctional furocoumarin, TMP, in combination with either 405 nm or 365 nm radiations. We have also investigated the extent to which the sensitivity of FA cells is related to their capacity to repair CL and cross-linkable MA_f.

- As mentionned above, in the range of doses used in biological experiments, TMP in combination with 405 nm radiation induces only MA. We show that FA cell lines from both complementation groups are more sensitive than normal cells to TMP photoinduced MA. FA (B) cells are about 5 fold more sensitive whereas (A) cells are only about 3 fold more sensitive than normal human cells. It should be recalled that after treatments inducing a mixture of CL and MA, FA (A) cells are more sensitive than FA (B) cells (Papadopoulo et al., 1987 ; Moustacchi et al., 1989). FA cells are only marginally susceptible to cytotoxicity due to DNA damage induced by UV (Klocker et al., 1985), ionizing radiation (Dritschillo et al., 1984 ; Duckworth-Rysiecki and Taylor, 1985) and monofunctional compounds such as decarbamoyl-mitomycin C, methylmethane sulfonate or 4-nitroquinoline oxide (Weksberg et al., 1979 ; Sasaki, 1978). The hypersensitivity of FA cells to N-methyl-N'-nitro-N-nitro-soguanidine (MNNG) (Ishida and Buchwald, 1982) and to TMP photoinduced MA appear to be exceptions to this general rule.

- It is established that only MA_f are susceptible to conversion into CL by additional exposure to 365 nm light. After incision, MA_f lose their convertibility. This property allows us to follow, as a function of time of incubation after treatment with 405 nm radiation, the proportion of MA_f which are incised ; at different time intervals after the first dose, cells are treated with a second dose of 365 nm radiation. Subsequent alkaline elution measurement of the number of CL's allows us to calculate the incision rate of the initial MA's. 24 hours after treatment, FA cells incise approximately 4 times less efficiently than normal cells cross-linkable MA_f (8% against 40% for normal cells). This is likely to be related to the higher sensitivity of FA cells to TMP plus 405 nm light-induced MA.

It can be asked why, in contrast to other monofunctional agents, FA cells turn out to be hypersensitive to TMP photoinduced MA. The excision repair pathway is assumed to operate on bulky DNA adducts that cause major helical modifications. The bacterial uvr ABC excinuclease participates in the repair of DNA lesions induced by MNNG (Van Houten and Sancar, 1987). In other words, beside the lesions repaired by the methyl transferase and glycosylase, MNNG produces lesions which are recognized and processed by the excision complex. The MA induced by TMP plus 405 nm light are likely to produce perturbations in the stability of the double helix. It can be proposed that minor perturbations produced by monofunctional agents and radiations are processed in FA cells as in normal ones. It would be only for high levels of helical perturbations that the incision complex in FA cells would manifest a defect. In other words, with respect to the relative sensitivity of FA cells to genotoxic agents, it is not the mono- or bifunctional nature of the DNA adducts which matters but the degree of helical modification induced.

2) Mutagenic response in FA cells. It is accepted that unrepaired damage can lead to mutation in the surviving cells. For instance, it has been demonstrated that the deficiency in excision of UV-induced lesions in XP is associated with an increase in the frequencies of induced-mutations (Maher et al., 1976). Similarly, it can be asked if the unprocessed lesions in FA cells lead to an increased number of mutants at specific loci. With respect to this question, it has been demonstrated that FA fibroblasts show no increase in mutation frequency at the HGPRT and Na^+/K^+ATPase loci when exposed to EMS or MMC (Buchwald, pers. commun.). Also hypomutability of FA cells to benzo(a)pyrenediolepoxide is mentionned in a report (Watanabe cited in Tabeke and Tatsumi, 1986). In contrast, at high survival level only, the frequency of mutation at the HGPRT locus was found to be higher in a FA lymphoblastic cell line (HSC 99) in comparison to that of normal lymphoblasts following treatment with diepoxybutane (Takebe et al., 1987).

In view of these conflicting results, we examined the frequency of mutations induced at two genetic loci (HGPRT and Na^+/K^+ATPase) in FA lymphoblastoïd cells from the genetic complementation groups A and B in comparison to that of normal cells. The agents used were the bifunctional psoralens 8-MOP or TMP associated with irradiation at 365 nm (UVA). It is shown that, for the two loci, the frequency of induced mutations is lower in FA cells compared to normal. This is true when the mutation frequencies are expressed as a function either of dose or of survival level (Papadopoulo et al., 1990).

In summary, in FA cells, the efficiency of CL incision and that of furan-side MA were found to be reduced in comparison to normal cells. In contrast to XP, such

poorly eliminated lesions do not appear to be chanelled via an error-prone mechanism, the FA cells demonstrating an hypomutability. In other words, the data suggest that FA cells are deficient in such an error-prone pathway and this could be associated to the high frequencies of induced chromosomal aberrations observed in FA cells. Indeed unrepaired or badly repaired lesions maintained after S phase, would trigger the production of chromosomal anomalies and may lead to the elongation of the G2 phase of the cycle, two characteristics of the FA phenotype. The notion of "error-prone pathway" could cover either a specific mechanism such as the translesion synthesis as reported in bacteria or specific alterations in the structure of the chromatine as in the rad6 mutation in yeast which is characterized by hypomutability.

Similarly to FA, ataxia telangiectasia (AT) is a genetic recessive disease associated with a predisposition to cancer and a marked elevation in levels of spontaneous and induced chromosome aberrations compared to normal cells. Cells derived from AT patients are hypersensitive to the cytotoxic effect of ionizing radiations and certain chemotherapeutic agents. As in FA cells, hypomutability has been reported for AT, in that case following treatment with X-rays (Arlett, 1980). Moreover, FA and AT cells not only share the property of being hypomutable but they also demonstrate aberrant enhanced viral reactivation when infected with psoralen-damaged Herpes virus as in the case of FA (Coppey et al., 1989) or with X or UV-damaged adenovirus as in the case of AT (Bennett and Rainbow, 1988). Since cellular oncogenes might be activated either by classical point mutation and/or by chromosomal rearrangements, it is proposed that the development of neoplasm in XP is predominantly related to the former process whereas in FA and AT it is associated to the later mechanism.

CONCLUSION

FA cells are hypersensitive to DNA cross-linking agents, including psoralen derivatives in combination with UVA. Although incision of CL following post-treatment incubation takes place in FA cells, the processing of these lesions is hampered compared to normal cells ; the incision kinetics is slower and the final amounts of CL incised are lower in FA relative to normal cells. FA complementation group A cells are more affected than group B cells both in terms of cell survival and in terms of CL repair. To our surprise in the absence of CL, FA cells showed a higher sensitivity than normal cells to the cytotoxicity of MA induced by TMP and 405 nm, in this case, group B cells being more sensitive than group A cells. Both cell lines have in fact a reduced incision capacity of MA relative to normal cells, this is especially true for MA_f. Thus, FA cells are altered in the

processing of both CL and MA of a specific structural type. In the presence of high amounts of MA_f, the incision of CL is blocked and it is only when the proportion of MA_f declines that incision of CL can take place. This phenomenon is even more pronounced in FA cells. Such alteration in processing of DNA lesions is associated to hypomutability which suggests a defect in an error-prone pathway. By attempting to clone the normal sequences correcting the FA sensitivity to cross-linking agents and by characterizing the correcting diffusible factor identified by cocultivation, we hope to unravel the nature of this pathway.

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V. Publications: 1989

(from 1986 to 1988, please see our annual reports)

1) In journals

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DNA ligase activity in human cell lines from normal donors and Bloom's syndrome patients.
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F. ROSSELLI and E. MOUSTACCHI.
Human Genet. (in press).

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D. PAPADOPOULO, B. PORFIRIO and E. MOUSTACCHI.
Cancer Res. (in press).

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S. ROUSSET, S. NOCENTINI, B. REVET and E. MOUSTACCHI.
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2. In books

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E. MOUSTACCHI, D. AVERBECK, C. DIATLOFF-ZITO and D. PAPADOPOULO.
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DNA ligase activity in human cells from normal donors and from patients with Bloom's syndrome and Fanconi's anemia.
M. MEZZINA, S. NOCENTINI, J. NARDELLI, I. SCOVASSI, U. BERTAZZONI and A. SARASIN.
In : "DNA repair mechanisms and their biological implications in mammalian cells". NATO Advanced Research Workshop, Fontevraud (France) October 2-7, 1988. Eds. : M. W. Lambert and J. Laval (1989) (in press).

Title of the project no.: **2 :** Repair functions in FA

Head(s) of project: **J. COPPEY**

Scientific staff
J. COPPEY
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M. SALA-TREPAT

I. Objectives of the project: The hypersensitivity of Fanconi's anemia (FA) cells to DNA cross-linking agents is likely be due to a defect in the processing of cross-links. Data on the recombinational repair pathway of cross-links in FA compared to normal fibroblast cell lines can be obtained by analysing the process of multiplicity reactivation (MR) of trimethylpsoralen photo-damaged Herpes virus and the fidelity of the MR. On the other hand, the use of in vitro systems allowing to monitor the steps of homologous recombination or of recombinational repair of double-strand breaks promoted by human nuclear proteins may allow to reveal possible alterations of these processes in normal and FA cells. The biological importance of the double-strand breaks induced by ionizing radiation is demonstrated for lower eukaryotes. Their role remains to be clearly established for human cells. The system developped by us aims at such a better understanding.

II. Objectives for the reporting period:

Manipulation of an in vitro system of phage DNA substrates to analyse :

1) the molecular characteristics (fidelity and DNase protection) of homologous recombination promoted by homologous recombination catalysed by human nuclear proteins
2) the recombinational repair of DNA double-strand break catalysed by human nuclear proteins.

A special emphasis was placed on :

- the constraints of homology
- the structural constraints
- the topological constraints

III. Progress achieved:

METHODOLOGY

We used the substrates depicted in Fig. 1-A :

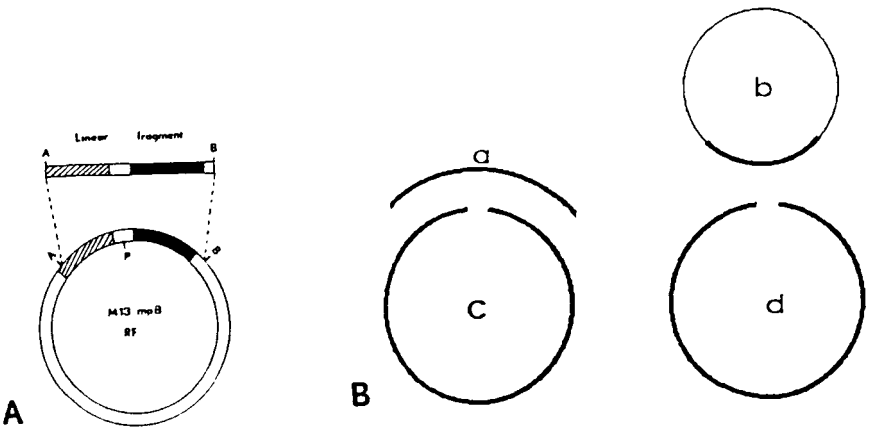

Fig. 1 - A : Recombination substrates used : one is the replicative form (RF) of M13 mp8. The other (AB) is a 1 kb restriction fragment isolated from M13 mp8 RF. Black box = lac Z gene ; hatched box = lac i' sequence ; P = primer annealing site
Fig. 1 - B : Substrates used for recombinational repair of a defined double strand break (dsb) : a = the 1 kb restriction fragment ; b = the 1 kb restriction fragment incorporated in pBR 322 plasmid ; c and d = M13 mp8 RF containing a dsb in the region of homology with the fragment.

DNA was labelled by nick translation with ^{32}P-dCTP. Sequencing was performed by the dideoxy method. For the in vitro recombination assays, the substrates were incubated in equimolar amounts as indicated previously (Lopez et al., 1987). The DNase protection assays were described in Lopez and Coppey (1989a). The strategy used for studying the recombinational repair of a double-strand break (DSB) introduced in M13 mp8 RF has been described previously (Lopez and Coppey, 1987). The use of appropriate restriction enzymes allowed to introduce a DSB at distances varying from 7 up to 950 bp from an heterology of 8 or 163 bp placed either on the RF (fig. 1-A, c or d) or on the fragment (fig. 1-B, a or b).

RESULTS

I. Molecular analysis of homologous recombination

We have shown that homologous recombination between two duplex DNA's (see fig. 1-A) can be promoted by nuclear proteins from human cells of different origin. Recombination intermediates (RI) were isolated and visualized by electron microscopy. Heteroduplex molecules were formed (Lopez et al., 1987). Restriction

analysis data on RI molecules (Lopez and Coppey, 1989b) allows to demonstrate that recombination proceeds via single-strand exchange which can be reciprocal.

Sequence analysis of 20 recombined clones (400 nucleotides per clone) in lac i' (see fig. 1-A), a genetically silent sequence surrounding the recombination initiation or termination sites shows no modification compared to the parental sequence.

On an other hand, in conditions of catalysed recombination, a protection against DNase I of a one kb long DNA fragment takes place. The protection is very efficient : the system appears to be saturated with 2 units of DNase I and no additional effect is observed with a large excess (100 u) of DNase I (fig. 2-A). The protection is sequence homology dependent. Moreover its extent is maximal when the ratio of the two substrates is one, which suggests that a stochiometric interaction between the substrates is involved.

The protective effect takes place rapidly since it is maximum after one minute incubation. Time course data (fig. 2-B) show that the protective effect is transient, occuring between 1 and 30 minutes. When DNase I digestion is performed after phenol extraction of the DNA's previously incubated with nuclear proteins, there appears a lack of protection (fig. 2-C). We conclude that protection against DNase I is afforded by factors present in the protein extract.

II. Characteristics of recombinational repair of a double strand break (DSB)

For analysing, the characteristics of recombinational DSB repair in human nuclear protein extracts, we used the system of substrates depicted in fig. 1-B : digested RF + 1 kb fragment (c + a) or digested RF + 1 kb fragment inserted in a heterologous plasmid, pBR322 (d + b). The use of the substrates (c) and (a) allowed to demonstrate that the repair of a DSB in presence of intact fragment preferentially occurs by homologous recombination, via a pathway initiated at the location of the DSB. The repair involves a multienzymatic complex for promoting the successive steps of this process : i) initiation at the location of the DSB and exonucleolytic degradation ; ii) strand exchange with the intact DNA sequence ; iii) polymerisation to fill in the single-stranded regions ; iiii) resolution of the cross-junction. The structure of the DSB has a critical effect. Indeed 5'-protruding or blunt ends are recombinogenic whereas 3'-protruding ends are not.

An heterology of 8 or of 163 nucleotides was introduced at various distances from the DSB (fig. 3-A). It can be seen (fig. 3-B) that, when the heterology is located at < 15 bp from the break, there is no reactivation, whereas when it is located at 27 bp, a significant reactivation occurs. The significance of this result was confirmed by carrying out experiments using a digested RF with dephosphorylated ends at the DSB. With such a substrate, the reactivation rate is

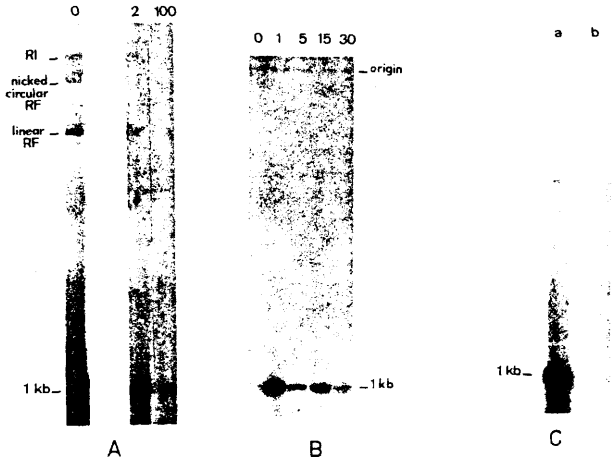

Fig. 2 : DNase I protection by the extract. A : effects of various quantities of DNase I 0, 2 or 100 units ; B : kinetics of protection. DNase I (100 units) was added at the time (min) indicated ; C : DNase I protection of the incubated (1 min) DNA's. a = normal conditions, b = after phenol extraction.

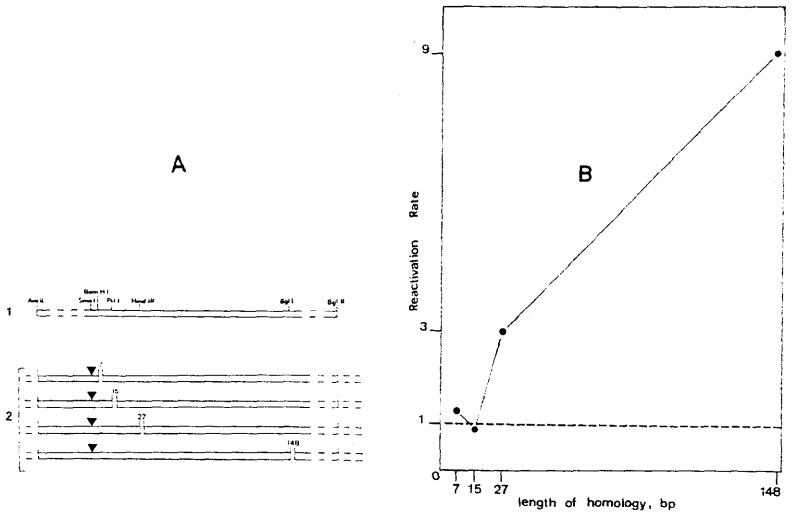

Fig. 3 - A : Scheme representing the different restriction cuts introduced in M13 mp8 RF at the indicated distances from an heterology of 8 or 163 bp (black triangles). B : Reactivation rate as a function of the length of homology adjacent to the dsb.

strongly increased because of the impossibility of ligation (Lopez and Coppey, 1987).

For 27 bp homology, the reactivation rate is close to 20. A maximum extent of reactivation is reached for a 148 bp homology (fig. 3 - B). Conversely, we have tested the configuration in which the heterology is now present in the fragment, i.e. in the donor molecule. For such a configuration, the constraints of homology described above are no more observed : whatever the length of homology adjacent to the DSB, the extent of reactivation remains the same. However, when the fragment bearing the heterology is inserted in a closed circular heterologous sequence pBR 322 (molecule b in fig. 1), it still represents the donor molecule, but constraints of homology similar to those depicted in fig. 3 - B are again seen. In addition, the introduction of a cut into the pBR 322 sequence (cut located far from the inserted fragment) abrogates the homology constraints. In other words, both configurations (a) and (b) with a cut in the heterologous sequence are equivalent with regards to the homology required for DSB recombinational repair.

III. Multiplicity reactivation and mutagenesis of trimethylpsoralen-damaged Herpes virus in normal and Fanconi's anemia cells

We used purified Herpes simplex virus (HSV) as a probe to monitor in FA cells recombinational repair of 4,5',8-trimethylpsoralen (TMP) damage created in the virus. We have chosen TMP because this psoralen has a high photoreactivity and forms a relatively high proportion of DNA interstrand cross-links. For testing recombinational repair activity in FA cells, we determined the extent of reactivation by multiple infection (= multiplicity reactivation, MR) by measuring single cycle viral yields in cells infected at high multiplicity of infection (moi) by TMP damaged HSV relatively to those infected at low moi. The survival of damaged virus at high moi relatively to that at low moi gives an estimate of repair by recombination. The experiments were carried out in skin fibroblast cells from three normal and five FA donors.

The data indicate that the survival of TMP damaged HSV is : i) similar at low moi in FA and in normal cell lines ; ii) significantly higher over a range of survival (greater than 10%) in FA than in normal cell lines. The resulting extent of MR is 2.5 to 5 times greater in FA than in normal cells (table 1).

We conclude that the process of host-cell reactivation of TMP-HSV is not impaired in FA cells and that in the range of survivals above 10%, the process of multiplicity reactivation is greater than the normal in the five FA lines tested. We next performed a mutagenesis study on Herpes virus grown in FA cells. Forward mutation in the thymidine kinase (tk) locus was chosen. Wild type HSV is tk^+. The acquired tk^- phenotype means genuine plaque formation in presence of

Table 1. Multiplicity effect on the single cycle viral yield by cells infected with psoralen-damaged[a] HSV

Cell line	Extent of multiplicity reactivation[b]
Pr . . ., N	70
Ja . . ., N	90
1BR/3,N	80
FA 71	220
FA 109	200
FA 145	250
FA 150	230
FA 402	400

[a]Treatment of HSV: 3.3 kJ/m^2 UV-A in the presence of 2×10^{-6} M TMP.
[b]The values of multiplicity reactivation were calculated as ratios: residual virus production at m.o.i. 10/residual virus production at m o.i. 0.1

iododeoxycytidine. The extent of spontaneous mutation towards a tk$^-$ phenotype was determined in the progeny from the different cell lines infected by HSV in the exponential growth phase. The results obtained after 2 and 3 full viral cycles show that the spontaneous HSV mutation rate is slightly but significantly lower in FA than in normal cells (table 2). This difference was repetitively obtained over separate experiments.

Table 2 . Extent of forward spontaneous mutation towards a *tk*$^-$ phenotype in progeny from HSV-infected normal and FA cells

Cell line	Time of harvest after infection[a] (h)	
	40 (2 cycles)	60 (3 cycles)
Ja . . .	1.5×10^{-4}[b]	3.1×10^{-4}
1BR/3	2.0×10^{-4}	4.0×10^{-4}
FA 71	4.2×10^{-5}	2.2×10^{-4}
FA 145	3.3×10^{-5}	1.7×10^{-4}
FA 150	4.5×10^{-5}	1.5×10^{-4}

[a]Growing cultures were infected at time 0 by 100 p.f.u. of virus.
[b]Average values of two separate titrations which gave results concordant within 15–20% limits.

The extent of mutation was then measured in the progeny from TMP-HSV reactivated by multiplicity of infection. The resulting proportion of mutant viruses was greater (normal cells) or unchanged (FA cells) in the progeny of TMP-HSV than in those of untreated virus (table 3).

Table 3 . Extent of mutation towards a *tk*⁻ phenotype in progeny virus from psoralen-HSV[a]-infected cells (24 h harvest)

Cell line	Infection (m.o.i. 10) by	
	Untreated virus	TMP virus[a]
Ja . .	2[b]	7.5
1BR/3	3.9	6.2
Pr . . .	2	3 5
FA 71	1.2	1 3
FA 109	1.7	1 4
FA 145	1.5	1 3
FA 150	2.5	2.2
FA 402	2.1	1.2

[a]Treatment of virus: 4,5',8-TMP 2×10^{-6} M + 3.3 kJ/m^2 UV-A
[b]Ratio of *tk*⁻ mutant ($\times 10^{-4}$) in the single cycle virus harvests.

These data show that mutagenesis in viral progeny from psoralen-damaged HSV reactivated by multiplicity is lower in FA than in normal host cells.

DISCUSSION

FA cells are hypersensitive to the lethal effect of agents which can form DNA interstrand cross-links (CL). The increased susceptibility of FA cells can be due to an abnormality in the processing of CL. In vitro studies demonstrate that the successive pathways of CL repair involve several enzymatic activities among which the strand transferase activity of the Rec A protein (Sladek et al., J. Biol. Chem., 264:1965, 1989 and ref. therein). Therefore it was logical to study various parameters of recombinational repair in human cells and to focus our work on FA cells. This aim led us to combine an in vivo study using a nuclear replicating double-stranded DNA virus (HSV) as a probe to an in vitro study allowing to determine the characteristics of recombination and recombinational repair catalysed by human nuclear extracts.

The extent of multiplicity reactivation (MR) of Herpes simplex virus (HSV) containing CL in its DNA was tested because MR has been demonstrated to involve recombination between coinfecting viruses and that one CL in viral DNA constitutes

a lethal lesion in condition of single infection (ref. in Coppey et al., 1989). The main result obtained is the fact that MR of cross-linked HSV is greater in FA than in normal cells. We interpret this as follows : i) CL in DNA gives rise to double strand breaks (DSB) following replication across it ; ii) recombination between co-transfected viral DNA is strongly stimulated by DSB. The increased MR of HSV in FA cells can reflect an accumulation of DSBs resulting from slowed down removal of CL from damaged DNA. The fact that the initial step of CL repair, i.e. incision, is slower and finally less efficient in FA than in normal cells (Papadopoulo et al., Mutation Res., 184:271, 1987) supports this possibility.

In addition, mutagenesis studies based upon measurements of the mutation rate of HSV towards a tk$^-$ phenotype show that : i) the mutation rate is lower for HSV replicated in FA cells than for that replicated in normal cells ; ii) in normal cells, the mutation rate is greater in the progeny from damaged HSV repaired by MR than in that from undamaged virus. In FA cells, it remains unchanged.

In accord with data obtained in our group for an endogenous gene such as HGPRT (Papadopoulo et al., 1989, in press), FA cells exhibit an hypomutator effect towards replicating HSV or reactivating by multiplicity psoralen-damaged HSV. Since recombination appears to be an error-free pathway in human cell extracts (Lopez and Coppey, 1989a), this may account for the hypomutator state towards HSV in FA cells inasmuch as recombination is relatively more involved in these cells than in the normal cells.

Our in vitro data show that human nuclear extracts contain proteins promoting recombination between two duplex DNA's via single strand exchange (Lopez et al., 1987) which can be reciprocal (Lopez and Coppey, 1989b) and also recombinational repair of DSB (Lopez and Coppey, 1987). Extracts from normal fibroblast cells, SV-40 transformed cells, lymphoblastoïd cells and also cell lines derived from human carcinoma (lung, cervix) exhibit comparable levels of activities promoting either recombination or recombinational repair of DSB's.

A comparison of these activities in different cell lines (including FA) and according to their genetic origin is planned. However, it seems to be still premature at this stage. Indeed our results indicate that numerous enzymes and proteins are involved in either recombination process. For instance, the catalysis of DSB recombinational repair uses at least endonuclease, helicase, exonuclease (polarity ?), possibly strand transferase, DNA polymerase, resolvase and ligase. We do not exactly know the precise participation of the recipient bacteria in the complete process (Lopez and Coppey, 1989b). In this context, it is important to point out that it was suggested that the "misrepair" of DSB could be due to an excess of

exonucleolytic activity in -rays hypersensitive ataxia telangiectasia cells (Debenham et al., J. Cell. Sci. Suppl. 6:177, 1987). Indeed the mere imbalance between the relative amounts of these enzymatic activities could heavily perturb either recombinational process.

In conclusion, the avaibility of more refined tests is required to enable us to precisely compare the physiological status of recombination enzymatic machineries in human cell strains expected to present recombination alterations such as FA cells. We have recently achieved a progress towards this direction by thinking out a method then developing a device according to which the different proteins of a human nuclear extract promoting the strand exchange reaction can be separated by gel electrophoresis and detected (Corteggiani et al., manuscript in preparation).

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

V. Publications:

1) In Journals

Effect of thymidine kinase activity on repair or enhanced reactivation of ultraviolet-irradiated Herpes virus in human cells.
B. LOPEZ and J. COPPEY.
Life Sci. Advances - Molecular Genetics, **6**, 67-70 (1987).

Homologous recombination intermediates between two duplex DNA catalysed by human cell extracts.
B. LOPEZ, S. ROUSSET and J. COPPEY.
Nucleic Acids Res., **15**, 5643-5655 (1987).

Promotion of double-strand break repair by human nuclear extracts preferentially involves recombination with intact homologous DNA.
B. LOPEZ and J. COPPEY.
Nucleic Acids Res., **15**, 6813-6826 (1987).

Multiplicity reactivation and mutagenesis of trimethylpsoralen damaged Herpes virus in normal and Fanconi's anemia cells.
J. COPPEY, M. SALA-TREPAT and B. LOPEZ.
Mutagenesis, **4**, 67-71 (1988).

Molecular analysis of homologous recombination catalysed by human nuclear extract : fidelity and DNase protection.
B. LOPEZ and J. COPPEY.
Biochem. Biophys. Res. Commun., **158**, 454-461 (1989a).

2) In books

Duplex-duplex homologous recombination catalyzed by human nuclear extract. Involvement in double-strand break repair.
B. LOPEZ and J. COPPEY.
In : "DNA repair mechanisms and their biological implications in mammalian cells". NATO Advanced Research Workshop, Fontevraud (France, October 2-7, 1988. Eds. : M. W. Lambert and J. Laval (1989b).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Consiglio Nazionale delle Ricerche
Piazzale Aldo Moro 7
I-00185 Roma

Contract no.: BI6-E-158-I

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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:

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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. Identification and mapping of human repair genes complementing the defects present in the UV sensitive (UV^S) mutants.

II. Objectives for the reporting period:

Study of the function of poly(ADP-ribose)polymerase (pADPRP) in DNA replication and repair by analyzing the activity, structure and expression of the enzyme in different cellular systems. Analysis of the activity and structure of DNA ligase from mammalian cells and from patients with Bloom's Syndrome and Fanconi's Anemia.

Cellular and genetic characterization of UV^S CHO mutants. Mapping of human genes involved in DNA repair. Analysis of genetic instability in patients with clinical features suggesting alterations in DNA repair. Genetic analysis of the defect conferring UV sensitivity in trichothiodystrophy (TTD). Study of the TTD/XP-D association.

III. Progress achieved: METHODOLOGY

The study of poly(ADP-ribose)polymerase (pADPRP) and DNA ligase in mammalian cells has been conducted by using the following techniques: enzyme purification, activity gel procedures, production of specific antisera, ELISA, immunoblotting, affinity chromatography, fast protein liquid chromatography, RNA isolation, Northern blotting using a cDNA probe obtained from Prof. M. Miwa (Tokyo), microinjection of 32P-NAD in mouse fertilized eggs.

The following cells were used in the experiments: human lymphocytes in Go phase and after mitogen stimulation, lymphoblastoid cell lines, fibroblasts, human and rodent heteroploid cell lines (HeLa, CHO); the various organisms analyzed to assess phylogeny of pADPRP were obtained from different sources.

Experimental models for in vivo carcinogenesis included the Teebor and Becker system of discontinuous 2-AAF treatment, the Solt and Farber model of mutagen treatment followed by partial hepatectomy and the Druckrey model based on diethylnitrosamine (DENA) treatment. Extent of damage to rat liver DNA was measured according to the alkaline elution technique.

Response to mutagens has been studied by measuring the level of DNA repair synthesis (UDS) both by autoradiography and liquid scintillation technique, survival, and DNA replication rate.

Baseline and UV-induced mutation frequency for resistance to ouabain (OUA) and 6-thioguanine (6TG) has been analyzed following standard procedures. 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.

Genetic analysis of the function defective in the UV sensitive (UV^S) cells has been performed by measuring the UDS or the survival in hybrids after fusion of UV^S cells with normal or UV^S cells belonging to the complementation groups so far identified.

RESULTS

The principal results achieved during the past five years (1985-1989) can be summarized as follows:

Study of mechanism of action of poly(ADP-ribose)polymerase (pADPRP) and of its role in DNA repair in mammalian cells and in rat liver carcinogenesis

To possibly understand the precise role of the enzyme poly(ADP-ribose) polymerase in DNA replication and repair, its variation in human lymphocytes stimulated with PHA and after treatment with DNA

damaging agents was followed. The intensity of the active band of the enzyme, visualized on activity gel, increased significantly one day after lymphocyte stimulation, reached its maximum at 3rd day and was mantained at very late times of stimulation, when DNA synthesis is low but capacity to perform repair synthesis is elevated. When the pADPRP protein band was analyzed on Western blot a similar pattern was obtained, thus indicating that the increase in pADPRP activity was correlated to an increase in enzyme protein.

The results obtained by treating human lymphocytes with DNA-damaging agents indicated that DMS is 10 fold more active than MMS in stimulating pADPRP activity and that, at very high doses, the activity of the enzyme tends to disappear.

The role of the enzyme in rat liver carcinogenesis was studied in collaboration with Dr. Cesarone and coworkers. The exposure of rats to a feeding regimen containing 2-AAF, following the experimental model of Teebor and Becker, causes an accumulation of lesions and a progressive impairment in DNA repair capacity. The catalytic band of 116 kDa of pADPRP, present in control rats, was undetectable after the 1st cycle of treatment with 2-AAF, returning progressively to a normal level within the 3rd and 4th cycle. The extent of DNA damage and repair, measured by the alkaline elution technique, showed that the number of alkali-labile sites in DNA was significantly enhanced after the first cycle and remained at high levels during following cycles.

Other models of rat hepatocarcinogenesis were also experimented, namely those of Solt and Farber and of Druckrey. In the first system a single injection of DENA resulted in a significant decrease of pADPRP activity and the subsequent administration of 2-AAF induced a further drop. Concerning the Druckrey model, no significant variation in enzyme band intensity was noted after several weeks of oral administration of DENA. The level of mRNA for pADPRP in liver of rats treated according to the Teebor and Becker system showed no significant variations during the four cycles of treatment.

The activity of pADPRP was analyzed in fibroblasts and lymphoblastoid cells from Fanconi's Anemia (FA) patients which are considered to be impaired in the NAD metabolism. The results obtained indicate that the enzyme in FA cells (c.g. A and B) presents the same characteristics as in control cells. Also the response of FA cells to mitomycin C treatment did not differ from that observed in normal cells.

To possibly understand further which nuclear proteins are ADP-ribosylated in physiological conditions in mammalian cells, different approaches were considered. The pattern of radiolabeled proteins was similar in all systems considered, showing main autoradiographic bands of about 170, 110, 85 and 30 kDa.

A phylogenetic analysis of the poly(ADP-ribose)polymerase was conducted in different classes of eukaryotes by means of biochemical, immunological and activity gel procedures. It appeared that the enzyme is present in multicellular organisms while is lacking in unicellular

eukaryotes.

Investigation on the activity and structure of DNA ligase from mammalian cells and from patients with Bloom's Syndrome and Fanconi's Anemia

In collaboration with M. Mezzina and A. Sarasin (Villejuif) a new method to detect DNA ligase in CV1-monkey kidney cells infected with SV40 or treated with mitomycin C was developed. The results obtained indicate that DNA ligase is heterogeneous in size and presents several high Mr peptides endowed with enzymatic activity. DNA ligase was purified from normal and regenerating rat liver and a major active polypeptide of 130 kDa was isolated from both types of cells.

The analysis of DNA ligase in human cells obtained from normal individuals and four Bloom's Syndrome (BS) and Fanconi's Anemia (FA) patients showed that the level of enzyme activity in untrasformed and transformed FA cell lines was identical to that of control and that it was significantly higher in BS cell lines. The structural properties of DNA ligase were the same in control and BS cell lysates. These results indicated that FA and BS cells do not appear to be deficient in DNA ligase activity.

Cellular and genetic analysis of UV sensitive CHO mutants.

Cellular analysis was performed in UV^S mutants by studying UV-induced DNA repair capacity, sensitivity to different mutagens, baseline and induced frequency of chromosomal aberrations, mutation frequency.

Seven mutants showing different UV sensitivity were analyzed for UV-induced chromosomal aberrations; only in the most sensitive clone the frequency of aberrant mitoses and breakage rate was higher than in wild-type cells. Similar results were obtained by analyzing the UV-induced frequency for 6TG and OUA resistance in three mutants: the response to UV mutagenesis at the two markers was enhanced only in the most sensitive clone.

Eight UV^S mutants analyzed for survival after exposure to mono- and bi-functional alkylating agents and to x-rays, showed a different pattern of cross-sensitivity.

Genetic analysis of the DNA repair defect in six UV^S clones indicated that three mutants (CHO43RO,CHO423PV,CHO3OPV) belong to group 1, one (CHO5OPV) belongs to group 5 whereas the last two (CHO4PV,CHO7PV) represent two new complementation groups. In fact CHO4PV and CHO7PV cells were able to complement each other and showed complementation after fusion with any of the eight groups so far identified.

Attempts to isolate the genes that correct the defect in these two mutants were so far unsuccessful since the low mutagen hypersensitivity of the cells does not provide an efficient selection system for cells transfected with human DNA.

To localize on human chromosomes the gene able to complement the repair defect, fourty hybrids obtained by fusing CHO7PV cells with human lymphocytes have been isolated. So far eighteen hybrids have been characterized for survival after UV irradiation and human chromosome content; human chromosomes were recognized by in situ hybridization with total human DNA and identified by banding procedures. Human chromosome 7 exhibits the strongest correlation with repair ability. To confirm the involvement of this chromosome in the complementation of the defect of CHO7PV cells, the characterization of subclones of different hybrids is in progress.

The homology, at the genetic level, between DNA repair deficient human and rodent mutants was investigated by complementation analysis. Restoration of the UV-induced DNA repair synthesis was found in heterokaryons after fusion of UV^S CHO cells belonging to complementation group 1 with xeroderma pigmentosum (XP) cells of groups A,B,C,D,F,G.

Analysis of genetic instability in patients with clinical features suggesting alterations in DNA repair

An abnormal response to UV was found in cells from a patient affected by precocious ageing. A decreased level of UDS was observed in 60% of Go lymphocytes and in fibroblasts after the fifth culture passage. After stimulation with mitogens, the patient lymphocytes were hypersensitive to UV whereas a normal sensitivity was observed after treatment with alkylating agents.

Cytogenetic instability expressed as spontaneous chromosome damage was observed in lymphocytes of "cancer family" subjects; SCE frequency, response to mutagens, expression of fragile sites were in the normal range.

No evidence of genetic instability was found in lymphocytes from four nevobasal cell carcinoma syndrome (NBCCS) patients. The level of UV-induced DNA repair synthesis, the DNA replication rate after treatment with mutagens inducing different kinds of damage in the DNA molecule, the baseline mutation frequency and the spontaneous chromosome breakage showed normal values; only the response to mitogens was delayed in comparison to that in normal donors.

In a patient affected by Werner syndrome (WS), cytogenetic studies indicated the presence of variegated translocation mosaicism. Conversely the baseline mutation frequency, the capability to perform UV-induced DNA repair synthesis and the sensitivity to various mutagens were in the normal range.

The study of efficiency of DNA repair mechanisms in trichothiodystrophy (TTD) patients with or without photosensitivity, performed in five Italian patients and in six patients from different countries (cells were kindly provided by Dr. A. Lehmann-Brighton, Dr. W. Kleijer-Rotterdam, and Dr. A. Sarasin-Villejuif indicated that TTD may

be associated with normal or enhanced cellular UV sensitivity. Among the patients showing cellular photosensitivity (nine out of the eleven so far analyzed), reduced levels of UDS ranging from 10% to 50% of normal values were observed.

Genetic analysis of the repair defect was carried out by analyzing UDS in hybrids obtained by fusion of TTD cells with XP cells belonging to different complementation groups. The results of complementation studies demonstrated that in all the repair-deficient TTD cells the alteration was due to the presence of XP group D (XP-D) mutation.

In three Italian families with four TTD/XP-D affected members we performed a search for consanguinity based on the reconstruction of genealogical trees, the study of blood genetic markers and the analysis of surnames. These studies led us to conclude that it is highly probable that the patients are carriers of a genetic defect identical by descent, a consequence of multiple remote inbreeding.

DISCUSSION

Study of function of poly(ADP-ribose)polymerase in DNA repair and carcinogenesis

The enzyme poly(ADP-ribose)polymerase is known to play a central role in the modulation of cellular response to DNA damage. The activation of pADPRP in cells treated with DNA-damaging agents is considered to be strictly dependent on the appearance of newly formed single-strand breaks in DNA. Our results, showing that DMS is 10 fold more effective than MMS in activating the enzyme in human lymphocytes, are in accordance with the rate of nick formation determined by these two mutagens. The increase in the intensity of the active band of the enzyme is reflecting a change in the intrinsic activity since the protein band produced the same signal in Western blots of control and treated cells. On the contrary, the enhancement of ADPRP activity in PHA stimulated human lymphocytes is concomitant to a sharp increase of the protein band, indicating that a de novo synthesis of the enzyme occurred.

The relationship between DNA repair and carcinogenesis was studied in (in collaboration with F. Cesarone) in liver of rats exposed to the carcinogen 2-AAF by measuring the extent of DNA damage and the activity of pADPRP during four cycles of treatment (model of Teebor and Becker). From the activity gel and immunoblot analysis it appeared that pADPRP activity is depleted after one cycle of 2-AAF treatment, possibly as a result of an inhibition of pADPRP de novo synthesis. The activity of pADPRP was also strongly decreased after treatment of rats with DENA, a carcinogenic initiator, followed by treatment with 2AAF, thus confirming the results previously obtained. On the contrary, when DENA alone was continously given to rats, the activity of the enzyme remained constant, suggesting that the 2-AAF is needed as promoter to produce an effect on

pADPRP activity.

The molecular basis of the reduction of repair capacity in Fanconi's Anemia (FA) is not well understood. Since other authors have reported that these cells are defective in NAD metabolism following DNA damage, we have analyzed pADPRP activity in the two FA complementation groups so far identified. We have not observed changes in the level and in the structure of pADPRP in FA cells compared to normal cells. These results could indicate that the molecular defect of FA is not strictly associated to the activity of pADPRP.

The distribution of in vivo ADP-ribosylated proteins was investigated by using different biological systems and by means of several experimental approaches. We have found the same pattern of ADP-ribosylated proteins in HeLa intact cells and isolated nuclei and in mouse fertilized eggs. Radiolabeled protein bands showed a typical pattern distribution at 170, 110, 85 and 30 kDa. These results suggest that the non-histone proteins ADP-ribosylated in vivo are very specific and their number is much reduced with respect to that observed by in vitro studies.

Analysis of DNA ligase structure in mammalian cells

An activity gel method was developed in collaboration with A. Sarasin and M. Mezzina which allows the in situ identification of the active protein bands and of their molecular size. The analysis of the enzyme in mammalian cells indicated that DNA ligase is heterogeneous since several high Mr active peptides were visualized in the activity gels.

DNA ligase was also analyzed in human cells obtained from normal individuals and from Bloom's syndrome (BS) and Fanconi's Anemia (FA) patients. The levels and the structural properties of the enzyme of control cells were identical to those of FA cells lines while the activity was significantly higher in BS cells. These results strongly suggest that FA and BS cells are not deficient in DNA ligase activity.

Cellular and genetic analysis of UV sensitive CHO mutants

The results of these studies indicate that:

- the excision repair in Chinese hamster is an error-free mechanism as suggested in man by mutation data obtained for XP;
- clastogenic and mutagenic effects of UV light are more marked in UV ^{S} rodent cells showing high cellular UV hypersensitivity;
- DNA repair patways involved in the restoration of different lesions have common steps;
- mutation in the ERCC1 gene may result in different degrees of phenotypic alterations, as demonstrated by the assignement to group 1 of three mutants showing different degree of mutagen sensitivity, UDS and chromosomal fragility;
- human and Chinese hamster repair functions cooperate in the XP-CHO

heterokaryons and the genetic defect in the CHO mutants belonging to group 1 is different from those present in XP groups A,B,C,D,F,G;

Two new mutations involved in the repair of DNA damage, in addition to the eight so far described in UV^S rodent cells, have been identified. The defect of one of this mutant (CHO 7PV) is complemented by a gene preliminary assigned to human chromosome 7.

Analysis of genetic instability in patients with clinical features suggesting alterations in DNA repair.

The results of DNA repair studies indicate that:

- the primary defect responsible for precocious aging can influence the capacity to repair DNA damage and probably involves common factors in the control of immunity and DNA repair processes;
- chromosomal instability and cellular UV hypersensitivity are not distinctive and constant features of NBCCS; the clinical radiosensitivity of NBCCS is not related to increased cellular mutability;
- the primary defect in WS does not influence the baseline mutation rate and it does not interfere with the DNA repair mechanisms involved in the restoration of lesions induced by UVC light, and mono- and bi-functional alkylating agents;
- a reduced cellular ability to repair UV-induced DNA damage, due to the presence of XP-D mutation was identified in 9 out of 11 patients affected by TTD. Neverthless, in all these patients tumors and severe skin alterations typical of XP are absent. This fact could be related to the young age of the patients, or it may be hypothesized that the association with TTD gives rise to a different phenotypic expression of the XP-D mutation. The elucidation of this aspect will be relevant in understanding the correlation between defective response to mutagens and tumor proneness.

The co-inheritance of XP-D and TTD in four Italian patients, probably related by remote multiple consanguinity, suggests that the two mutations responsible for TTD and XP-D should be at loci closely linked on the same chromosome or affect the same gene. On the other hand, the concurrence of TTD and XP-D in several unrelated patients can not be considered fortuitous and suggests that the genetic event leading to the TTD/XP-D association should involve a genetically unstable region.

The results of our investigations on patients with different pathologic conditions stress how complex is the genetic control of DNA repair processes in man and how intriguing the relationship is between DNA repair efficiency, chromosomal instability, ageing and tendency to neoplasia.

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

1) A. Sarasin and M. Mezzina, Institut de Recherche Scientifiques sur le Cancer, Villejuif (France).

2) E. Moustacchi, Institut Curie, Paris (France).

3) A.R. Lehmann, MRC Cell Mutation Unit, University of Sussex, Brighton, U.K.

V. Publications

PUBLICATIONS IN SCIENTIFIC JOURNALS (1985-1989)

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: B16-E-223-D

Universität - GSH Essen
Universitätsstr. 2
D-4300 Essen 1

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

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Universität - GSH Essen
Universitätsstr. 5
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Telephone number: 0201 183-1 2100

Title of the research contract:

The production of chromosome aberrations in human lymphocytes by low doses of X-rays.

List of projects:

1. The production of chromosome aberrations in human lymphocytes by low doses of X-rays.

Title of the project no.:

The production of chromosomal aberrations in human lymphocytes by low doses of X-rays

Head(s) of project: Prof. Dr. G Obe

Scientific staff: Dr. U Weissenborn
Mr C Johannes

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 chromosomal aberrations. The primary objective was to verify the existence of any low dose plateau in the response over the range zero to a few tens of milligrays. Blood from a number of donors was irradiated and cultured in one laboratory and the microscope analysis was done in six collaborating laboratories. The extent of inter-donor and inter-laboratory variations in the data was also examined.

II. Objectives for the reporting period:

The report covers the whole study which has extended over 5 years. In interim reports each year`s results have been summarized and during the final year the most recent work added data from blood of 20 donors irradiated to 5 and 300 mGy.

Results and Discussion:

This is a multi-laboratory project. For a common report on the project, see the report of Contract no. B.16-E-225-UK (NRBP).

RADIATION PROTECTION PROGRAMME
Final Report

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
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Università di Roma "La Sapienza"
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Telephone number: 49.56.205

Title of the research contract:

Adaptive response to low doses of radiation: studies in human cells of a possible radiation-stimulated repair.

List of projects:

1. Study of the effect of a low pre-dose of radiation on the level of chromosomal damage induced by a subsequent acute dose in human cells.

Title of the project no : B16-E-186-I
Study of the effect of a low pre-dose of radiation on the level of chromosomal damage induced by a subsequent acute dose in human cells.

Head(s) of project:

Prof. Gregorio OLIVIERI

Scientific staff.

F. PELLICCIA, A. MICHELI, A. BOSI, S. PIETROSANTI, C.TRINCHESE

I. Objectives of the project:

To study the mechanism by which low levels of chronic radiation can trigger or induce increased repair of radiation induced chromosome breaks.

II Objectives for the reporting period:

We have called "adaptive response" (AR) the reduction of clastostogenic damage observed in human lymphocytes cultivated in tritiated thymidine (^{3}HdThd) and subsequently exposed to high doses of X-rays (Olivieri et al. 1984). In the past four years, it was deemed interesting to study several features of this "adaptive response" in human lymphocytes.

III. Progress achieved:

During the contractual period (86-89) the attention was focussed on a) possibility of artifacts b) involvement in the AR (adaptive response) of poly (ADP-ribose) synthesis c) clastogenic cross-adaptation d) individual variability of the donors.

METHODOLOGY

Experiments were carried out using cultures of blood from donors of different sexes. Whole blood (0.5 ml) was added to 4.5 ml of RPMI 1640 medium containing 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin and 2% phytohemagglutinin M (Gibco). The experiments consisted of first exposing cultured human lymphocytes to adapting treatments and subsequently challenging the cells with high doses of X-rays or other mutagens. The cells were scored to see whether the prior exposure reduced the number of chromatid and isochromatid breaks induced by the challenging doses.

RESULTS AND DISCUSSION

a) To determine if ^{3}H dThd selects against a radiosensitive subpopulation of lymphocytes and prevents their progression to mitosis, ^{3}H dThd-labelled female cells were co-cultured with unlabelled male cells and the proportions of female to male cells were determined at metaphase. In control experiments unlabelled female cells were co-cultured with unlabelled male cells. In both cases the same proportions of female to male cells were recovered at metaphase as were initially established in the cultures. Additionally, autoradiograms of the metaphase preparations showed that all the cells observed at metaphase at 53 h of culture were labelled. There was no selection against cells labelled with ^{3}H dThd. Consequently, the adaptive response cannot be attributed to selective killing of a radiosensitive population of cells by the incorporated radioisotope; which then would lead to an apparent decrease in aberration yield.

In experiments designed to see if the adaptive response was restricted to the cells containing ^{3}H dThd, or if it involved the induction of a diffusible factor that could bring about the response in neighbouring cells, mixtures of labelled female cells and unlabelled male cells were cultured and then irradiated. Again, after the cells reached metaphase, cells from both sexes were scored separately on the same slide. The results show that after X-irradiation the adaptive response is restricted to those cells exposed to radiation from the

incorporated ^{3}H dThd and is not mediated by factors that diffuse from these cells to neighbouring unlabelled cells.

b) Because poly (ADP-ribose) polymerase activity may be required for the efficient repair of chromosomal damage, experiments were carried out to see if 3AB, an inhibitor of poly (ADP-ribosyl)ation, would prevent the repair. When lymphocytes were irradiated at 48 h of culture and fixed 6 h later, those cells that had incorporated ^{3}H dThd had fewer aberrations than the sum of those induced by ^{3}H dThd and X-rays separately. However, when 3AB was present during the entire culture period, or even just after irradiation, the effect was abolished and the yield of aberrations was simply the sum of the individual effects of ^{3}H dThd and X-rays. On the other hand, if the 3AB treatment was restricted to the time before X-ray exposure, there was no effect on the adaptive response. The results indicate that inhibition of poly (ADP-ribose) polymerase by 3AB treatment after irradiation reverses the adaptive response. Whether poly(ADP-ribosyl)ation itself is directly involved, or whether the response is a secondary effect is as yet unknown. Inhibition of poly(ADP-ribose) polymerase, which prevents the repair of chromosome breaks in G_1, prevents the S/G_2 adaptive response as well.

c) 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), approximately twice as much damage was found than was induced by MMS alone.

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 occured. 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 alkykating 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.
Because MMS is unlike N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), in that the two alkylating agents give a different spectrum of methylated bases (Laval and Laval, 1984), with MMS producing a far higher ratio of N^7-methylguanine to O^6-methylguanine than does MNNG, experiments were carried out with MNNG to see if the synergism obtained between X rays and a subsequent exposure to the simple alkylating agent MMS was obtained with another simple methylating agent that produces a different spectrum of lesions. The experiments showed, as had been found previously that a conditioning treatment with 1 cGy of X rays was synergistic with a subsequent high dose of MMS. When 0.018 mM MMS or 600 ng/ml MNNG was used as a conditioning treatment, they, too were found to be synergistic with a subsequent exposure to a high dose of MMS. When, however, the challenge treatment was X rays, then a pretreatment or conditioning with 1 cGy of X rays 0.018 mM MMS, or 600 ng/ml of MNNG decreased the numbers of aberrations induced by the subsequent challenge with 150 cGy of X rays, showing that conditioning with simple alkylating agents, can reduce the yield of aberrations induced subsequently by a high dose of X rays. It was also found that X rays, MMS, or MNNG can reduce the numbers of aberrations induced by subsequent exposures to a high dose of MNNG.
To date the results indicate that all of the agents,including MMS, can induce an adaptive response in human lymphocytes that makes the cells less susceptible to damage induced by higher doses of some of the agents. The only exception to this occurs when the challenge is with MMS. Here it is found that pre-exposure either to X rays or to MMS itself can render the cells more susceptible to damage induced by a higher dose. These results, therefore, indicate that MMS, with its different spectrum of lesions, produces enough damage in DNA of the type necessary to induce the adaptive response, but that the predominant lesion that leads to the production of chromosomal breaks after a challenge with MMS differs from that found with the other agents. This synergim is difficult to interpret.
Conceivably the constitutive and induced DNA repair system network is very complex and, as a result of the induction of several of these enzymes, is possibly liable to imbalances affecting the final result of damage repair. This is a

further indication that the dangerousness of low doses of mutagens is likely to be strongly increased in such circumstances.

d) To determine whether these is individual variability in the adaptive response to ionizing radiations we exposed human lymphocytes from 18 different healthy donors to "adapting" doses of ^{3}H dThd (0.01 µCi/ml) or X-rays (0.01 Gy) and subsequently to a "challenge" treatment of 0.75 Gy of X-rays delivered 2 h before fixation.

Four of the 18 donors did not show an adaptive response; in some cases in these individuals a synergistic response of increased, rather than decreased, damage was found. Subsequently we carried out experiments using lymphocytes from 7 different donors, who had already been used in the preceding work. The 7 donors included the 4 donors who had not shown any adaptive response (AR- donors) and 3 who had (AR+ donors). During these experiments, in which the lymphocytes were pretreated with low doses of X-rays or MMS and then challenged with 0.40, 0.75 or 1.5 Gy of X-rays, two AR- donors showed an adaptive response and two AR+ donors did not. Furthermore, it was attempted to modify the response of AR- donors by adding different compounds to the cultures. It was found that the addition of Adrenal Cortex extracts, Hydrocortisone, Thymus extracts and IL-2, although not modifying the adaptive response of one AR+ donor, elicited an adaptive response from donors who had not previously shown any. The addition of two other substances, Insulin and INF-γ had no effect on the response of AR- donors.

As far as the AR in human lymphocytes is concerned, the results of the experiments described herein indicate that the variability in AR found in the various donors is not linked to their genetic constitution but is dependent on some transient physiological parameters. Two donors, who were AR+ in previous experiments displayed no adaptive response. On the other hand, two AR- donors displayed a clear-cut AR in experiment I. Furthermore, durign the experiments, cases were observed in which, under standard culture conditions the donor displayed no AR, and yet an AR could be evidenced by modifying the culture conditions.

Therefore, our evidence seems to point to the great importance for AR of the metabolic state of cells during conditioning. We do not know exactly what modifications are produced in the metabolism of the lymphocytes by the compounds which elicited an AR also in donors who displayed none under standard conditions. However, in all probability, the compounds used by us all have the power to stimulate protein synthesis in cells. Considerable evidence points to the fact

that the presence of an AR in lymphocytes or in other biological systems needs a certain level of cellular metabolic activity and an unimpaired protein synthesis.

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

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Laboratory of Radiobiology, University of California,
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V. Publications:

WIENCKE J.K., AFZAL V., OLIVIERI G. and WOLFF S., 1986: "Evidence that the ^{3}H thymidine-induce adaptive response of human lymphocytes to subsequent doses of X rays involves the induction of a chromosomal repair mechanism Mutagenesis 1: 375-380.

WOLFF S., AFZAL V., WIENCKE J.K., OLIVIERI G. and MICHELI A., 1988: "Human lymphocytes exposed to low doses of ionizing radiations become refractory to high doses of radiation as well as to chemical mutagens that induce doublestrand breaks in DNA. Int. J. Radiat. Biol. 53: 39-48.

PELLICCIA F., BOSI A., BELLONI G., MICHELI A. and OLIVIERI G., 1988: "Studies on chromosome aberrations induced by incorporated tritium: effects of post-treatment with hydroxyurea and caffeine in G_2 Mutation Res. 199: 139-144.

BOSI A., G. OLIVIERI, 1989: "Variability of the adaptive response to ionizing radiations in humans" Mutation Res. 211: 13-17.

WOLFF S, OLIVIERI G. and AFZAL V.: "Adaptation of human lymphocytes to Radiation or Chemical mutagens: differences in Cytogenetic repair". Symposium on Chromosomal Aberrations Essen 1989 (in press).

OLIVIERI G. and BOSI A.: "Possible causes of variability of the adaptive response in humans lymphocytes". Symphosium on Chromosomal Aberrations Essen 1989 (in press).

WOLFF S., OLIVIERI G. and AFZAL V.: "The adaptive response of human lymphocytes to radiation or chemical mutagens: cross-adaptation and synergism in Mechanisms of Environmental Mutagenesis-carcinogenesis (ed. A. Kappas) 1989 (in press).

RADIATION PROTECTION PROGRAMME
Final Report

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)]:

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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:

II Objectives for the reporting period:

Results and Discussion:

This is a multi-laboratory project. For a common report on the project, see the report of Contract no. B.16-E-225-UK (NRBP).

RADIATION PROTECTION PROGRAMME
Final Report

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
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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.

CEC FINAL REPORT 1984-1988

Contrat BI-6-154 F (CD)

Head of projet : M. RADMAN

Title of the project n° : MOLECULAR BASIS OF RADIATION INDUCED MUTAGENESIS IN PROKARYOTES AND EUKARYOTES : NEW EXPERIMENTAL SYSTEMS.

- GENERAL COMMENTS.

The Reseach programme proposed by this laboratory for the period of 1984-88 has been accomplished for the largest part. However, the most valuable contribution is due to unexpected new discoveries describes below. This has happened in spite of the initial arbitrary cut of about 50% of the requested funding, yet this cut has severely decreased our research capacity and our international competitiveness in the research domains opened up by our laboratory.

The reduction in the funding has slowed down most severely the realisation of the proposed development project towards establishment of a new molecular methodology to diagnose mutations in the genome of live human subjects using mismatch correction enzymes. Whereas all fundamental and technical problems to achieve this have been solved (including the demonstration that mutations in a defined DNA molecule can be detected and localised by the mismatch repair activity from bacterium E. coli and frogg Xenopus laevis egg cells), the methodology has not yet been elaborated as a routine, generally applicable procedure. Below is the summary of the research achievements in the two initially proposed areas.

PROJECT A : BIOCHEMISTRY OF MUTAGENESIS INDUCED BY DNA DAMAGE, INCLUDING RADIATION DAMAGE.

This group has been involved in the study of mechanisms of radiation and chemically induced mutagenesis since the first CEC Radiation Protection Programme contract in 1974, the year when we have discovered the cellular SOS system in bacteria. It is now well established that DNA damage-induced mutagenesis proceeds through a transient induction of a cellular mutator effect (1). The SOS mutator effect is caused by a decrease in the fidelity of DNA synthesis allowing the copying of damaged DNA (normally stalled at sites of damage) and hence mutation fixation.

In a three years long collaborative effort with the laboratory of Dr M. Goodman (University of Southern California), we have succeeded in identifying an error-prone SOS-inducible DNA polymerase capable of copying a typical non-coding DNA damage. This damage is the abasic site which is known to be produced by ionizing radiation both directly (depurination) and following the activity of repair enzymes (N-glycosylases) removing the damaged base (e.g., thymine glycol), which was incorporated into a specific site of a synthetic oligonucleotide substrate. The inducible mutagenic polymerase is the E. coli DNA polymerase II (2).

PROJECT B : CHARACTERISATION OF MOLECULAR MECHANISMS AND BIOLOGICAL EFFECTS OF THE MISMATCH REPAIR SYSTEMS. USE OF MISMATCH REPAIR ENZYMES FOR PHYSICAL DETECTION OF MUTATIONS.

Our laboratory made the major contribution in this area of molecular genetics (3, 4). Here are the principal conclusions of our work in the period 1984-88 :

I. MISMATCH REPAIR AND THE FIDELITY OF DNA REPLICATION.

(i) Methyl-directed correction of DNA replication errors has been well characterized in bacterium *E. coli* . The multienzyme system removes mismatched bases (errors) from the newly synthesized undermethylated DNA strands in several steps including : recognition of the mismatch by the MutS protein, communication (even at long distances) with the nearby unmethylated GATC sequence which is recognized and nicked by the MutH protein, the MutL protein mediates the MutH and MutU (helicase II) activity to remove the nicked mismatch-bearing unmethylated strand (5, 6).

(ii) Over 99% of replication errors are corrected (4) and the specificity of mismatch repair (7, 8, 9) is such that it counteracts DNA polymerase errors, thus uniformizing the frequency of diverse types of spontaneous mutations along the DNA (10).

(iii) The molecular structure of repaired (intrahelical) and unrepaired (extrahelical) mismatches has been determined by NMR studies of synthetic oligonucleotides (11).

II. *IN VITRO* MISMATCH REPAIR IN *XENOPUS LAEVIS* EGG EXTRACTS.

The work of Dr P. Brooks and Ms C. Dohet has provided the first biochemical evidence for mismatch repair in a vertebrate (12, 13). DNA repair synthesis has been physically mapped : its peak corresponds to the position of the mismatch, but it extends as much as 1kb. The demonstration that a nick stimulated and directs mismatch repair *in vitro*, suggests that the discontinuous DNA synthesis could provide adequat signal for mismatch correction of replication errors in eukaryotes.

III. MISMATCH REPAIR OF DEAMINATED 5-METHYL-CYTOSINE IN *E. COLI* AND *XENOPUS* EGGS.

We have characterized a specialised mismatch repair system in E. coli that corrects the G:T mismatch arising from 5-meC-->T deamination, back t othe G:C pair (14). Similar enzymatic activity was found in Xenopus egg extracts (12).

IV. RECOMBINATION AS A MISMATCH REPAIR PROCESS.

The work of Ms M.P. Doutriaux has shown that the mismatch repair enzymes can inactivate a mismatch-bearing DNA molecule. It appears that only recombination with a sister molecule can repair the broken DNA, hence a biological role for the phenomenon of SCE (15).

V. GENETIC CONSERVATION AND DIVERSIFICATION BY TWO MODES OF MISMATCH REPAIR.

It was demonstrated experimentally that the general (methyl-directed) long-patch mismatch repair (LPMR) system conserves genetic information both in replication and recombination of DNA (16), whereas the specialized very-short patch mismatch repair (VSPMR) systems diversify genetic information in genetic recombination by creating patchwork sequences in hybrid DNA (17, 18). It was suggested that the immunoglobulin and histocompatibility gene diversity can be the result of hyperconversion involving VSPMR mechanisms (19).

VI. MISMATCH REPAIR AND THE FIDELITY OF GENETIC RECOMBINATION : A ROLE FOR REPETITIVE SEQUENCES IN CHROMOSOMAL STABILITY.

We made recently an unexpected discovery that the LPMR system "proofreads" the heteroduplex recombination intermediates by aborting strand exchange between the nonidentical (diverged, partially homologous DNA sequences (17, 18). If all recombination involving

nonidentical sequences is "edited" by LPMR, then, given the high sequence polymorphism among human individuals (about 0.3%), only the sister chromatid exchange would be permitted, as indeed observed. Since the sequence polymorphism is concentrated in repeated and other noncoding sequences (e.g., SINES, LINES, introns and enhancers/promoters), which are usually about 80-90% homologous, we propose that they serve to prevent mitotic recombination in mammalian cells (and to stabilize the chromosome structure in general). Mitotic recombination would destroy the advantage of diploidy as a means of phenotypic suppression of deleterious recessive mutations in somatic cells.

VII. MISMATCH REPAIR, INTERSPECIFIC RECOMBINATION, SPECIATION AND EVOLUTION.

Our eecent work has shown that, in the absence of LPMR system, we can mate and recombine two different genera, *Escherichia* and *Salmonella*, over million-fold more efficiently than in wild type strains. These two genera have separated genetically about 140 million years ago and are only about 80% homologous at DNA level. Thus, our work defines the LPMR as a molecular mechanism for genetic separation in the process of speciation, without the need for geographical separation. The LPMR probably prevents recombination between the interspersed repetitive sequences thus preventing excessive, probably lethal, chromosomal rearrangements. The practical impact of our discovery cannot yet be fully assessed, e.g., formation or new hybrid species bearing mosaic chromosomes and novel genes by recombination and pathworking of any cloned diverged genes coding for the same function in different organisms, including human cells, by simple *in vivo* recombination with relaxed homology requirement. The latter corresponds to the most efficient and promissing "protein engineering" technique.

VIII. PUBLICATIONS.

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RADIATION PROTECTION PROGRAMME
Final Report

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Contract no.: BI6-E-155-B

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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 G. MAENHAUT-MICHEL

Scientific staff: P. CAILLET-FAUQUET
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I. Objectives of the project:

This project aims at studying the mechanisms and regulation of mutagenic processes and in particular the SOS mechanism which is triggered by ionizing radiations and radiomimetic chemicals in bacteria. The correlation between the spectrum of induced mutations and different DNA damaging agents has been studied extensively in several laboratories these last years. But the molecular mechanism of mutagenesis is not yet elucidated. The mutation spectra induced in human cells by different DNA damaging agents that provoke mutations via the SOS mechanism in E.coli bacteria, showed close similarities with the spectra obtained in E.coli. This may mean that basically similar mutagenic mechanisms operate in most organisms and encouraged us to consider E.coli as a pertinent model and a convenient tool to study the mechanisms of mutagenesis.

II. Objectives for the reporting period:

The objectives 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, iii) the replication and mutagenesis of damaged DNA using in vitro damaged heteroduplex DNA, iv) the study of the conversion of premutagenic lesions into mutations, in specific sequences of the DNA. Several factors govern the conversion of premutagenic lesions into mutations among them, reactivity of DNA bases, sequence context and local configuration of the DNA helix, v) the identification of E.coli proteins involved in mutagenesis and able to recognize specific local configuration.

III. Progress achieved:

1 Introduction

Most chemical and physical agents that initiate carcinogenesis interact with cellular DNA either directly or, indirectly through one or more metabolic activation(s) They provoke different types of genetic modifications. base substitutions, addition or deletion of one or several nucleotides, genetic recombination and larger genetic changes. DNA lesions that block the replication fork induce a series of cellular genes, the products of which are involved in the mechanisms of repair, replication and cellular division. We have studied this phenomenon called the SOS response (Radman, 1974) in *E.coli* for several years. In these bacteria, induction of the SOS functions is under the control of *recA* and *lexA* genes (for review; Walker, 1984) The presence, inside the DNA, of non coding lesions that prevent the elongation of the newly synthesized strand, generates a signal activating the RecA protein in RecA*. Activated RecA* protein plays a key role in the conversion of premutagenic lesions into mutations. It stimulates the cleavage of the LexA protein, which is the repressor of a series of genes that code for proteins involved in DNA repair, mutagenesis, recombination, cellular division etc... Among these proteins, UmuD and UmuC are the only proteins to be identified, at present, as being necessary for the expression of mutagenesis induced by ultraviolet light (UV) and ionizing radiations. It has recently been shown that cleavage of the UmuD protein by RecA* is necessary for UV mutagenesis (Nohmi et Al, 1988). In addition to these regulatory roles, RecA protein seems to play another role in SOS mutagenesis. This role as well as the molecular mechanism by which mutation fixation occurs are still unknown.

2 Methodology

2.1 Different systems were developed to detect mutations induced by chemical or physical agents in *E.coli*:

1°) Mutagenesis of the *E.coli* chromosome was determined by measuring either the appearance 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. The second system allows to detect frameshift mutations as the *lac*⁻ mutant used is a (+1) frameshift mutant in the *lacZ* gene

2°) Mutagenesis of phages with single or double stranded DNA was measured in two different mutation systems: i) a forward mutation system detecting all types of mutations in the *cI* gene of phage lambda(i.e.: Base substitutions, frameshifts and others) and ii) a reversion mutation assay detecting base substitutions by looking for revertants of an amber mutation.

3°) Frameshift Mutagenesis of plasmids. The plasmid pX2 is a multicopy plasmid derived from pBR322. It contains two additional GpC base pairs inserted by genetic engineering between base pairs 435 and 436 within the NarI site in the tetracycline resistance gene (Burnouf and Fuchs,1985). This addition generates a new restriction site, BssHII. The plasmid pX2 confers ampicillin resistant and tetracycline sensitive phenotypes to bacteria The mutation assay detects plasmids having reverted from the tetracycline sensitive phenotype to tetracycline resistance. The

selection of mutants is based on the ability of the revertants to grow on tetracycline containing plates The mutation event is dominant and the target multiple (average of 20 copies per cell). It is very specific as it occurs by the loss of two GC base pairs located within the BssHII restriction site. This reversion was shown to be efficiently induced by the ultimate carcinogen Acetoxy-N2-Acetylaminofluorène (N-Aco-AAF).

2.2 The genetic control and the mechanisms of SOS mutagenesis

They were analysed using different mutants of *E.coli.* For this purpose, a set of isogenic strains was constructed with mutations in genes involved either in the control of SOS functions or in the mechanisms of repair and mutagenesis. The use of phages and bacteria for analysing UV-induced mutagenesis has permitted a distinction to be made between the mechanism of targeted and untargeted mutagenesis

The search for new mutants involved in mechanisms of frameshift mutagenesis was undertaken in collaboration with Dr Fuchs' laboratory (Institut de biologie moléculaire et cellulaire, IBMC, CNRS, Strasbourg, France). A culture of *E coli* transformed with the plasmid pX2 was mutagenised either with UV light or with methyl methane sulfonate (MMS), and screened for mutants that are unmutable by N-Aco-AAF

3 Results

3.1 The genetic control of SOS mutagenesis. (P. Caillet-Fauquet, F. Laengle-Rouault and G. Maenhaut-Michel.)

3.1.1 Involvement of RecA and UmuD, UmuC proteins in SOS mutagenesis.

We have shown previously that UmuD and UmuC proteins are not necessary for mutation fixation of all mutations induced by UV (Maenhaut-Michel and Caillet-Fauquet, 1984) and gamma radiations (see EEC progress report n°B10-E-548-83-B) in bacteriophage lambda We have undertaken new experiments to understand which type of mutations requires the UmuDC proteins. It has been shown that induction of the SOS mechanism enhances mutagenesis of undamaged phage, generating untargeted mutations. It has been proposed that untargeted mutagenesis in phage DNA is the consequence of an SOS-induced decrease in the fidelity of the replication complex (Caillet-Fauquet et Al., 1977). It has also been suggested that inhibition of the proofreading function, the exonuclease 3' to 5' associated with DNA polymerase in *E. coli,* may allow bypass synthesis at blocking lesions in damaged DNA and, consequently also produce errors in undamaged DNA by reducing DNA polymerase fidelity (Villani et Al , 1978) We have shown that UV-induced mutagenesis of undamaged phage by SOS induction of the host bacteria does require UmuDC proteins when phage is single-stranded DNA (as is the case for phages ϕX174 and M13 , Maenhaut-Michel and Caillet-Fauquet, 1990) This suggests that the involvement of UmuDC proteins in mutation fixation depends on the structure of the DNA.

The SOS mutator effect can also be promoted by mutation in the *recA* gene. Modification of the RecA protein by the *recA730* mutation increases the level of spontaneous mutations in the bacterial DNA The *recA730* mutation has a very small effect on spontaneous mutagenesis of phage lambda. On the contrary UV-irradiation of *recA730* hosts increases phage untargeted mutagenesis up to the level observed in the *recA*$^+$ bacteria The UV-induced mutator effect in *recA730* mutants is not suppressed by an *umuC* mutation whereas the *recA730*-induced mutator effect is totally abolished by mutation in this gene. Therefore UV and the recA730 mutation seem to activate different SOS mutator activities both generating untargeted mutations (Caillet-Fauquet et Maenhaut-Michel, 1988)

We have also shown that, in phage lambda, untargeted mutations (probably frameshift mutations), could be produced in the absence of RecA protein if genes under the control of the LexA repressor are expressed (Brotcorne-Lannoye and Maenhaut-Michel, 1986) This is not the case for base substitution mutations and therefore suggests that different modes of mutation fixation exist (Maenhaut-Michel and Caillet-Fauquet, 1990).

3.1.2 Mismatch repair and the SOS mutator effect.

Methyl-directed mismatch repair in *E. coli* appears to remove mispaired and unpaired bases from newly synthesized DNA strands, thus correcting replication errors (for review: Radman and Wagner, 1986) The discrimination between the parental and the newly synthesized strand is based on the transient undermethylation of GATC sequences in nascent strands (Radman *et al.*,1980). We have previously shown that, as compared to UV, irradiation of host bacteria with gamma-rays, induces less untargeted mutations in phage lambda (EEC progress report n° B10-E-548-83-B). We have also shown that a greater number of untargeted mutations is produced in UV-irradiated mismatch repair deficient host bacteria (Caillet-Fauquet *et Al* , 1984) We have suggested that the higher proportion of untargeted mutations found in phage lambda DNA as compared to bacterial DNA, is due to the fact that replication errors are corrected with a low efficiency in phage DNA than in bacterial DNA. The 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 in the newly synthesized strand as correctable, replication errors (i e. untargeted)

We have studied the interaction between mismatch repair and DNA lesions using *in vitro* constructed DNA heteroduplexes irradiated by UV or gamma rays or treated with DNA endonuclease I. Preliminary results indicated that some type of single strand breaks could stimulate the efficiency of mismatch repair. We also showed that a nick in one strand of the DNA could replace the role of the *MutH* protein which recognizes unmethylated GATC sequences and cuts unmethylated DNA strands (Laengle-Rouault et Al , 1987)

3.1.3 Involvement of DNA polymerase I in SOS mutagenesis.

DNA polymerase I which is involved in DNA repair mechanisms and in the scealing of Okazaki fragments in DNA replication, is generally considered not to be essential for SOS

mutagenesis However, an altered form of DNA polymerasel, Pol I', was purified from extracts of SOS-induced cells (Lackey et Al , 1982) There was an apparent striking similarity in the genetic requirements for the expression of Pol I' and of phage lambda untargeted mutagenesis in SOS-induced cells. Previous data on SOS mutagenesis was obtained in *polA* mutants that are not completely deficient in polymerase activity New, well characterised, mutants have been recently isolated in which either the polymerase or the exonuclease activities are totally defective (Joyce *et Al.*, 1984) We used these mutants to study untargeted mutagenesis of phages with single or double stranded DNA. The results are consistent with the interpretation that the expression of untargeted mutagenesis in the double stranded DNA phage lambda as well as in the single stranded DNA phage M13 depends on the polymerase but not the exonuclease 5' → 3' activities of Pol I However, UV-induced mutagenesis of the bacterial chromosome was not affected in either *polA* mutant (Maenhaut-Michel and Caillet-Fauquet, 1990).

3.2 Specific strand loss in damaged DNA. (G. Maenhaut-Michel, in collaboration with N. Koffel-Schwartz and R.P.P. Fuchs, IBMC Strasbourg)

We analysed the effects of gamma- or UV-irradiation on the expression of both strands of heteroduplex DNA molecules in phages lambda and ϕX174. The principle of the method consists in the use of a double stranded DNA genetically labelled on each strand by means of a single base-pair mismatch conferring a wild type phenotype to one strand and an easily identified mutant phenotype to the other We have shown that UV lesions in DNA generate the loss of one of the two strands when both strands are UV-irradiated When only one strand has been UV-irradiated, only the genotype of the intact strand was found among the descendants (Z Trogocevic and M Radman, unpublished results) This UV-damage stimulated strand loss seems to be less important in *umuC*⁻ mutants suggesting that the *umuC* gene product might be involved in this process.

We found a similar result using ϕX174 DNA However, when gamma rays are used in place of UV, no obvious strand loss was observed, at least in the range of doses investigated. For similar survival of ϕX174 heteroduplex DNA, UV light generates strand loss with a higher efficiency than gamma rays

We have undertaken a collaboration with the R.P.P Fuchs'laboratory (IBMC, CNRS, Strasbourg, France) N-2-Acetylaminofluorene (AAF), a well-known chemical carcinogen, when covalently linked to guanine residues, constitutes a premutagenic lesion that is converted into frameshift mutations *in vivo* In *E coli*, it is thought that -AAF adducts block the replication fork and that the mutagenic processing of the -AAF adducts is mediated by the SOS response. The *in vitro* construction of plasmids containing -AAF adducts in one strand only of the double stranded DNA molecule enabled us to investigate both strand segregation and lesion mutagenicity *in vivo* When -AAF adducts are present in one strand only, in 90% of the transformants there is a consistent loss of the information contained on the parental strand that contains the AAF adducts. In the constructions having -AAF adducts in both strands, the transformed bacteria carry exclusively either one or the other allele. Our results suggest that when blocking lesions (pyrimine dimers or -AAF adducts) are present in one strand only, they trigger the specific loss of that strand

On the other hand, when lesions are present in both strands, the progeny has the pure phenotype of one or the other allele. We suggest that the presence of a blocking lesion in one strand leads to the uncoupling of concurrent replication (Koffel-Schwartz et Al., 1987)

3.3 Mechanism of frameshift mutagenesis induced by an ultimate model carcinogen.(G. Maenhaut-Michel, in collaboration with R. Bintz and R.P.P. Fuchs, IBMC Strasbourg)

AAF-induced mutations are not distributed randomly along DNA but are found to be clustered within specific sequences referred to as "hot spots" The correlation between AAF-induced mutation hot spots and DNA sequences has been extensively studied (Fuchs, 1983) More than 90% of mutations induced by AAF adducts are frameshifts whose induction depends on UV-irradiation of the bacteria These mutations appear either within monotonous runs of guanine residues (repetitive sequences) or within short stretches of alternating GC sequences The mechanism inducing mutations in repetitive sequences involves the participation of *umuDC* gene products and RecA protein, whereas the mechanism inducing mutations in alternating GC sequences does not. A strategy to study this specific pathway of mutagenesis was developed involving the search for new mutants of *E.coli*, suppressing specifically this type of mutagenesis Several mutants were isolated in this way. They were mapped by Hfr crosse, P1 transduction and complementation with F' episomes. One of them is thermosensitive, maps between 63' and 68' and is sensitive to UV. It is suggested that it could be in the primase, i e in the *dnaG* gene. Further precise mapping is under investigation. Another mutant was found to map in the *recA* gene This new *recA* mutant is defective in recombination and in UV-induced mutagenesis Further characterization of this mutant, including protein purification is under investigation.

3.4 Development of a method to purified mismatch recognizing proteins from *E.coli*. (A. Brandenburger, in collaboration with J.D.Franssen)

The proteins that are involved in the methyl-directed mismatch repair are coded by the *mutH, mutL, mutS* and *mutU* genes We have tried to isolate Mut proteins by immunoprecipitation from bacterial extracts. Purified Mut proteins were to be used as a probe for the detection of divergences in nucleotide sequences between two homologous genomes. We benefited from the help of Dr. J.D. Franssen (Laboratory of Immunology, D.B.M., Université libre de Bruxelles) for the immunological part of this project

Experimental approach:

1) *In vivo* fusion between the *E.coli lacZ* gene, coding for ß-galactosidase and each of the *mut* genes (Casadaban *et Al.*, 1980). The expression of the hybrid proteins is controlled by the *mut* promotors.

2) *mutL/lacZ* and *mutS/lacZ* fusions were cloned onto a plasmid to amplify the production of hybrid proteins This step was necessary because *mut* promotors were found to be very weak and the level of hybrid protein synthesis (10 to 20 molecules per cell) was too low to allow their purification from crude extracts (Brandenburger *et Al* , 1984)

3) Purification, by affinity chromatography, of hybrid Mut/ß-gal proteins from extracts of bacteria that carry the fusions on a plasmid (Ullman,1984) The yield with this method was very low with our hybrid proteins and did not allow their purification

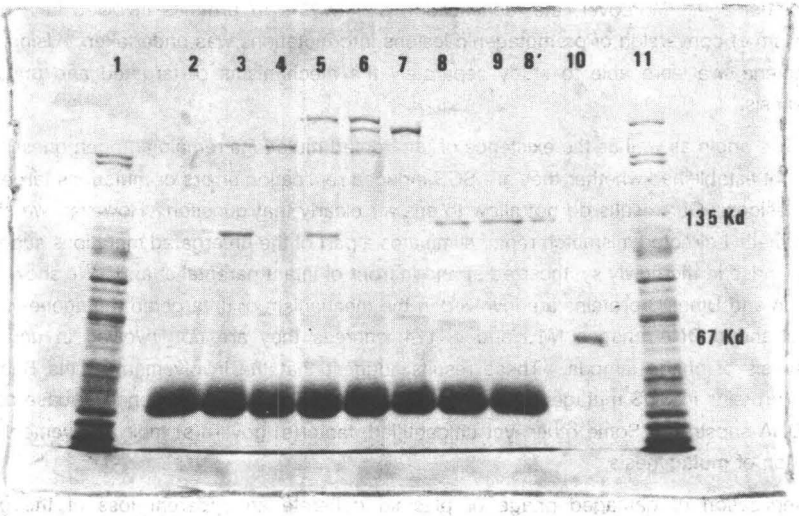
4) Immunization of mice with the MutS/ß-gal protein and cell fusion to obtain monoclonal anti-MutS antibodies. In this first fusion, we only obtained anti-ß antibodies (J D. Franssen, unpublished results). However we isolated from this fusion anti-ß-gal antibodies that allowed to elute ß-galactosidase at a lower, non denaturing pH, than usual anti-ß-gal antibodies.

5) Purification of hybrid proteins with these monoclonal anti-ß-gal antibodies. We could in this way purify the two hybrid proteins with a much higher yield than that obtained by chromatography.

6) The purified proteins were used to immunize mice We have injected the mice three times with 14 day intervals. Repeated injections have been reported to stimulate the immune response against the non-ß-gal part of a hybrid protein in rabbits (Jacq *et al.*, 1984) However, we did not obtain the same results in our system 100 out of 1000 hybridomas secreted anti-ß-gal antibodies but none secreted anti-Mut antibodies. We have concluded from these results that the immune response against ß-galactosidase is very strong in mice.

We propose two solutions to resolve this problem. i) to immunize rats or rabbits instead of mice and ii) to replace the ß-gal part of the hybrid Mut/ß-gal proteins by the mouse immunoglobulin lambda light chain gene Mouse immunoglobulins are proteins of the self and, therefore will not trigger the immune system of the mouse. A plasmid carrying the mouse immunoglobulin lambda gene as well as antibodies directed against this protein are available in our institute (Laboratory of Immunology)

Figure: SDS-PAGE (5%) of immunoprecipitated extracts containing different plasmids: lane 3; pULB3000 (L1 fusion on pBR325), lane 4; pULB3010 (L1 fusion on pMC1403), lane 5; pULB3005 (S4 fusion on pBR325), lane 6 "up" mutant derived from pULB3005, lane 7; pULB3011 (S4 fusion on pMC1403). Host strains are RH4630(Del*lac* F' *lacZ* DelM15) and RH4641(Del*lac*) for plasmids derived from pBR325 and pMC1403 respectively. Lanes 2, 8 and 9; controls RH4630, ß-gal 1μg and 2μg respectively of pure ß-galactosidase were precipitated with anti-ß-gal antibodies in the same conditions as the other extracts. Lane 8'; 1μg pure ß-gal was directly applied on the gel. Lane 10; 1μg BSA. Lanes 1 and 11; 15 μg crude extracts of bacteria containing pULB3010 and pULB3011 respectively.



4 Discussion.

We have slightly modified our initial objectives taking into account progress achieved in the related field. The spectrum of mutagenesis in response to specific damage has been extensively studied in several laboratories these last years. The analysis of mutations induced by several SOS-dependent mutagens revealed that each treatment generated its own characteristic site-specific and mutation-specific spectrum. But the molecular mechanisms of mutagenesis are not yet elucidated.

We have focused our efforts on the study of the mechanisms by which DNA damage are converted into mutations by looking for cellular functions affecting mutagenesis. Mutations are not randomly distributed along DNA. Several factors govern the conversion of premutagenic lesions into mutations among them, reactivity of DNA bases, sequence context, local configuration of the DNA helix...We have tried to determine what favours a mutagenic response following some types of damage. Moreover, the identification of new *E.coli* proteins involved in a specific mechanism of conversion of premutagenic lesions into mutations was undertaken. Using phage and bacteria, we were able to study separately the mechanisms of targeted and untargeted mutagenesis.

The origin as well as the existence of untargeted mutations remain an open question. So far it is not established whether they are SOS-induced replication errors or mutations targeted on cryptic lesions. Our results do not allow to answer clearly that question. However, we showed that the methyl-directed mismatch repair eliminates a part of the untargeted mutations suggesting that they arise in the newly synthesized strand in front of intact parental strand. We showed also that RecA and UmuDC proteins are involved in the mechanism of untargeted mutagenesis of the single stranded DNA phages M13 and ϕX174 whereas they are not involved in untargeted mutagenesis of phage lambda. These results suggest that the involvement of the RecA and UmuDC proteins in SOS mutagenesis may be related not only to the presence of base damage in the DNA substrate. Some other, yet unidentified, factor(s) govern(s) their involvement in the expression of mutagenesis.

Replication of damaged phage or plasmid generate an apparent loss of the genetic informations contained in one of the two parental strand. We hypothesized that lesions blocking the replication fork, might provoke uncoupling of the replication complex allowing the replication to continue only on one of the two strands.

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Maenhaut-Michel, G. (1988) Genetic characterization of the SOS mutator effect in E.coli. In "DNA Replication and MUtagenesis" eds. R.E. Moses and W.C. Summers, pp. 332-338.

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Centre National de la
Recherche Scientifique
Quai A. France, 15
F-75700 Paris

Contract no.: BI6-E-163-F

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

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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.: BI 6 - E - 163 - F

Molecular analysis of mutagenesis in mammalian cells treated by radiations and chemical carcinogens.
Use of specific genes as probes for mutagenesis, oncogenesis in xeroderma pigmentosum patients, and characterization of DNA ligase activities in human cells.

Head(s) of project:
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Scientific staff:

Alain SARASIN, Alain GENTIL, Mauro MEZZINA, François BOURRE, Leela GROSJEAN, Michael R. JAMES, Catherine MADZAK, Anne STARY, Anne LICHTENBERGER, Horacio G. SUAREZ.

I. Objectives of the project:

a) Characterization at the molecular level of the type of mutation induced by UV-light or by different chemical carcinogens in mammalian cells : development of modified SV40 vectors and of several shuttle vectors.

b) Analysis of oncogenesis in the xeroderma pigmentosum disease.

c) Characterization of DNA ligase(s) in mammalian cells.

II. Objectives for the reporting period:

III. Progress achieved:

I - Initial aims of the project.

The general theme of the research I proposed in 1985 was the study of relationships between the existence of DNA lesions induced by radiations or carcinogens and their consequences on the initiation of carcinogenesis. DNA repair processes are very important in this respect since a mutation event could either come from an unrepaired lesion or a lesion repaired by an error-prone process. This latter process, called SOS repair in bacteria, has been shown to occur in specific conditions in mammalian cells.

Exogeneous DNA is very useful to analyze inducible SOS functions since it permits one to discriminate between the "inducing" treatment carried out on the cell, and the DNA lesions to be repaired. Animal DNA viruses or shuttle vectors can be used as biological probes for studying SOS repair processes in eukaryotes. Cells are first treated by UV-radiation or chemical carcinogen, then infected with virus pre-irradiated in vitro. The way irradiated virus is repaired or mutated in treated cells can tell us how SOS repair works. Such experiments are carried out with monkey kidney cells or human cells and simian virus 40 (SV40). Numerous mutants of SV40 being well characterised, the virus permits the study of mutagenic properties of SOS repair.

Complementary to that topic, several processes linked to DNA repair were proposed to be analyzed by studying some human cells derived from cancer-prone patients and some enzymes involved in the repair pathway such as DNA ligases.

II - Results obtained during the reporting period.

a. Use of specific genes as probes for mutagenesis.

1 - SV40

A mammalian genetic system composed of a reversion assay from a temperature-sensitive ts phenotype towards a wild-type phenotype has been developed in our laboratory. Two types of viral genes have been selected as target : the early gene of the large T antigen and the late gene of the VP1 capsid protein. The main two ts mutants we studied (tsA58 and tsB201 respectively) are due to a single nucleotide change which modified a single amino-acid compared to the wild type sequence. These two systems have been worked out in order to follow mutagenesis efficiency after virus or naked DNA-treatment by ultraviolet-light (UV), acetoxy-acetylaminofluorene (AAAF) or heat under acidic conditions, in order to induce apurinic sites. Mutation spectra were also produced from sequencing revertant genes after mapping mutations by using the marcker rescue technique.

UV-mutagenesis is essentially due to point substitutions located opposite pyrimidine-pyrimidine sequences. It is known that UV-light induces pyrimidine-dimers (Py-Py) and pyrimidine (6-4) pyrimidone photoproducts. By using the photolyase of E. coli which remove specifically Py-Py from UV-irradiated SV40 DNA but does not react with Py(6-4)Py, we showed that pyrimidine dimers are essentially responsible for virus lethality after UV-irradiation. Both lesions are mutagenic in this assay giving rise to base substitutions opposite them. However, the mutation spectra are

partially different indicating that both lesions are mutagenic but do not induce the same type of mutations. The direct comparison of the two spectra tells us the respective role of these two lesions and eventually of a new lesion we have found present on the ACA sequence. Moreover, mutation spectra in general were found to be sensitive to the mode of DNA transfer into the host cell. DNA lesions are more lethal and more mutagenic when virus infection is used as compared to naked DNA transfection. Some mutation hot-spots are common to the two protocols whereas other hot spots are found with only one or the other. Interestingly enough, several scattered base substitutions were found after DNA transfection while usually only one mutation was found after virus infection. Similarly, the spontaneous mutation spectra are different according to the experimental way to introduce the DNA into the cells. The spontaneous hot spots appeared to be essentially present on putative secondary structures after DNA transfection.

We have continued using the simian virus 40 as a biological probe for analysing mutations in mammalian cells induced by chemical carcinogens such as the N-acetoxy-N-2-acetylaminofluorene. In vitro treatment by AAAF of the SV40 virion increases mutagenesis and decreases survival in the viral progeny. A lethal hit of approximately eighty five acetylaminofluorene-adducts per SV40 genome has been calculated. The molecular analysis of independent SV40 revertants showed that N-acetoxy-N-2 acetylaminofluorene induces base substitutions which are located not opposite putative acetylaminofluorene adducts but next to them. Moreover, a hot spot of mutation restoring a true wild type genotype

is observed in 65 % of the revertants analyzed. This hot spot, not targeted opposite a major DNA lesion, was not observed using UV-light as damaging agent in the same genetic assay. Two models involving the stabilization by N-acetoxy-N-2-acetylaminofluorene of a secondary structure of a specific quasi-palindromic SV40 sequence have been proposed to explain this sequence-specific hot spot.

2 - Shuttle vectors

To avoid some limitations observed with the SV40 system in which only reversion assay can be performed, we have developed new alternative probes such as Epstein-Barr virus-based shuttle vectors and SV40-based shuttle virus.

Shuttle vectors are plasmids able to replicate and to be selected in both mammalian cells and bacteria. Mutations are produced in mammalian cells, then screened and analysed in bacteria. Several targets to study mutation spectra have been inserted such as lacO, lacZ' and supF sequences. We also developed the shuttle virus system in which the genome can be packaged as SV40 pseudo-virion without excision of plasmid sequences. Some of these virus exhibited a low spontaneous mutation frequency and are able to infect and to replicate in monkey COS cells. In order to do that, we have cloned in the SV40 genome and in place of the large T antigen gene, the smallest bacterial plasmid ($\pi\Delta$lac). Such vector replicates in COS cells which bring in trans the large T antigen and produces SV40 virions due to the presence of the late viral genes. Specific sizes of the shuttle vector genomes can only allow

encapsidation. Sizes about 800 bp smaller than the SV40 wild type genome have the best efficiency of encapsidation. The use of SV40-based shuttle virus can be as useful as SV40 with the advantage to have mutation targets selected in bacteria.

More recently, we have developed an SV40-based shuttle vector able to be produced as single-stranded DNA in bacteria due to the insertion in either orientation of the f1 replication origin sequences. This vector, containing the supF gene, has been used to produce UV-induced mutation spectra after treatment of a given strand. Results clearly showed that mutations were targeted opposite py-py sites (and not opposite Pu-Pu sites) and a very high propotion of targeted tandem mutations or closely-close mutations were recovered. Several scattered mutations were found along the supF sequences as we reported for SV40 when naked DNA was used instead of encapsidated DNA. These mutations could be due to DNA lesions produced during the transfection process and to the presence of an error-prone DNA polymerase.

This single-stranded probe allowed us to produce heteroduplex molecules in which a given DNA strand has been UV-irradiated while the other did not contain DNA damages. These molecules were replicated by COS7 cells but the damaged strand was completely lost during replication and only the undamaged one was recovered in the progeny. This results demonstrates the existence of strand loss in heteroduplex molecules as it was reported in bacteria and may indicate technical problems when one wants to study mutagenesis of unique DNA lesion.

We were also interested in Epstein-Bar virus based shuttle

vectors which replicate episomally in human cells. Since the copy number of these vectors is usually very low (between 10 and 100 per cell) we tried to increase it by cloning in such vectors the SV40 replication origin. Therefore in conditions where the SV40 T antigen is expressed, this vector (p205-GTI) is replicated on the SV40 mode which should lead to a high copy number per cell. Interesting results appeared when we studied the replication of this vector in cells expressing the SV40 T antigen. Indeed, a very high level of intramolecular recombination was detected. DNA sequences of the recombinational hot spots indicated that the SV40 enhancer and promoter sequences were directly involved in the recombination sites.

b - Oncogenesis in Xeroderma Pigmentosum patients.

Xeroderma pigmentosum (XP) is a rare hereditary disease transmitted as an autosomal recessive trait. The classical XP cells are deficient in the excision DNA repair pathway and can be classified into 8 complementation groups. This syndrome is characterized by a very high incidence of epithelioma produced only on sun-exposed skin. This disease was the first example and still the best one to demonstrate the direct correlation between the presence of unrepaired DNA lesions and the induction of tumours. We therefore decided to determine if and how oncogene activation may take place in tumours isolated from XP patients in order to correlate the presence of unrepaired lesions and the type of oncogene modifications. The aim of this study was to determine if a specific pattern of oncogene activation could be found in these

tumours and could be correlated with the specific types of UV-induced DNA lesions.

Several baso-cellular and squamous cell carcinomas from XP patients were collected and tumoral DNA or RNA were prepared. We have isolated by the NIH 3T3 assay three activated oncogenes derived from three different XP tumours among 12 tumours tested. Two activations were due to a base substitution on the 61 codon of N-ras and one was found on the 12 codon of Ki-ras. In all cases, the mutation arrived at a position where a UV-induced DNA lesion (CC or TT) can be made after sun-light exposure. In these tumours containing an activated ras, amplified and overexpressed C-myc or Ha-ras were found. More characteristically, we found that 30-40 % of XP epithelioma harbored an amplified Ha-ras. These results appeared to be either specific of XP tumors or specific of UV-induced skin epithelioma. It is interesting to note that the pattern of oncogene activation reflects quite exactly what could be anticipated from mutation spectra derived from model systems such as bacteria or shuttle vectors. This result confirms nicely that the UV-induced skin tumors in XP are essentially due to the presence of unrepaired DNA lesions. UV-induced DNA lesions are known to give rise to gene amplification in some treated cells probably due to the blockage of DNA polymerases and multiple reinitiations at the same replication origin. It is therefore interesting to note that a high level of oncogene amplification was found in XP skin tumors which could be due to the presence of unrepaired lesions as shown in cultured cells.

c. Characterisation of DNA ligase activities.

In our laboratory and in collaboration with Dr. U. Bertazzoni (Pavia), new assays to qualitatively and quantitatively measure DNA ligase activities both in crude extracts and in purified fractions have been developed. These assays were used to determine the relationships and the characteristics between DNA ligase I and DNA ligase II found in mammalian cells. The technologies were worked out in rat liver enzymes to benefit from a great amount of material and then, applied to cultured human cells isolated from various patients in order to detect if there is any DNA ligase-defective disease in man. In particular, 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 labelling 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.

We have also analyzed qualitatively and quantitatively DNA ligase activities in cultured fibroblasts and lymphoblasts derived from normal, XP, ataxia telangiectasia, Fanconi's anemia and Bloom's syndrome (BS) patients. No significant differences were found in ligase activity between these different cell lines. However for ligase I isolated from BS cells we found it was precipitated

during the polymin P precipitation step in the pellet with nucleic acids, while the enzyme from the other cells stayed in the supernatants. This result suggests that the amount of enzyme is normal in BS compared to control cells, but the structure of the enzyme could well be partially different between the two types of cells. This divergent behaviour for the BS ligase may explain the discrepancy published in the literature with this syndrome.

III - Conclusions.

We have developed most of the techniques we proposed five years ago in the application and during the course of this project we even carried out new experiments and constructed more interesting vectors. Particularly, our vectors producing a single-stranded genome are very useful for analyzing at the molecular level the initial role of DNA lesions in the mutagenic process. These vectors are potential probes for studying the effect of various genotoxic drugs and eventually various protecting agents. In general, shuttle vectors could constitute an elegant probe for evaluating the role and the effect of radioprotection compounds. Cells can be treated by the protecting agents and then DNA-damaged shuttle vectors can be transfected into these cells allowing the screening of positive effect. Such protocol exhibits the enormous advantage that the target used for measuring biological effect (here shuttle vector) is different from the target of the compound to be tested.

Finally, this European project has made possible for us to collaborate and publish with other European laboratories and

constitutes therefore an obvious help to extend our scientific network.

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

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V. Publications:

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-E-164-UK

Medical Research Council
20 Park Crescent
GB-London W1N 4AL

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

Dr. J.R.K. Savage
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Harwell, Didcot
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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.:

Head(s) of project:

Dr J R K Savage

Scientific staff:

Dr Z S Aghamohammadi

I. Objectives of the project:

To refine and apply replication-banding techniques to *in vitro* cultures as a means of assigning cells, cytologically, to their position in the cell cycle at the time of radiation or other clastogenic treatment. Chromosomal aberrations can then be scored in marked cells which can later be "unscrambled" and replaced into cohorts in correct chronological order irrespective of perturbation and mitotic delay, to provide much more reliable quantification.

II. Objectives for the reporting period:

Final report

III. Progress achieved:

Introduction

The cell replicates the bulk of its chromatin within a discrete period during the interphase between successive divisions, conventionally termed "S-phase". This phase lasts 6-7h in most "normal" mammalian cells cultured *in vitro*.

At the level of chromosome bands the process of replication can be seen to be discontinuous and the pattern of bands operating changes during the transit of S-phase. The overall programme is, however, extremely precise, each pair of autosomes having their own independent programme (defined by order and temporal spacing of band activity) yet keeping in step with other chromosomes within the same cell. Thus, given the pattern reached by one pair, that for any other pair can be predicted with reasonable accuracy.

The patterns and programmes can be readily investigated using the thymidine analogue, bromodeoxyuridine (BrdU). When added to a culture of growing cells, all subsequently replicated chromatin incorporates bromouracil. Consequently, all cells in S-phase at the time of addition arrive at the next metaphase with two types of chromatin; that made before BrdU arrives (TT) and that made after (TB). These two types can be made to stain differentially. The relative amounts of TT and TB will depend upon the position in S-phase of the cell when BrdU arrived, and detailed evaluation of the patterns displayed can be used for a variety of purposes as demonstrated in this report.

Initially, we developed keys to provide a finer analysis of the cell cycle than is obtainable with the usual method using tritiated thymidine. These keys have been used to investigate replication programmes in a variety of cultured cell types and then to "unscramble" populations subject to perturbation and delay after radiation or chemical clastogen treatment. This "unscrambling" facility allows us to study the production of chromosomal aberrations in relation to the cell cycle in both normal and abnormal cells.

By studying the replication programmes we have developed a novel method for comparing programmes in different cultures and after different treatments. This method overcomes many of the inherent problems imposed by having to work with a dynamic situation using "still" measurements.

Finally, in the light of certain limitations which arise from the standard "terminal" BrdU protocols, we have developed a reverse staining method which allows us to detect "pulses" of BrdU so that we are now in the position to answer such questions as "What is replicating at this point in time?" and also to make cohorts of cells that can be identified and scored later in time irrespective of radiation induced perturbation.

All these applications help to overcome difficulties in the quantification of induced aberrations which are inherent in work with asynchronous cell populations.

Cell position assignment keys

The time of appearance of replication bands during S-phase is so precise, that specific bands can be used to delimit objective borders or stages within S. Judicious selection of bands allows sub-division of S into 4-6 sub-phases. These sub-divisions are very consistent as has been shown in primary cells of both Syrian hamster and human origin (1,3).

Of the keys tested in human cells, two have proved particularly reliable and useful. Key 4, based on bands in chromosomes 3 and 4, which produces 4 sub-phases and key 5 which is based entirely on bands in chromosome 5 and produces 6 sub-phases.

Both of these keys rely on the "terminal" application of BrdU (ie, BrdU added to the culture remains until sampling time) and a staining method (KG) which renders TT chromatin dark and TB chromatin pale. By definition then, early replicating regions (relative to the time of arrival of BrdU) appear as dark zones. If BrdU is added at or close to the time of treatment (eg, radiation) cells are "marked" as to cycle position when treatment was given and can be located and assigned, cytologically, at the time of scoring.

Application to experimental situations

Low-folate growth conditions are frequently used to produce cell synchrony and provide extra long chromosomes for high-resolution banding studies. It is said that substances which produce such conditions (eg, methotrexate) generally block cell progression at the G_1/S border causing accumulation there. Upon release, the accumulated cells progress as a synchronous wave to division. Reference to the literature, however, indicates some confusion as to the exact position of the block.

Using the principle that BrdU can overcome a methotrexate block, we have used an S-phase sub-division system to investigate the precise position of the block in Syrian hamster cells treated with the standard 16h methotrexate. We found that cells do not accumulate at G_1/S but appear to halt where they are in S, and on release, continue in unchanged order (like a column of marching soldiers) (5,8).

Low-folate conditions play an important role in the investigatiion of inherited chromosome fragile sites. These are specific regions that show excessive sensitivity to spontaneous breakage and in one case at least (fra Xq27) are associated with a form of mental retardation. In collaboration with the Wessex Regional Cytogenetics Unit in Salisbury, we used the sub-phasing system to investigate two problems, a) The nature of the "fragile" aberration by comparison with other low-folate induced chromatid-type aberrations present in the same cells, b) The time of production of fra-X in relation to the cell cycle.

Using BrdU to mitigate the effect of low-folate elevation of fra-X, we studied the fall in frequency of all aberrations in relation to cycle stage determined by sub-phasing and were able to show that the fra-X event is S-dependent, and probably occurs fairly early in S, and before the visible time of replication of Xq27. The fra-X aberration displays very variable expression and cannot be categorised to any known conventional

structural chromatid intrachange (6,9).

In a previous contract we began to investigate the formation of aberrations in relation to the cell cycle by various agents. For the S-dependent sulphur-mustard we showed by sub-phasing that the observed localization of aberrations to late-replicating chromatin was an artefact produced by the selective interphase death of early S cells. We termed the phenomenon "localization by default" (2). The most localized aberration distribution known is that produced by mitomycin C (MMC) in human lymphocytes which would be consistent with the interphase death of all but the last period of S-phase. In conjunction with visiting workers we tested this hypothesis using sub-phasing and found this not to be so. Although a large amount of cell killing took place, it did not appear to be preferential for early S, and localization was found in pre-S cells to the same degree. Less localization appeared to be present in cells from a Fanconi anaemia source (4,7).

Since the early sulphur mustard work had been done in Syrian hamsters, which have a lot of late-replicating chromatin we have begun to repeat the experiments using nitrogen mustard (a close structural relative of sulphur mustard) on human blood. Preliminary results suggest that preferential selection may be a concentration-dependent phenomenon.

Sub-phase analysis has also been used to investigate induction by ultrasoft X-rays of chromosome aberrations at different phases of the cell cycle in BHK Syrian hamster cells (Contract BI6-A-009-UK) (14).

Between culture comparisons

Good general comparisons between different cultures, cell types and treatments can be made by superimposing full sub-phase analysis curves, but this is very time-consuming. Comparisons at single sample times are confounded by differences in cell kinetics, bearing in mind that replication is a dynamic process.

The time of appearance of specific bands is very precise and, with a terminal BrdU protocol, the band appearance curves (BAD curves) approximate very well to cumulative normal distributions. Using known mathematical properties of such curves, it is possible to make a legitimate comparison (for a sub-set of bands) between two cultures at a single time (which does not have to be identical for each culture). If the programmes of replication (defined by order and temporal spacing) are identical in the two cultures, then the probits of the band frequencies will lie on a straight line at 45^0. Departures from this situation indicate differences between cultures, the nature of which can often be determined by inspection.

We have applied this method to several problems, showing, for example, that for early replicating bands on several chromosomes, replication programmes are identical in normal and ataxia telangiectasia primary fibroblasts (10). The same is true for early and late-replicating X chromosomes in normal human lymphocytes and fibroblasts (11).

Reverse staining and pulse BrdU protocols

"Terminal" BrdU protocols have a number of disadvantages. One is that the band criteria for selecting sub-phase borders change from discrete dark bands in early-S phase cells to unfilled "gaps" in late-S cells, and the former is much more objective for the human eye. Furthermore, whilst detailed study of the earliest bands to replicate and the latest to finish is easy, the precision for mid-S is rather poor.

The usual (KG) staining gives pre-BrdU bands dark and special BrdU/thymidine protocols are needed to study late-replicating bands.

Staining methods do exist for "harlequin" chromosomes which will reverse the reaction, giving BB chromatin dark and TB light. We have adapted one of these to give TB dark and TT light. Not only does this allow us to examine late-replicating bands in detail with a terminal BrdU protocol, but more interestingly, it allows us to detect (as dark bands) pulses of BrdU as short as 5 mins.

We are now in a position to develop sub-division keys using a constant criterion (the presence of a dark band) throughout S.

So far we have applied the "pulse" protocol in two areas. First, a transit of S using 10 min pulse to investigate the mid region. We have shown that, contrary to popular belief, there is no clear break between the replication of R-zones and G-zones but a confused transition region exists where activity can be found in both within the same cell (13).

Second, using longer pulses (2h) we have isolated, cytologically, a cohort of S cells which can be identified at second division for the purposes of scoring sister-chromatid exchanges (SCE), thus purifying the very heterogeneous second-division population usually scored and so improving quantification (12).

Pulse protocols are much more versatile for experimental work, and we propose to concentrate future research in this direction.

1. Savage, J.R.K. & Bhunya, S.P. (1980). Chromosoma 77: 169-180.

2. Savage, J.R.K. & Breckon, G. (1981). Mutation Res. 84: 375-387.

3. Savage, J.R.K. & Prasad, R. (1984). J. Med. genet. 21: 204-212.

4. Savage, J.R.K. & Cao, S. (1985). Mutation Res. 150: 307-312.

14. Wilkinson, R.E., Goodhead, D.T. & Thacker, J. (1985). Rad. Protec. Dosim. 13: 161-165.

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

V. Publications:

5. Savage, J.R.K. & Prasad, R. (1986). J. Med. Genet. 23: 80 (ABS).

6. Dewdney, R.S., Savage, J.R.K. & Fitchett, M. (1986). J. Med. Genet. 23: 80 (ABS).

7. Savage, J.R.K. & Reddy, K.S. (1987). Mutation Res. 178: 65-71

8. Savage, J.R.K. & Prasad, R. (1988). Mutation Res. 201: 15-201

9. Savage, J.R.K. & Fitchett, M. (1988). Chromosoma 96: 391-396.

10. Savage, J.R.K. & Papworth, D.G. (1988). J. Theoret, Biol. 134: 365-377.

11. Reddy, K.S., Savage, J.R.K. & Papworth, D.G. (1988). Human Genetics 79: 44-48.

12. Aghamohammadi, S.Z. & Savage, J.R.K. (1989). Mutation Res. 216: 259-266.

13. Aghamohammadi, S.Z. & Savage, J.R.K. (1990). Chromosoma, (in press).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Greek Atomic Energy Commission
National Research Center for
Natural Sciences "Demokritos"
Aghia Paraskevi POB 60228
GR-153 10 Athens

Contract no : BI6-E-224-GR

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

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Telephone number: 301-6513111

Title of the research contract:

Radiobiological damage induced into mammalian and human cells by low energy moncenergetic 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 calculacions 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. 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 radiaiton in biological systems.

II. Objectives for the reporting period:

Continuation of the work on determining RBE values for low energy monoenergetic protons. Estimation of RBE values from DSB induction and correlation of these values to RBE values from other end points. Study of the "repair effect" through the use of repair inhibitors following exposure to monoenergetic protons. Parallel orientation work on the use of inverse gas chromatography, and γ-γ perturbed correlation method developed by our group (Kalfas et al. 1984, Int. J. Appl. Rad. Isot. 35:889-893) will be used for the study of radiation effects of monoenergetic protons.

III. Progress achieved:

INTRODUCTION

The study of the radiobiological properties of protons is particularly interesting because their use in radiotherapeutical applications and because their properties concerning energy deposition in subcellular level make them useful in understanding the mechanisms of induction of biological damage. Thus knowledge gained from these studies is directly related to the optimization of clinical procedures and risk estimation probing focusing on genetic damage and related stochastic effects of charged particles. The purpose of this study was to estimate RBE values from different end-points of the radiobiological damage induced by protons that is the end-point of chromosome aberrations, DNA breaks and survival. Though survival is frequently used for the estimation of RBE values of protons and charged ionizing radiation particles, risk estimates for genetic damage seems more meaningful to be based on RBE values estimated from the study of chromosome or DNA damage.

METHODOLOGY

The biological system used in this work was the V-79 line from Chinese hamster cells incubated in polysterene petri dishes in the presence of Eagle's minimal medium with Earle's salts supplemented with foetal bovine serum. The medium was removed before irradiation and the cells were covered with a 8 μm thick mylar foil to maintain suitable and sterile atmosphere for the cells. Accelerated protons of 7.4 MeV (LET 5.8 keV/μm) and 3.0 MeV (LET 12.1 keV/μm) were used in this work. V-79 cells were exposed to Cobalt-60 gamma rays (LET 3.0 keV/μm).

The absorbed dose, in the case of protons, was estimated from the equation

$$D = k.j_c.L$$

where $k=1.602\times10^{-8}$, j_c=number of protons per 1 cm² of surface of the exposed cells and L=LET value for the accelerated protons in MeV/cm. The energy of protons crossing the cells and thus the corresponding LET values can be considered to remain constant as the protons pass through the cells due to the relatively small depth d of the attached cells. The d was estimated from equation

$$d = \frac{(4/3).\pi.r^3}{s}$$

where s = the mean surface area of the cells in the form of tapetium and r=the mean radius of detached cells following trypsimization. The d was estimated from these calculations, as low as 6.3 μm.

The number of protons j_c crossing 1 cm² of cell's tapetium was calculated from the equation

$$j_c = \left(\frac{d\sigma}{d\Omega}\right)_{lab} \cdot N\Omega$$

where N = the number of scattering nuclei per cm² of the cell target, Ω = the solid angle in sterads subtended by 1 cm² of cell surface relative to the gold target centre of the scattering chamber. The $(d\sigma/d\Omega)_{lab}$ factor is the Rutherford scattering cross section in the laboratory setup. The laboratory experimental set-up with a proton scattering gold foil of 5 μm thickness was attached to one exit of a Tandem van der Graaff accelerator.

More details on the experimental set up, the exposure to proton beam and the calculations involved are given in a previous publication (Peris, Pialoglou, Katsanos and Sideris, 1986, Int. J. Radiat. Biol. 50:1093-1100). The estimated dose distribution on the cell tapetia in the petri dishes was verified by the exposure of X-ray sensitive film placed at the exit of the beam. Survival data were adjusted by the least square method through the use of a specific algorithm (Marqard, 1963, J. Soc. Industr. Appl. Math. 2:431-441). The RBE values and other related parameters were estimated from these fitted functions.

The frequency of radiation induced dicentrics to which the tricentrics were added to the rings and the excess acentrics was calculated from material fixed and stained in situ in the petri dishes (Sideris et al., 1984, Stain Technology 59:187-192). The cells were exposed to radiation at tapetium stage when contact inhibition phenomena keep most of the cells at the G_1 phase. The colchemized cells were fixed following 17-19 h incubation so that most of the observed metaphases were at the first post-irradiation mitosis (Zoelelief and Baredsen 1983, Intern. J. Radiat. Biol.43:349-362). Abberant chromosomes classification was carried out according to the IAEA suggestions (IAEA 1986, Tech. Rep. Series no. 260).

Double Strand Breaks (DSB) of the DNA were measured with the neutral elution technique (cf. Bradley and Kahn 1979, Nucleic Acid Research 7:793-804) following labelling in situ with ^{14}C-Thymidine. The aliquots of the eluant as well as the filters were finally transferred in Aquasol-2 and their radioactivity was measured.

The method of Inverse Gas Chromatography (Gilbert 1984 In "Advances in Chromatography" 23) was introduced to study the relationship between radiation dose and the destruction of hydrogen bonds between the chains of the DNA double helix. This destruction of hydrogen bonds in the regions of near by induced single strand breaks (SSB) has been associated

damage of electric circuities at the Tandem Accelerator of the NCSR "Demokritos" during last year did not permit the completion of the work at the molecular level (DNA, DSBs, Tm, PAC) in material exposed to protons.

RESULTS AND DISCUSSION

The RBE_D-values related to reproductive death as they are estimated from survival following exposure to different doses of gamma rays and protons of 7.4 and 3.0 MeV (LET 3.0, 5.8 and 12.1 keV/m respectively) were calculated equal to 1.68 and 2.37 for protons of 7.4 and 3.0 MeV energy. The RBE^{D37} values were calculated equal to 1.60 and 2.10, while the RBE_a values were equal to 1.87 and 3.37 for the two kinds of proton beams. The a and b coefficients of the equation

$$S = e^{-(aD+bD^2)}$$

where S=survival fraction of exposed cells and D=dose are given below:

Irradiation	a (Gy^{-1})	b(Gy^{-2})
gamma rays	0.117	0.012
protons 7.4 MeV	0.219	0.024
protons 3.0 MeV	0.394	0.000

The RBE_D values estimated from the mean inactivation dose are of the order expected from previous work (Bettega et al. 1979, Radiat. Res. 7785-97 and 1983 Il Nuevo Cimento 2D:907-916) for EUE cells exposed to monoenergetic protons of 12 and 8 MeV. The respective RBE_D values calculated in this case were 1.4 and 1.6. In the same work the RBE_{D37} the RBE_{D37} for 8 MeV protons was calculated to be 1.20.

The increase of coefficient a as a function of the LET (keV/μm) follows a linear pattern. This increase is attributed to higher deposition of energy in the vicinity of DNA molecule (Chadwick KH and Leenhouts HP, 1981, The Molecular Theory of Radiation Biology, Springer-Verlag).

The RBE coefficient is decreasing with an increase of dose D as it is expected from the theory of Dual Action (Kellerer AM and Rossi HH, 1978, Radiat. Res. 75:471-488). The interaction distance (estimated from the a and b coefficients) d=0.37 μm. On the basis of Dual Action Theory it is expected to be less than 1 μm.

The RBE_D values estimated from the frequency of radiation induced chromosome aberrations was calculated to be 1.3±0.2 from the distribution of dicentrics and 1.4±0.4 from the distribution of acentrics that is lower from the RBE_D calculated on the basis of survival data. The RBE_a for 7.4 MeV

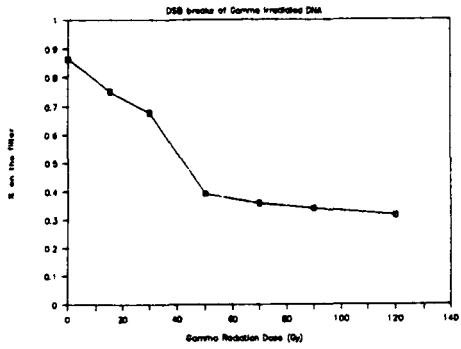

Fig.1 Retention of DNA molecules on polycarbonate filters measuring the induced by ionizing radiation (0 to 120Gy) Double Strand Breaks.

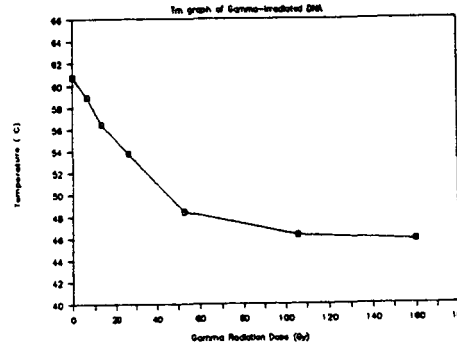

Fig.2 Annealing temperatures (T_M) related to the breakage of hydrogen bonds between the two chains of native DNA exposed to ionizing radiation.

(cf. Hagen 1967, Bioch. Biophys. Acta 134:45-58) with the appearance of double strand breaks which finally might evolve into chromosome breaks. To this effect the Gibbs Free Energy, at 25°C, of sorption between volatile and non-volatile organic compounds and DNA molecule was studied with the above mentioned method. The number of bonds is estimated by the function:

$$Y = G + n\{1 - e^{f\left(\frac{\theta-\theta_O)^{N+1}}{N+1}\right.}\}$$

where θ_O=Gibbs Free Energy.

The strength of bonding between the two chains of the DNA helix was studied in parallel with the classical method of Thermal Transition Spectrophotometry while the rotational correlation times and the corresponding quadrupole interaction frequencies in irradiated and non-irradiated DNA were measured with the Perturbed Angular Correlations (PAC) method (Kalfas, Sideris and Martin, 1984, Intern. J. Appl. Radiat. Isot. 35:889-893 and Sideris, Kalfas and Katsaros, 1986, Inorg. Chimica Acta 123:1-4). Finally the method of supercoiled to circular form transition of plasmid DNA, studied by gel electrophoresis was added to the methods currently used in the laboratory (Burchuladze, Sideris et al., 1990, submitted to Photochem. Photobiol.). It should be noted that an extensive

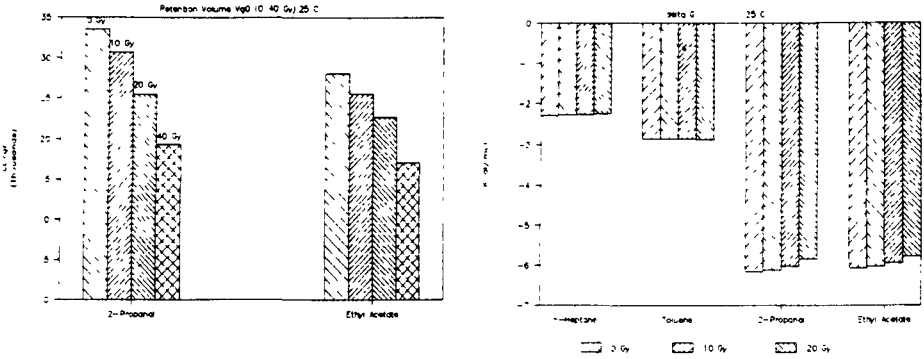

Fig. 3 (left) Retention volume (cc/g) during Inverse Gas Chromatography of two polar solvents by native DNA exposed to ionizing radiation (0-40 Gy) related to the number of existing hydrogen bonds and (right) Changes in Gibbs Free Energy (kcal/mol) in the presence of polar (2-Propanol and Ethyl Acetate) or non polar (n-Heptane and Toluene) solvents, of native DNA molecules exposed to ionizing radiation (0-40 Gy).

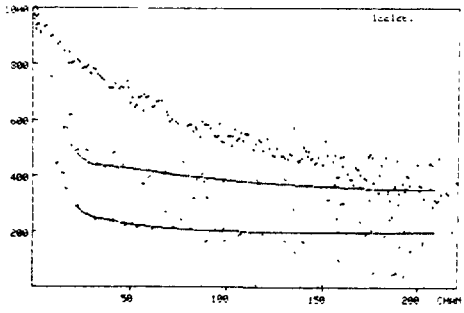

Fig. 4 Exponential $G_2(t)$ Perturbation Factors from PAC measurements and associated theoretical fits for buffer (upper curve), native DNA (lower curve) and native DNA exposed to 20 Gy (middlecurve). These Factors are related to the apparent rigidity of the DNA macromolecule.

protons was calculated equal to 1.0 the dicentrics and 0.7 for the acentrics that is considerably lower that the value calculated from survival data. These results indicate that RBE coefficients for low at least energy protons estimated from the frequency of induced chromosome aberrations do not necessarily correspond to those estimated from survival data. On the other hand should not be overlooked that the RBE_D and RBE_a values calculated from the chromosome aberrations data are not in agreement with the Dual Action Theory and the results from work with human lymphocytes (Edwards et al. 1986, Intern. J. Radiat. Biol. 41:654-656). On the basis of the chromosome aberration data the interaction distance (d) between two initial lesions and hence the target diameter seems to be of the order of 0.5 μm, which is of the same order

of magnitude with that estimated for human lymphocytes (Bauchinger et al. 1973, Mutat. Res. 20:107-113) irradiated with X-rays of 220 keV.

As it was mentioned in the methodology section of this report due to extensive damage of the Tandem Accelerator the work for the estimation of RBE on the basis of DSBs and the related areas of hydrogen bond destruction was not completed since the work with protons could not be carried out. The radiation controls, though, were completed and the results are given in Figures 1,2,3 and 4.

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

Gesellschaft fur Schwerionenforschung (G.S.I.) Biophysics Group (Dr. G. Kraft). Darmstadt

V. Publications:

PIALOGLOU P., E.G. SIDERIS, A. PERRIS (in press) Enhancement of cellular damage induced by gamma rays after inhibition of poly-(ADP-Ribose)-Polymerase by 3-Aminobenzimide. In Frontiers in Radiation Biology, Verlagsgesellschaft Publishers, pg 001-008.

PIALOGLOU P., E.G. SIDERIS, A. PERRIS (in press). Cell survival and chromosomal aberration frequency in V79 Chinese hamster cells exposed to low energy protons. In Frontiers in Radiation Biology, Verlagsgesellschaft Publishers, pg 001-007.

PIALOGLOU P. 1989 Genetic lesions induced in mammalian cells exposed to low energy proton radiation Ph.D. Thesis presented at the Department of Medicine. University of Athens.

RADIATION PROTECTION PROGRAMME
Final Report

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)]:

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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.:

The production of chromosomal 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 chormosomal aberrations. The primary objective was to verify the existence of any low dose plateau in the response over the range zero to a few tens of milligrays. Blood from a number of donors was irradiated and cultured in one laboratory and the microscope analysis was done in six collaborating laboratories. The extent of inter-donor and inter-laboratory variations in the data was also examined.

II. Objectives for the reporting period:

This report covers the whole study which has extended over 5 years. In interim reports each year's results have been summarised and during the final year the most recent work added data from blood of 20 donors irradiated to 5 and 300 mGy.

III. Progress achieved:

Methods

Expt 1. Blood from 4 healthy male donors was irradiated at 37°C to 8 doses (shown in table 1) with a internationally defined quality of x-rays; 169 keV isowide series. This was obtained from 250 kVp x-rays with a HVL of 4.3 mm Cu and gave a peak energy to the blood of 171 keV. Exposures were acute, ranging from 1-7 min and afterwards the blood was held at 37° for 2h. Lymphocytes were then cultured for 48h and harvested for metaphase preparations which were checked by FPG staining for low contamination with second division spreads. Microscope slides were coded and distributed to the collaborating laboratories.

Expt 2. Blood from 10 male and 10 female additional donors was irradiated and cultured with the same conditions as before. Fewer doses (shown in table 3) were used. Throughout the tables the numbered laboratories are as follows:

1. Chilton
2. Essen
3. Leiden
4. Mol
5. Rome
6. Sellafield

Results and Discussion

Table 1 shows the scoring results from expt 1 for dicentrics plus centric rings (D+R) and excess acentric fragments (AF) noted by each laboratory for the 4 donors and 8 doses. The pooled data have been presented previously (Lloyd et al Int. J. Radiat. Biol. 53, 49 1988) but the values given here differ slightly. This is mainly because on reassessment we have chosen to omit a few exchange aberrations seen in incomplete cells.

In table 2 we present the results of an analysis by the chi^2 method for variability between the 4 donors. For D+R there is significant variation at 5.8 and 290 mGy. At 5.8 mGy this is mainly due to one laboratory that scored 5 dicentrics for donor 4; four of the dicentics were contained in 2 cells. At 290 mGy the yields for donor 1 is considerably higher than the other subjects. This, taken together with the yields at 47.7 and 28.7 mGy, suggests that donor 1 may be more sensitive than the others. This possibility prompted experiment 2 which was designed to look for donor variation among a larger number of subjects.

Table 3 (a-d) lists the results of expt 2 and in table 4 donor variability has again been examined. As there were more donors studied it was possible to use the dispersion index test. For D+R there is some evidence of variability at 4.8 and 280 mGy but for the latter it is marginal. Overall the D+R do not exhibit a very marked inter-donor variability. Probably most of the observed variations arise from the occasional odd result. For example inspection of table 3b reveals that at 4.8 mGy the variation can be ascribed to labs 2 and 3's results for donor 7 which are inconsistent with the scoring from the other labs. At other doses however the data for donor 7 are unremarkable. If there were a strong donor effect one would expect some subjects to appear as either high or low at each or most doses and this is not immediately apparant in table 3. This has been examined more thoroughly in table 5 where for D+R a Friedman two-way analysis of variance ranking order test on the 20 donors has been done.

For each dose the donors have been ranked in order. Where two or more were equal the ranking has been averaged which accounts for some non-integral values. For each donor the rankings have been summed (R). The test states that if there is no correlation of rankings the statistic

$$\frac{12}{NK(K+1)} \sum_{i=1}^{K} R_i^2 - 3N(K+1)$$

should be distributed as chi^2 with K-1 degrees of freedom. For these data K = 20 (the no. of donors) and N = 4 (the no. of doses). The resultant value of chi^2 is 27.55. On 19 DF P = 0.093. This is a reasonable fit but could be interpreted as indicating a slight degree of donor variability. Donors 10 and 11 tend to have high yields whilst 19, 21 and 22 are low. The test was also performed for AF where Chi^2 = 37.8. P = 0.01 which shows definite donor variability with subjects 11 and 13 being high and 23 and 24 low.

Inter-laboratory variation has been examined in tables 6 and 7. In table 6 data from the 4 donors in table 1 have been combined and for D+R significant variability is seen at 19.3 and 290 mGy. Reference to table 1 shows that at both doses lab 3 has scored higher than the rest. Inspection of the other doses in table 1 leads to the conclusion that lab 3 has scored generally high. In table 7, inter-laboratory variation for expt 2 is considered and here there are more marked differences. Lab 3 again appears to be among the higher scorers. Lab 4 by contrast has tended to score low although this is not so apparant in expt 1. Inter-laboratory variability in scoring AF is much more marked in both experiments and this conforms with the general experience of many researchers that scoring for AF is less accurate than for exchange aberrations.

Despite the inter-laboratory variations and the marginal inter-donor effect we feel that it is permissible to pool the data provided equal weight is given to each lab/donor contribution. This permits any overall trends to be discerned and as each lab scored the same number of cells per point this does not introduce bias into the overall result.

Fig 1 shows the D+R and AF yields in expt 1 plotted against dose. The plotted uncertainties (SE) are such that the familiar $Y = \alpha D + \beta D^2$ model can be fitted to the points. The fitted coefficients obtained by the iteratively reweighted least squares method and the parameters of probability of fit are given in table 8. The data have been fitted to the quadratic model for all doses and the linear model over the range 0 - 47.7 mGy.

While the fits for D+R appear reasonable it should be noted that at 9.6 mGy and below the observed yields lie below the zero dose control value. This is shown as histograms in Fig 2 where dicentrics and rings are presented separately. Even with the large amount of scoring for each point the SEs are such that the yields are not inconsistent with a linear extrapolation down to the origin. Nevertheless the possibility of a low dose plateau in response over the range 0 - 9.6 mGy needs to be considered particularly in the light of a similar observation over a comparable range of x-ray doses by Pohl-Rüling et al (Mutat. Res. 110, 71 1983).

In Fig 3 the D+R and AF data from expt 2 are again shown as histograms. Again there is a suggestion that particularly for dicentrics the lowest dose dips below the control value. If rings are included with dicentrics the suggestion of a dip in yield is much weaker. The lowered

value at 4.8 mGy is mainly caused by the scoring from labs 1 and 4 where the yields of D+R appear exceptionally low (table 3b). Thus it has been decided that the two labs will reexamine this material suitably recoded but at the time of writing this report results are not available. Likewise the yield by lab 2 at 28.5 mGy will be checked.

Conclusion

Despite the considerable amount of scoring that has gone into this study the statistical uncertainties on the data points are quite considerable. The study appears to have reached the limit of resolution for the cytogenetic end point at low doses using analysis by conventional microscopy. Any improvement must await advances in automated systems that would permit orders of magnitude increases in the numbers of cells that may be examined and also reduce scorer variations. With present techniques it appears impossible to measure cytogenetically whole body doses below about 20 mGy and doses as low as this would require a reliable control measurement.

If the low dose response is really linear as has been the usual assumption in radiobiology, it is surprising that three separate experiments (Pohl-Rüling et al, and the two described here) have all shown, albeit each non-significantly, evidence for a low dose plateau in repsonse and a reduction below the control value at 10 mGy or less. In the present work this applies certainly for dicentrics and is still just evident if rings are included. For AF however there is no suggestion of a reduced yield below the control. Because AF predominate at low doses there is no reduced yield when all aberration types are combined. This contrasts with Pohl-Rüling et al who showed the effect at 4 mGy for all aberrations.

Dose (mGy)	Donor	Laboratory 1	2	3	4	5	6	Total
0	1	0/1	1/5	4/5	1/2	1/3	1/3	8/19
	2	1/0	2/2	0/1	2/0	0/0	0/1	5/4
	3	0/0	0/4	0/1	1/1	1/2	1/1	3/9
	4	0/0	0/0	1/1	1/0	2/2	0/0	4/3
	Tot	1/1	3/11	5/8	5/3	4/7	2/5	20/35
3.13	1	0/0	1/4	2/3	0/2	0/1	2/0	5/10
	2	0/0	0/4	0/1	0/0	1/1	0/1	1/7
	3	0/0	0/1	0/5	0/4	2/4	0/0	2/14
	4	0/0	1/0	1/5	3/3	0/7	0/1	5/16
	Tot	0/0	2/9	3/14	3/9	3/13	2/2	13/47
5.80	1	0/1	0/2	1/5	0/1	0/3	0/2	1/14
	2	0/0	2/3	0/2	0/0	1/1	0/1	3/7
	3	0/0	+1/3	0/1	0/1	1/1	0/1	2/7
	4	1/3	0/5	0/3	0/1	3/3	5/2	9/17
	Tot	1/4	3/13	1/11	0/3	5/8	5/6	15/45
9.65	1	0/0	0/4	1/2	0/1	1/4	1/3	3/14
	2	1/0	+2/1	1/5	0/2	0/4	0/0	4/12
	3	0/0	0/2	0/1	1/2	1/6	1/0	3/11
	4	0/1	0/1	2/1	0/1	1/3	1/1	4/8
	Tot	1/1	2/8	4/9	1/6	3/17	3/4	14/45
19.3	1	1/2	0/2	3/4	0/1	0/3	0/0	4/12
	2	3/1	0/8	1/3	+1/14	1/4	0/1	6/21
	3	1/0	0/4	2/1	0/2	1/1	0/0	4/8
	4	2/0	0/3	+8/6	0/1	0/3	1/2	11/15
	Tot	7/3	0/17	14/14	1/8	2/11	1/3	25/56
28.7	1	3/1	0/3	2/5	1/3	+5/6	1/0	12/18
	2	0/1	0/3	2/1	1/4	2/2	+2/0	7/11
	3	3/1	1/3	0/2	1/2	1/4	1/0	7/12
	4	1/2	+1/6	2/3	1/0	1/4	+1/1	7/16
	Tot	7/5	2/15	6/11	4/9	9/16	5/1	33/57
47.7	1	1/1	0/6	4/2	3/3	—	2/3	10/15
	2	0/0	1/2	0/5	0/1	2/5	0/2	3/15
	3	0/0	2/4	0/0	0/1	3/2	0/1	5/8
	4	3/2	1/2	2/0	1/1	3/9	0/0	10/14
	Tot	4/3	4/14	6/7	4/6	8/16	2/6	28/52
290	1	+7/7	+5/9	+17/4	11/6	7/7	+15/3	62/36
	2	3/5	3/13	+8/7	3/6	2/10	4/6	23/47
	3	6/6	5/8	10/8	3/6	7/6	4/5	35/39
	4	7/5	5/5	8/4	5/3	6/5	6/8	37/30
	Tot	23/23	18/35	43/23	22/21	22/28	29/22	157/152

Table 1

Experiment 1 Aberrations scored in 500 cells by 6 laboratories in 4 donors at 8 doses. Dicentrics and rings are on the left and acentrics are on the right. A dagger (+) shows that a centric ring was scored. At each dose the totals for all laboratories and donors are given.

Table 2 Chi^2 analysis for variability among the four donors in expt. 1; pooling the scoring (Table 1) of the 6 laboratories.

	Dose mGy	Mean Yield	x^2	DF	P
D + R	0	5.0	2.8	3	0.42
	3.13	3.25	3.9	3	0.27
	5.80	3.75	10.3	3	0.02
	9.65	3.50	0.3	3	0.96
	19.3	6.25	5.2	3	0.16
	28.7	8.25	3.0	3	0.39
	47.7	6.00	4.3	2	0.12
	290	39.25	20.5	3	0
AF	0	8.75	18.4	3	0
	3.13	11.75	4.15	3	0.25
	5.80	11.25	6.8	3	0.08
	9.65	11.25	1.7	3	0.64
	19.3	14.0	6.4	3	0.09
	28.7	14.25	2.3	3	0.51
	47.7	12.3	2.3	2	0.32
	290	38	3.9	3	0.27

Table 3(a) 0 mGy

	Laboratory						
Donor	1	2	3	4	5	6	Total
5	0/0	1/3	1/1	0/0	0/0	1/0	3/4
6	0/0	0/4	0/1	1/4	0/0	0/1	1/10
7	1/0	1/3	0/0	0/0	0/1	0/0	2/4
8	0/0	2/7	1/8	0/1	0/0	0/1	3/17
9	1/0	0/5	3/4	0/1	0/2	0/0	4/12
10	0/0	5/3	1/0	0/3	0/0	0/0	6/6
11	1/0	1/3	1/1	2/0	0/4	0/1	5/9
12	2/0	0/1	0/1	0/1	1/0	2/0	5/3
13	1/0	0/6	0/6	0/1	0/1	1/0	2/14
14	1/1	0/1	1/0	0/2	0/1	0/1	2/6
15	0/0	0/2	0/2	0/1	1/0	0/1	1/6
16	0/0	1/1	0/1	0/1	2/1	0/0	3/4
17	0/0	0/2	0/0	0/0	1/2	1/0	2/4
18	0/0	1/2	0/0	1/0	0/2	0/0	2/4
19	0/0	†1/4	0/0	0/0	0/1	0/0	1/5
20	1/0	1/1	1/2	0/0	0/0	0/1	3/4
21	1/0	0/5	0/0	0/1	1/0	0/1	2/7
22	0/1	0/4	1/1	0/0	0/1	0/0	1/7
23	0/0	0/1	0/0	0/0	0/0	0/1	0/2
24	0/0	1/3	0/0	0/0	1/0	0/1	2/4
Total	9/2	15/61	10/28	4/16	7/16	5/9	50/132

Table 3(b) 4.82 mGy

	Laboratory						
Donor	1	2	3	4	5	6	Total
5	0/1	3/2	0/0	1/1	0/0	0/1	4/5
6	0/0	0/3	0/3	0/0	0/2	0/4	0/12
7	0/1	†5/4	3/1	0/1	0/3	0/5	8/15
8	0/0	0/0	1/2	0/0	0/2	0/0	1/4
9	0/1	0/3	2/0	0/0	0/5	0/1	2/10
10	0/3	0/4	0/0	0/0	0/4	††2/3	2/14
11	1/2	†1/5	1/5	0/1	0/4	1/1	4/18
12	0/2	0/3	0/0	0/0	0/3	0/2	0/10
13	†1/3	1/4	0/0	0/2	1/3	3/2	6/14
14	0/3	1/9	1/0	0/0	0/4	0/0	2/16
15	0/0	1/1	0/0	0/1	1/2	0/0	2/4
16	0/0	††2/1	2/0	0/0	0/3	0/0	4/4
17	0/1	0/3	0/1	0/1	0/2	1/1	1/9
18	0/1	1/0	0/1	0/0	†2/3	0/0	3/5
19	1/2	0/2	0/0	0/0	1/2	0/0	2/6
20	0/1	0/3	0/2	0/3	0/5	0/1	0/15
21	0/1	0/5	0/0	0/0	0/0	0/3	0/9
22	0/0	0/1	1/0	0/0	0/2	0/2	1/5
23	0/1	0/1	0/0	1/2	1/2	1/2	3/8
24	0/0	0/0	0/1	0/0	0/1	†1/1	1/3
Total	3/23	15/54	11/16	2/12	6/52	9/29	46/186

Table 3(c) 28.5 mGy

	Laboratory						
Donor	1	2	3	4	5	6	Total
5	1/0	†2/2	1/0	0/0	1/0	0/1	5/3
6	2/0	0/4	4/2	0/0	3/3	0/0	9/9
7	2/0	0/1	0/3	1/2	2/2	0/1	5/9
8	1/0	0/3	3/4	0/0	1/1	1/0	6/8
9	2/0	0/2	†3/5	0/0	0/1	2/1	7/9
10	0/0	†1/9	†3/3	3/0	0/1	0/0	7/13
11	0/0	0/3	1/4	1/3	2/4	1/8	5/22
12	0/0	1/4	1/1	0/1	1/5	0/4	3/15
13	0/0	0/4	0/2	1/1	2/3	0/0	3/10
14	1/0	0/1	0/2	2/1	0/1	2/1	5/6
15	0/1	1/2	2/1	0/3	0/3	0/0	3/10
16	†1/2	0/2	0/0	0/0	0/0	2/2	3/6
17	1/1	0/3	0/1	1/0	0/1	0/1	2/7
18	0/1	0/1	3/1	0/1	1/2	0/1	4/7
19	0/0	0/9	0/2	1/1	0/1	0/1	1/14
20	0/1	0/3	1/3	0/0	0/0	0/1	1/8
21	0/1	0/2	1/1	†1/1	0/0	0/2	2/7
22	1/0	0/5	0/2	1/2	1/4	0/2	3/15
23	0/1	0/4	2/2	0/0	0/1	1/0	3/8
24	0/0	0/1	0/0	0/1	2/1	0/0	2/3
Total	12/8	5/65	25/39	12/17	16/34	9/26	79/189

Table 3(d) 280 mGy

	Laboratory						
Donor	1	2	3	4	5	6	Total
5	1/1	1/1	1/3	0/0	0/0	3/3	6/8
6	1/2	0/2	3/1	0/1	1/5	5/3	10/14
7	1/6	3/1	1/0	2/1	1/3	2/3	10/14
8	3/2	†5/1	2/9	0/2	3/3	4/6	17/23
9	2/1	1/0	4/2	2/2	2/1	1/4	12/10
10	2/0	1/4	†5/2	1/0	0/3	†8/6	17/15
11	1/5	†4/4	2/4	1/2	1/3	3/4	12/22
12	3/2	1/6	5/3	4/2	4/2	5/4	22/19
13	2/5	2/4	4/0	0/4	1/2	2/3	11/18
14	1/1	3/1	1/1	0/2	0/1	0/3	5/9
15	2/7	2/1	1/3	1/3	0/5	5/9	11/28
16	2/3	1/1	3/1	0/1	1/1	1/1	8/8
17	2/0	1/2	3/1	0/0	1/2	1/2	8/7
18	0/1	2/1	0/3	2/3	0/3	3/6	7/17
19	2/5	†1/1	0/2	1/3	0/3	†3/4	7/18
20	2/1	0/1	†2/1	2/0	†5/3	††2/1	13/7
21	2/0	2/1	1/0	4/1	0/2	0/4	9/9
22	2/2	1/1	3/0	0/1	†1/4	0/6	7/14
23	2/0	1/1	3/2	0/0	1/0	1/1	8/4
24	2/1	2/0	4/0	3/0	0/1	1/2	12/4
Total	35/45	34/34	48/38	23/28	22/47	50/75	212/267

Table 3. Experiment 2. Aberrations scored in 500 cells at 0, 4.82 and 28.5 mGy and in 200 cells at 280 mGy per laboratory per donor for 20 donors. As in Table 1 D + R are on the left and AF on the right. A dagger indicates a centric ring.

Table 4

Dispersion index test (Papworth, Curr. Top. Radiat Res. 6, 129, 1970) to examine donor variability, among the 20 donors in expt.2. The scoring (Table 3) of the 6 laboratories has been pooled.

	mGy Dose	Mean Yield in no. of cells	σ^2/Y	u	P
D + R	0	2.5 / 3000	0.95	-0.16	0.56
	4.82	2.3 / 3000	1.93	2.89	0.002
	28.5	3.95/ 3000	1.16	0.49	0.31
	280	10.6 / 1200	1.68	2.11	0.02
AF	0	6.6 / 3000	2.35	4.18	0
	4.82	9.3 / 3000	2.44	4.43	0
	28.5	9.45/ 3000	2.14	3.54	0.0002
	280	13.35/ 1200	3.34	7.22	0

Table 5

A rank order test for donor variation using the D + R data from Table 3.

Donor	0	4.8	28.5	280 mGy	R
5	14.5	17	14.5	2	48
6	3.5	2.5	20	10.5	36.5
7	9	20	14.5	10.5	54
8	14.5	6.5	17	18.5	56.5
9	17	11	18.5	15	61.5
10	20	11	18.5	18.5	68
11	18.5	17	14.5	15	65
12	18.5	2.5	8.5	20	49.5
13	9	19	8.5	12.5	49
14	9	11	14.5	1	35.5
15	3.5	11	8.5	12.5	35.5
16	14.5	17	8.5	7	47
17	9	6.5	4	7	26.5
18	9	14.5	12	4	39.5
19	3.5	11	1.5	4	20
20	14.5	2.5	1.5	17	35.5
21	9	2.5	4	9	24.5
22	3.5	6.5	8.5	4	22.5
23	1	14.5	8.5	7	31
24	9	6.5	4	15	34.5

Table 6

Results of a chi^2 test for homogeneity between laboratories. The data in Table 1 for each donor have been pooled.

	Dose mGy	Mean	Chi^2	DF	P
D + R	0	3.33	4.00	5	0.55
	3.13	2.17	3.14	5	0.68
	5.80	2.50	9.4	5	0.09
	9.65	2.33	3.15	5	0.68
	19.3	4.17	35.2	5	0
	28.7	5.5	5.4	5	0.37
	47.7	4.0	2.0	4	0.74
	290	26.17	15.4	5	0.009
AF	0	5.83	11.1	5	0.05
	3.13	7.83	20.8	5	0.001
	5.80	7.5	10.3	5	0.07
	9.65	7.5	19.9	5	0.001
	19.3	9.33	17.7	5	0.003
	28.7	9.5	17.6	5	0.003
	47.7	7.2	9.3	4	0.05
	290	25.3	5.6	5	0.35

Table 7

Results of a homogeneity test between laboratories for data in Table 3. Results for the donors have been pooled.

	Dose	Mean	Chi^2	DF	P
D + R	0	8.3	9.6	5	0.09
	4.82	7.67	16.1	5	0.007
	28.5	13.2	17.8	5	0.003
	280	35.3	19.3	5	0.002
AF	0	22	99.9	5	0
	4.82	31	52.4	5	0
	28.5	31.5	62.8	5	0
	280	44.5	30.6	5	0

Table 8

Fits of the data in expt. 1 (Table 1) to the quadratic model over the dose range 0-290 mGy and linear model for doses 0-47.7 mGy.

Model	Aberrations	Fitted Control ± SE		α ± SE		β ± SE		DF	chi^2	P
		($\times 10^{-3}$)		($\times 10^{-5}$ mGy^{-1})		($\times 10^{-8}$ mGy^{-2})				
Quadratic	D + R	1.25	0.22	3.12	1.36	*3.28	4.81	5	6.4	0.27
Quadratic	AF	3.51	0.33	3.31	1.84	-0.54	6.21	5	3.2	0.66
Linear	D + R	1.25	0.21	3.23	1.16	-	-	5	6.4	0.27
Linear	AF	3.53	0.33	3.16	1.62	-	-	5	3.3	0.66

* This value of β is not significantly different from a value of 7×10^{-8} mGy^{-2} which is obtained from higher dose data for radiations of similar quality.

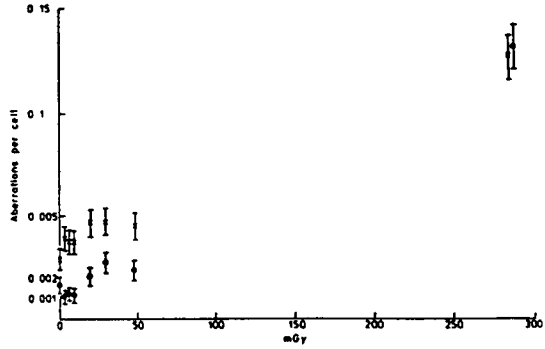

Figure 1. Pooled dose response data from Expt. 1.
• dicentrics plus rings, x excess acentrics

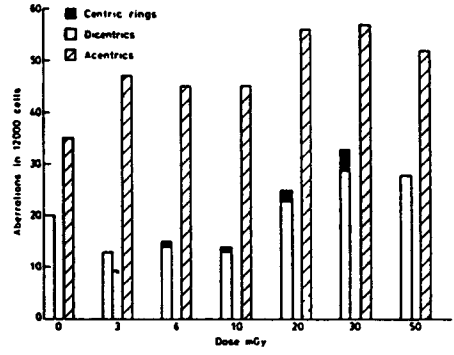

Figure 2. Data from Expt. 1. Histograms showing a dip in aberration yield at doses less than 10 mGy.

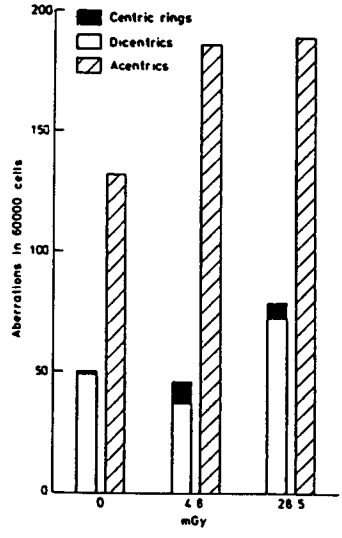

Figure 3. Data from Expt. 2. Histogram showing a possible dip below control value in exchange aberrations at 4.8 mGy.

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

1. State University of Leiden, Netherlands (Prof A Natarajan)
2. University of Essen, Germany (Prof G Obe)
3. CEN/SCK Mol, Belgium (Dr L Verschaeve)
4. BNF plc Sellafield, UK (Dr J Tawn)
5. University of Rome, Italy (D F Palitti)

V. Publications:

D C Lloyd et al (1988) Frequencies of chromosomal aberrations induced in human blood lymphocytes by low doses of x-rays. Int. J. Radiat. Biol. 53 49-55.

RADIATION PROTECTION PROGRAMME

Progress Report

1989

Contractor:

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Contract no.: BI6-E-312-D

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Title of the research contract:

Formation of micronuclei in human lymphocytes after partial and whole body irradiation

List of projects:

1. Development of reliable methods to determine radiation dose in accidentally exposed persons using the micronucleus technique

Title of the project no.: BI6-E-312-D

Formation of micronuclei in human lymphocytes after partial and whole body irradiation

Head(s) of project:

Prof. Dr. C. Streffer, Privatdozent Dr. W.-U. Müller

Scientific staff:

H.-W. Gantenberg

I. Objectives of the project:

Development of reliable methods to determine radiation dose in accidentally exposed persons using the micronucleus technique.

II. Objectives for the reporting period:

1. Establishing a micro-culture technique for human lymphocytes.
2. Introducing the cytochalasin B method and fixation of cells with preservation of cytoplasm.
3. Proliferation kinetics of stimulated lymphocytes.
4. Comparison of spontaneous micronucleus frequency in unexposed people.

III. Progress achieved:

METHODOLOGY

1. Culture conditions: An aliquot of 15.000 cells obtained from heparinized blood was transferred to 1 ml RPMI 1640 medium supplemented with 15% fetal calf serum, L-glutamine, antibiotics, and phytohemagglutinin. The cultures were incubated in small tubes in a water bath at 37 degrees centigrade.
2. Fixation of cells: In the case of proliferation studies, cells were fixed on a glass slide using a standard protocol (hypotonic treatment with 75 mM KCl, fixation in ethanol/acetic acid 3:1). In the case of application of cytochalasin B, this inhibitor of cytokinesis was added to the medium, when the simultaneously performed proliferation studies signalled the successful stimulation of lymphocytes (= when the sum of the S- and G2-cells exceeded 10%). Cells were fixed much more cautiously in order to preserve the cytoplasm (hypotonic treatment with 100 mM KCl, fixation with ethanol/acetic acid 5:1).
3. Staining of fixed lymphocytes: For the proliferation studies, the nuclei of the lymphocytes were stained with ethidium or propidium iodide and the relative DNA content was determined by quantitative fluorescence measurements with a microscope photometer. For counting micronuclei in binucleated cells, fixed lymphocytes were stained with Giemsa.

RESULTS

1. Methodological improvements

Usually, lymphocyte culturing is carried out in macrocultures (about 8 drops of blood in 8 ml of medium). In order to save material, microcultures (1 drop of blood in 1 ml of medium) were established in our lab, and it could be shown that stimulation and growth of lymphocytes was the same in both culture systems. Due to the now lower numbers of lymphocytes available, we could no longer follow cell proliferation by using flow cytometry (as it was announced in the proposal). Thus, we switched over to microscope photometry which allows control of cell proliferation at much lower cell numbers than flow cytometry.

A second very important improvement was introduced when a publication of Fenech and Morley came to our attention, in which they described a method for identification of those lymphocytes that divided just once after stimulation by PHA. An exact knowledge of the fraction of lymphocytes that has divided is crucial, because micronucleus expression is strongly dependent on the number of mitoses carried out

after radiation exposure. We therefore adopted the method of Fenech and Morley, in which cytochalasin B is used to prevent cytokinesis, whereas karyokinesis takes place unimpaired. Those lymphocytes that have completed a mitosis therefore show two cell nuclei per cell.

2. Proliferation of lymphocytes in vitro

Study of proliferation of lymphocytes in vitro after stimulation with phytohemagglutinin revealed, that even under exactly identical conditions proliferation varies considerably from individual to individual. This was true for the fraction of proliferating cells (between 15 and 50% of all lymphocytes), as well as for the start of proliferation (between 24 and 40 hours after stimulation). This means, that it is very dangerous to use a standard protocol in a way, that cytochalasin B is added and the cells are harvested at constant time points. Thus, continuous monitoring of proliferation is required in order to add cytochalasin B and to harvest the cells at the optimal time points.

3. Spontaneous frequency of micronuclei in healthy donors and patients

One of the prerequisites of the use of micronuclei as biological dosemeter is a spontaneous micronucleus frequency with low variability. Table 1 shows that indeed variability is rather small, at least in the two populations evaluated up to now. In the healthy donor group the mean value amounts to 0.014 micronuclei per binucleated cell with a standard deviation of 0.008; in the patient group the mean is 0.024 micronuclei per binucleated cell and the standard deviation 0.019. The slightly higher spontaneous frequency in patients is almost exclusively due to patient number 2. If this patient is excluded, the mean is 0.018 with a standard deviation of 0.008.

Table 1: Comparison of the spontaneous micronucleus frequency in binucleated lymphocytes of healthy donors and of patients with various lympho- and myeloproliferative diseases

a) Healthy donors

Donor	Binucleated cells scored	Binucleated cells with micronuclei	Number of micronuclei	Micronuclei per cell
1	1042	14	16	0.015
2	1018	18	18	0.018
3	1015	10	10	0.010
4	721	17	20	0.028
5	563	2	2	0.004
6	1389	11	15	0.011

b) Patients

Donor	Binucleated cells scored	Binucleated cells with micronuclei	Number of micronuclei	Micronuclei per cell
1	850	12	16	0.019
2	1093	64	72	0.066
3	1100	13	18	0.016
4	608	5	5	0.008
5	595	18	18	0.030
6	2008	14	16	0.008
7	864	17	18	0.021
8	1165	23	30	0.026

IV. Objectives for the next reporting period:

1. Additional data of spontaneous micronucleus frequency.

2. Radiation induced micronuclei in lymphocytes of healthy donors exposed in vitro and in lymphocytes of patients exposed either in vivo or in vitro.

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

VI. Publications:

2.

Gantenberg, H.-W.; Boecker, W.; Müller, W.-U.; Streffer, C.
Control of cell proliferation using an on-line microscope photometer/computer system.
Poster presented at the 2nd European Symposium on impulse cytophotometry and image analysis, Gent, 4.6.-6.6.1989

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Medical Research Council
20 Park Crescent
GB-London W1N 4AL

Contract no.: BI6-E-173-UK

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

Dr. C. Tease
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Medical Research Council
Harwell, Didcot
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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:

To compare the incidences of chromosome anomalies, induced by irradiation of mouse oocytes, at various stages of pre- and post-implantation embryonic development.

II. Objectives for the reporting period:

As above.

III. Progress achieved:

Methodology

Two experiments were carried out.

Expt. 1

Young female mice of the F_1 hybrid type C3H/HeH x 101/H (abbreviated hereafter to 3H1) were given a half-body, caudal dose of 4 Gy of X-rays (250 kV, 14 mA; 0.25 mm Cu; H.V.T. 1.2 mm Cu; 0.72 Gy/min). Unirradiated females of the same age were used as controls. One week after treatment, they were mated to male mice homozygous for 3 Robertsonian translocations (Rb(6.15)1Ald; Rb(9.19)163H; Rb(11.13)4Bnr) and examined daily for vaginal plugs. Pregnant females were killed on either day 7.5 or 8.5 of gestation, and post-implantation embryos were collected. Chromosome preparations were made using the technique described by Evans et al. (1972).

Expt. 2

Young 3H1 female mice were induced to ovulate using 5 iu PMS followed 48 hours later by 5 iu HCG (females used for the collection of post-implantation embryos were given 1 iu of each hormone). Three hours after HCG, the females were given a half-body dose of 1 Gy of X-rays (irradiation conditions were as for Expt. 1). Unirradiated, age-matched females served as controls. One group of females was used for the collection of metaphase II oocytes, the remainder were mated overnight and used for the collection of embryos at different stages of development. From one group of females, fertilized eggs were collected the day after irradiation and cultured in vitro to metaphase of the first-cleavage division; the routine used was identical to that in earlier experiments in which one-cell embryos were analyzed (Tease, 1982, 1985). A second group of females was killed on day 3.5 of gestation and morulae/blastocyst stage embryos collected for chromosome preparations using the routine described by Mikamo and Kamiguchi (1983). Post-implantation embryos were recovered from the remaining females on day 8.5 of gestation, and chromosome preparations made as in Expt. 1.

Results

Expt. 1

Embryos were screened for numerical and structural chromosome anomalies (Table 1). One trisomic and 14 apparently mosaic embryos were found among those from unirradiated females. In the irradiated group of females, 1 trisomic, 1 tetraploid, 3 monosomic and 30 apparently mosaic embryos were found. There was clearly no increase in the rate of trisomy following X-irradiation, however, the proportion of chromosomally abnormal embryos was significantly larger ($\chi^2_1 = 4.42$, $p = 0.04$) principally due to the increased incidence of mosaicism.

Table 1. Karyotype analysis of 7.5 and 8.5 day embryos from control and X-irradiated female mice

Treatment group	Chromosome number						% chromosomally abnormal	Total embryos analyzed
	36	36/37	37	37/38	38	74		
control	0	12	115	2	1	0	11.5	130
4 Gy	3	30	125	0	1	1	21.9	160

Expt. 2

The data obtained from the analyses of the different oocyte and embryo stages are presented in summary in Table 2.

a) Metaphase II oocytes

A small proportion of cells from unirradiated females were found either to be hyperhaploid or to contain a structural chromosome anomaly. After X-irradiation, however, the incidence of hyperhaploidy increased significantly ($p = 0.009$, 1-sided Fisher exact test). In addition, there was a very large increase in the proportion of cells containing 1 or more structural anomalies.

b) 1-cell embryos

A small proportion of the embryos from unirradiated females were either hyperploid or contained a structural chromosome anomaly. The incidence of hyperploidy rose after X-irradiation although the increase was on the borderline of significance ($p = 0.051$, 1-sided Fisher exact

test). The increase in the proportion of cells with structural chromosome anomalies was, however, very marked.

c) Morulae/blastocysts

None of the embryos from control females were hyperploid or contained a structural chromosome anomaly. After X-irradiation, however, the incidences of both types of anomaly increased significantly ($p = 0.03$ and 0.0008 respectively).

d) 8.5 day embryos

No hyperploid embryos were present among those sampled from control and irradiated females. Similarly, no examples were found of embryos with structural chromosome anomalies. The data summary in Table 2 excludes mosaicism: 5/120 embryos from control females and 15/105 embryos from irradiated females were apparently mosaic. This difference was significant ($\chi^2_1 = 6.53$, $P = 0.01$).

Table 2

Table 2. The proportions of cells and embryos that were hyperploid or contained 1 or more structural chromosome anomalies from control and X-irradiated female mice

Cell/ embryo stage	Control			1 Gy		
	% hyper-ploidy	% with structural anomalies	Total cells/ embryos analyzed	% hyper-ploidy	% with structural anomalies	Total cells/ embryos analyzed
metaphase II	0.4*	0.4	243	3.5*	60.8	339
1-cell embryo	2.1	2.1	235	5.4	30.6	242
morulae/ blastocysts	0**	0	223	2.3**	5.9	221
8.5 day embryos	0**	0	120	0**	0	105

* excludes cells with a single, additional chromatid
** excludes apparent mosaics

Discussion

Expt. 1

Tease (1985) reported that approximately 8% of 1-cell embryos from female mice given 4 Gy of X-rays 1 to 2 weeks prior to ovulation, contained hyperploid maternal pronuclei. C-banding was not used in that study to distinguish extra whole chromosomes from dicentric chromosomes and large acentric fragments. Nevertheless, the data suggested a substantial induction of trisomy under this irradiation regime. In the experiment described here, oocytes at a similar stage of follicular development would have been treated as in the experiment of Tease (1985). The data obtained from the analysis of post-implantation embryos revealed no increase in trisomy following X-irradiation, contrary to what might have been anticipated from Tease's (1985) observations. The lack of any trisomic, post-implantation embryos suggested that embryos with radiation-induced chromosome gains were not surviving to this stage of early post-implantation development.

Expt. 2

Tease and Fisher (1986) found that approximately 59% of metaphase I oocytes contained 1 or more structural chromosome anomalies when 3H1 females were given 1 Gy of X-rays 3 hours post HCG. The metaphase II oocytes from females similarly treated were found here to have a similar, overall incidence of cells with structural chromosome anomalies. The effect of the treatment on chromosome segregation was, however, much more difficult to ascertain. Approximately 5.6% of the cells contained a single, additional chromatid. The fate of such chromatids, in relation to contribution to embryonic aneuploidy, is uncertain and so they were not included in the hyperploidy category of Table 2. Nevertheless, the somewhat conservative approach adopted here of including cells with additional whole chromosomes or their equivalent, showed that the incidence of hyperploidy increased significantly following 1 Gy of X-rays.

It would be anticipated that the rate of hyperploidy should be similar in 1-cell embryos to that in metaphase II oocytes, providing there was little nondisjunction at anaphase II and no loss of hyperploid cells through death or selective fertilization of euploid oocytes. In

the present experiment, the incidence of hyperploidy was slightly larger in 1-cell embryos than in metaphase II oocytes after 1 Gy of X-rays. This might have resulted either from a contribution to embryonic hyperploidy by metaphase II oocytes containing a single additional chromatid or simply a chance sampling effect. The comparison does not suggest any loss of hyperploid cells between metaphase II and the first cleavage division.

Tease (1982), in an examination of radiation-induced aneuploidy in 1-cell embryos, estimated a rate of induced hyperploidy of 0.06/Gy following irradiation of the same stage of oocyte development. This rate predicts induction of approximately 6% hyperploidy after 1 Gy. Although the rate obtained here was slightly smaller, and must also have included spontaneous events, the data in this and the earlier experiment are not irreconcilable. Tease (1982) did not use C-banding to distinguish additional whole chromosomes from dicentric and acentric chromosomes. In the present study, the latter chromosome types were excluded from the hyperploidy total. On this basis, the earlier experiment should have overestimated the rate of induced hyperploidy.

The incidence of hyperploidy after irradiation was lower in morulae/blastocysts compared to 1-cell embryos. A similar phenomenon occurred with regard to structural chromosome anomalies. The decrease in relative numbers of embryos with chromosome anomalies suggests a progressive loss of embryos with induced genetic damage as gestation proceeds. This interpretation is supported by the paucity of post-implantation embryos with induced hyperploidy or detectable structural chromosome changes. It will be recalled that the data in Expt. 1 likewise failed to demonstrate the occurrence of radiation-induced trisomies in post-implantation embryos. Taken together, the data in the experiments here and previously (e.g. Tease, 1982, 1985) indicate that although hyperploidy is induced by irradiation of mouse oocytes, the resultant embryos do not appear to be capable of progression even to a relatively early stage of post-implantation development. The reason for this is uncertain. Possibly, the simultaneous occurrence of other types of induced genetic damage cause the death of the embryo. In Expt. 2, for example, approximately 60% of the metaphase II oocytes contained visible damage to chromosome structure. Such damage, rather than the aneuploidy itself, may be the principal cause of death of hyperploid embryos.

References

Evans, E.P., Burtenshaw, M.D., Ford, C.E. (1972) Stain Technology 47, 229-234.

Mikamo, K., Kamiguchi, Y. (1983) In: Radiation-Induced Chromosome Damage in Man (eds Ishihara, T., Sasaki, M.S.) Alan R. Liss, New York, pp. 411-432.

Tease, C. (1982) Mutation Research 95, 287-296.

Tease, C. (1985) Mutation Research 151, 109-119.

Tease, C., Fisher, G. (1986) Mutation Research 173, 211-215.

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

None

V. Publications:

Tease, C. (1989) Radiation-induced aneuploidy in mammalian germ cells. In: Somatic and Genetic Effects of Ionizing Radiation. Berzelius Symposium XV, pp. 113-117.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no : BI6-E-144-UK

Medical Research Council
20 Park Crescent
GB-London W1N 4AL

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

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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 Dr. P.G. Debenham
Dr. A. Hamilton

I. Objectives of the project:

To devise and implement methods for the molecular analysis of mutation and recombination of DNA in cultured mammalian cells, including 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:

To construct and test vectors carrying one or two small dominant genes, for stability, copy-number and expression after introduction into mammalian cells. To assess quantitatively and by molecular analysis the mutation or recombination (the latter using paired vectors carrying specific non-overlapping deletions) of introduced genes in both normal and radiation-sensitive cell lines. To measure the effect of DNA double-strand breaks in the region of homology on recombination frequency of introduced vector DNA.

Molecular analysis of radiation-induced (gamma-ray and alpha-particle) mutations in an endogenous gene (hprt) by examination of DNA and RNA using cloned gene probes.

III. Progress achieved:

1. Methodology

Vectors carrying one gene (gpt; Mulligan and Berg 1980, Science, **209**, 1422-1427) or two genes (gpt neo) were used as 'targets' for these studies. The 2-gene vectors were constructed in various orientations using standard molecular-biology methods to assess their usefulness (see also project 2); paired single-or 2-gene vectors carrying non-overlapping deletions were similarly made to give short regions of homology for recombination studies. Vectors were transferred using calcium phosphate co-precipitation, in some cases using HPRT-deficient cell lines. Radiation-sensitive cell lines were also used as recipients, either lines generated in this laboratory (irs mutants; Jones et al. 1987, Mutation Res., **183**, 279-286) or kindly supplied by Dr. P.A. Jeggo (xrs mutants; Jeggo and Kemp 1983, Mutation Res., **112**, 313-327). Cells transformed by the vectors were isolated from selective medium and stability checked by long-term growth in non-selective conditions. Mutation or recombination frequency was measured by colony-forming ability, using drug selection for expression (or lack of it) of the introduced gene(s).

High molecular-weight DNA was isolated from selected transformants, mutants or recombinants, cut with appropriate enzymes and separated by gel electrophoresis, before blotting onto membranes and hybridization to cloned probes. Total cell RNA was isolated and used for dot or Northern blots to analyse expression of mutant hprt genes.

2. Results

(i) Mutation studies on introduced 2-gene vector DNA integrated as a single copy in the mammalian genome.

Cells transformed with different versions of the 2-gene (gpt neo) vector were selected from various lines, including radiation-sensitive irs mutants. However, few of these transformants proved to be useful for further study because of instability or multiple vector copy-numbers integrated into the genome. Only one transformant was studied extensively:

when retention of the neo gene was selected for, this line had a very low frequency of spontaneous mutation to loss of gpt activity. However, this frequency increased with culture time and the cells grew relatively slowly in culture medium. We had previously established (Thacker et al., 1983, Mutation Res., 111, 9-23) that use of the gpt gene alone for mutation studies gave a relatively high mutant frequency and resulted in complete loss of the integrated vector DNA. Quantitative data for the line carrying a 2-gene vector showed that X-rays induced mutants in this line and molecular analysis revealed, in contrast to the single-gene vector studies, that part or all of the vector DNA was retained when selection for one of the linked genes was maintained.

(ii) Recombination analysis using introduced vector DNA. Pairs of vectors carrying non-overlapping deletions were transferred into wild-type and radiation sensitive (xrs) mutants to assess recombination. It was established that the quantitative measurement of recombination, while showing some variability between experiments, was feasible; and that selected recombinants carry a gene of wild-type size and function. Vector copy-number and uptake experiments were also made to show that these aspects were unlikely to influence the quantitation of data for the different cell lines. However, it was found that the major difference between the xrs mutants and their wild-type counterparts was not in their ability to recombine vector DNA, but in the reduced frequency with which vector DNA transformed the radiation-sensitive mutants.

(iii) Molecular analysis of radiation-induced mutants of the hprt gene.

In a continuing study of large numbers of gamma-ray and alpha-particle induced mutants, compared to mutants induced by the chemical agent ethyl methane sulphonate (EMS) or occurring spontaneously, DNA and RNA analysis was used to refine understanding of the nature of mammalian cell mutations. We had shown previously (Brown and Thacker 1984, Mutation Res., 129, 269-281; Brown et al 1986, ibid, 160, 111-120) by biochemical and reversion tests that radiation-induced mutants

had phenotypes indicating mutations of large effect, in contrast to most spontaneous and EMS-induced mutants. Molecular analysis revealed that this inference was correct, in that most radiation-induced mutants were found to carry large deletions which in many cases remove the whole hprt gene. The reverse situation was found for spontaneous mutants (the majority have small mutations, below the limits of detection of Southern DNA analysis) while no large mutational changes were found for EMS-induced mutants. RNA isolated from 59 mutants with presumptive point mutations (13 spontaneous, 46 EMS-induced) showed a wide range of hprt mRNA amounts, from mutants with undetectable amounts to those with more than wild-type amounts. However, Northern blots of all these mutant RNAs revealed only one (EMS-induced) with an alteration in mRNA size.

The final classification of this series of 163 mutants is shown in the Table below, bringing together the various analyses made over a number of years.

TABLE 1 CLASSIFICATION OF THIOGUANINE-RESISTANT MUTANTS OF V79 HAMSTER CELLS FROM ANALYSIS OF HPRT ENZYME ACTIVITY, REVERTIBILITY AND hprt GENE STRUCTURE

Agent	No. mutants analysed	Type of genetic change	
		Small ('point') mutations	Large (deletion/ rearrangement)
None	44	36 (82%)	8 (18%)
gamma-rays	48	18 (37.5%)*	30 (62.5%)*
alpha-particles	15	4 (27%)	11 (73%)
EMS	56	56 (100%)	0 (0%)

* data include a fraction of spontaneous mutants; if allowance is made for these, the correct proportions of small:large mutations becomes 29%:71%.

Recent studies have focussed on putative partial deletion/rearrangement mutations induced by ionising radiations, for which multiple enzyme profiles have been

produced on Southern blots. These have allowed, in conjunction with a map of the hamster hprt gene (Rossiter 1987, Ph.D. Thesis, Manchester University; H. Vrieling, pers.comm.), the mapping of the breakpoints of deletions within the hprt gene. The sites of these breakpoints show some evidence of clustering in the region of exons 4-6 of the gene. In addition, one duplication mutation was identified.

3. Discussion

(i) Mutation of integrated vector genes.

The original ideas behind the introduction of small defined genes as mutation 'targets' (Thacker et al. 1983, op. cit.) have been realized in part: establishment of single copies of such genes in mammalian cells and their use to give reproducible quantitative data were attained. However, difficulties were encountered with stability and copy-number in many of the selected transformants, and the goal of using the same gene at different genomic sites (to yield comparative mutation data) was not fully realized. The use of single-gene vectors was superseded by 2-gene vectors where one gene could be used to counter-select for complete loss of the vector DNA. The latter system yielded a mutational spectrum not unlike that found for the hprt gene [see (iii) above] insofar as a mixture of apparent point mutations and deletions/rearrangements were seen after X-ray induction. This distinction evidently point to the main mode of action of ionising radiations in mutagenesis: if the system allows (e.g., the single-gene vector transformants) large deletions will prevail so that the whole vector sequence is usually lost, but if the damage processing is constrained (e.g., 2-gene vector transformants with selection for the retention of one gene) then smaller mutations are found. Thus, ionising radiations can be said to induce predominantly large genetic changes unless the genetic region 'targeted' does not allow these to survive.

(ii) Recombination of transferred DNA sequences.

The major finding in this study was that the xrs mutants,

known to have reduced DNA double-strand break repair capacity, had a reduced frequency of transformation by introduced DNA. From the control experiments made, it seems likely that the defect lies in their ability to integrate exogenous DNA into the genome. Integration is presumed to occur by a process of illegitimate recombination, about which little is known but which will involve some form of breakage and reunion of DNA strands. It may be therefore that the biochemical process which underlies the defect in the radiosensitive xrs mutants is normally involved in recombination/repair processes. It is interesting in this context that ionising radiation is well-known as an inducer of events leading to illegitimate recombination (e.g., chromosomal translocations); taken with the present result it may be suggested that a major pathway for radiation damage repair is through non-homologous recombination enzymes.

(iii) Mutation of the mammalian hprt gene.

The extended study of a large number of hprt gene mutants induced by ionising radiation and other agents shows that this gene is able to detect a broad spectrum of mutation types, vindicating earlier predictions (Caskey and Kruh 1979, Cell, **16**, 1-9; Thacker and Cox 1983, in 'Radiation-Induced Chromosome Damage in Man', Liss:New York, pp.235-275). In particular, it is seen that ionising radiations induce a mixture of mutation types, with large deletions predominating, while agents such as EMS produce only point mutations. Little difference was found between the spectrum of mutations induced by gamma-rays and alpha-particles, perhaps indicating a limitation on the size of changes detected by the hprt gene. It should be noted that hprt is X-linked and therefore monosomic in male cells (such as V79) so that genetic damage extending to vital genes linked to hprt will not be tolerated and the cell will die. Exploration of potential differences between high and low LET radiation damage may, therefore, require the use of gene mutation systems which shield the cell from the lethal effects of very large genetic changes. The localization of breakpoints of partial gene deletions opens

the way to their cloning and sequencing, to yield information on the sequence specificity of sites of radiation damage. The combined results of this project identify the major type of genetic change induced by ionising radiations, but emphasise that the changes detected are also dependent on the gene mutation system used. Comparable data were obtained for the hprt gene system and for an introduced gene system when one part of the target DNA was constrained while the other part was mutated. Thus, in comparison to chemical mutagens inducing mainly 'point' mutations, ionising radiations may induce changes in the DNA covering a substantial region of the genome and potentially having more deleterious effects (e.g., heterozygosity of flanking genes). It will be important to identify the molecular processes which effect these changes to understand fully the mechanism of action of radiation on DNA. It is also of interest in the present study that a process of non-homologous recombination was implicated in the phenotype of radiation-sensitive mutants, suggesting a mechanism for the repair of radiation damage.

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

V. Publications

1. Major publications

Thacker, J. (1985) The molecular nature of mutations in cultured mammalian cells: A review. Mutation Res., 150, 431-442.

Brown, R., A. Stretch & J. Thacker (1986) The nature of mutants induced by ionising radiation in cultured hamster cells II. Antigenic response and reverse mutation of HPRT-deficient mutants induced by gamma-rays or ethyl methanesulphonate. Mutation Res., 160, 111-120.

Thacker, J. (1986) The use of recombinant DNA techniques to study radiation-induced damage, repair and genetic change in mammalian cells. Int. J. Radiat. Biol., 50, 1-30.

Thacker, J. (1986) The nature of mutants induced by ionising radiation in cultured hamster cells III. Molecular characterization of HPRT-deficient mutants induced by gamma-rays or alpha-particles showing that the majority have deletions of all or part of the hprt gene. Mutation Res., 160, 267-275.

Hamilton, A., & J. Thacker (1987) Gene recombination in X-ray sensitive hamster cells. Molecular Cell. Biol., 7, 1409-1414.

Debenham, P.G., M.B.T. Webb, A. Stretch & J.Thacker (1988) Examination of vectors with two dominant selectable genes for DNA repair and mutation studies in mammalian cells. Mutation Res., 199, 145-158.

Thacker, J. & A. Ganesh (1989) Molecular analysis of spontaneous and ethyl methanesulphonate-induce mutations of the hprt gene in hamster cells. Mutation Res., 210, 103-112.

Thacker, J. (1989) The use of integrating DNA vectors to analyse the molecular defects in ionising radiation-sensitive mutants of mammalian cells including ataxia-telangiectasia. Mutation Res., 220, 187-204.

Thacker, J. (1989) Use of recombinant DNA molecules to study the repair of DNA damage and radiation mutagenesis. In Somatic

and Genetic Effects of Ionising Radiation. Scientific Communications Inc., Umea, Sweden, pp.39-46.

2. Short Communications

Thacker, J., A. Stretch, A. Hamilton & N. Jones (1985) Cellular studies of repair and recombination in normal and radiosensitive hamster cells. Brit. J. Cancer, 51, 609.

Thacker, J., R. Brown, A.H. Cawood and A. Stretch (1985) The nature of mutations induced by ionising radiation in cultured mammalian cells. Int. J. Radiat. Biol., 48, 453.

Cox, R., and J. Thacker (1986) Some implications of molecular studies on DNA repair and mutagenesis for radiation carcinogenesis. Int. J. Radiat. Biol., 49, 543-544.

Hamilton, A., and J. Thacker (1986) Gene recombination in X-ray sensitive CHO cells. Brit. J. Cancer, 54, 356.

Thacker, J. (1987) Radiation mutagenesis in bacteria and mammalian cells. In Radiation Research: Proc. 8th. Int. Congr. Radiat. Res., Taylor & Francis: London, Vol.2, pp. 544-549.

Title of the project no.: 2

The isolation and cellular and molecular characterization of repair-deficient mammalian cell lines

Head(s) of project: Dr. J. Thacker

Scientific staff: Dr. J. Thacker Dr. P.G. Debenham
Dr. R. Cox Dr. N.J. Jones Dr. T.C. Brown

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 to apply both cellular and recombinant DNA techniques to the characterization 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 that damage.

II. Objectives for the reporting period:

To isolate new radiation-sensitive mutants of cultured mammalian cells, and to characterize their differences from other known mutant phenotypes (by complementation testing and measurement of a variety of responses after irradiation, such as DNA break repair, DNA synthesis response and mutation induction).

To apply a novel recombinant DNA assay for DNA break repair to normal human and radiosensitive ataxia-telangiectasia cells, as well as new hamster cell mutants, with particular emphasis on measuring the fidelity of repair.

To pursue methods of identifying and cloning DNA repair genes of mammalian cells.

III. Progress achieved:

1. Methodology

Radiosensitive mutant isolation was accomplished by screening mutagenized populations of V79 hamster cells, using a multiwell replica technique (Jones et al. 1987, Mutation Res., 183, 279-286). Mutants were selected for resistance to both thioguanine and ouabain before their use to create fusion hybrids for complementation testing with other known radiosensitive mutants (kindly supplied by those who isolated these). DNA single- and double-strand break repair was measured using sucrose gradient sedimentation, while the radioresistance of DNA synthesis was measured from dual-labelled cells using TCA-precipitable material (Thacker and Ganesh 1989, Mutation Res.,in press). Other studies on radiosensitive mutants were made with standard clonogenic methods, including hprt mutation assay.

A 2-gene vector (pPMH16) was constructed using standard molecular biology methods and used for repair fidelity measurement. The vector was broken at a specific site within one of its genes (gpt), transferred into normal and radiosensitive cells using calcium phosphate co-precipitation, and selection imposed for the unbroken gene (neo). Repair of the broken gene was assessed by backselection for the expression of gpt gene function. A further vector was constructed to allow the reversal of this procedure (i.e., selection first for the unbroken gpt gene followed by backselection for the broken neo gene).

To assay rejoining of specific DNA strand-breaks by cell-free extracts, the vector pUC18 was used. Extracts were prepared from washed nuclei, and put into a simple reaction mixture with broken vector DNA. Rejoining was assessed using gel electrophoresis and Southern blotting, followed by bacterial transformation. The ratio of blue:white colonies on specific media gave a measure of the fidelity of rejoining.

Various attempts were made to establish methods for the identification and cloning of DNA repair genes:

- the use of human DNA ligated to a selectable cosmid vector

and transferred into radiosensitive mutant cells (especially irs1);

- exploration of retrovirus-transformed cells as a potential means of rapid isolation of genes involved in radiation damage repair;

- isolation of SV40 minichromosomes from cell nuclei after treatment with DNA-damaging agents, to identify proteins associated with damaged DNA.

2. Results

(i) Isolation and characterization of new radiosensitive mutants.

Screening about 5000 individual colonies revealed 4 mutants of hamster cells with reproducible differences in sensitivity: one of the (irs4) had only a slight increase in sensitivity, while the other 3 (irs1, irs2, and irs3) had 2-3 fold increases. These mutants responded differently to other DNA-damaging agents, with irs1 especially showing a 60-fold increase in sensitivity to mitomycin-C (MMC) as well as increases for UV light and ethyl methane sulphonate. irs2 was the most sensitive to X-rays, at least at high doses, but was least sensitive to MMC. These mutants also appeared to be unlike any previously isolated, and complementation testing of the 3 more sensitive mutants against a battery of other mutants showed that this was true. Tests with each other and with the mutants EM7, XR1 and xrs showed that all were in different complementation groups. Subsequently these mutants have been tested against other new mutants, and only one (irs2) has been found to be in the same group as any other (in this case, a set of mutants recently isolated by M. Zdzienicka, Leiden).

Each of the mutants irs1-3 was examined for DNA break repair and post-irradiation DNA synthesis characteristics. No reduction in ability to repair DNA single- or double-strand breaks after irradiation was found in any of these mutants, compared to the parental cells, but differences were found in the radioresistance of DNA synthesis. In particular, DNA

synthesis in irs2 showed a considerably greater resistance than parental cells, similar to the difference found between normal human and ataxia-telangiectasia (A-T) cells, while irs1 showed some resistance at high irradiation doses.

Mutation frequency measurements in irs2 and irs3 showed that neither was altered in mutability per unit X-ray dose when compared to parental cells. However, irs1 appears to have an enhanced mutability, although further experiments are necessary to establish a reproducible body of data.

Complementation studies were extended to A-T cells in an effort to classify these in relation to the new hamster mutants (especially irs2) and to check different transformed A-T lines with each other. The former goal was not achieved despite considerable effort, because good hybrids were not found. Crosses between A-T lines from different patients gave good hybrids in some instances, as checked chromosomally and by DNA fingerprinting (Thacker et al. 1988, Somatic Cell Genet., **14**, 519-525), and were used for the first time to examine radiosensitivity by clonogenic survival. Hybrids between transformed lines of AT5BI and AT4BI showed no complementation, despite earlier evidence (using other techniques) to the contrary, while crosses of either A-T line to a normal human cell line showed the expected (A-T recessive) phenotype. Similarly measurement of the radioresistance of DNA synthesis showed no complementation for AT5BI and AT4BI.

(ii) Measurement of the fidelity of DNA break rejoining with a novel recombinant DNA assay.

Transfer of the vector pPMH16, broken with an endonuclease at a unique site in a selectable gene, into normal and radiosensitive cells identified a reduction in repair fidelity in two cases. Firstly, using the transformed A-T cell line AT5BIVA a reduction of about 6-fold in fidelity was found relative to a normal human cell line (MRC5V1). This reduction was very reproducible, and was also found when the reverse assay was used (i.e. breaking the neo gene instead of the gpt

gene) suggesting that it was not an artefact of the selective conditions. Secondly, a similar although less extreme reduction in fidelity was found for the mutant irs1 relative to the parental cells and the other irs mutants. To verify the A-T data, an attempt was made to use two other transformed A-T cell lines (AT4BI/NE1 and AT7BI/CA1) but for different reasons these proved refractive to the techniques used.

To overcome difficulties of DNA transfer and to allow further analysis of the break-rejoin process, we developed an in vitro assay based on treatment of recombinant DNA molecules with cell extracts. Using nuclear extracts and the lacZ-carrying vector pUC18 we established the feasibility of rejoining specific endonuclease-induced breaks in vitro. Comparison of extracts of normal MRC5V1 and radiosensitive AT5BIVA cells reinforced the result found with the DNA transfer technique: while the quantity of rejoined DNA was approximately the same for the two cell extracts, the frequency of misrepaired vector DNA molecules was about 30-times higher for the A-T extract under the conditions imposed. As yet these data have been obtained only with transformed A-T cells and using simple endonuclease (EcoRI)-induced breaks.

In a separate study, the effects of damage induced by X-rays or with the enzyme DNase I was assessed using recombinant vector DNA molecules transferred into hamster cells. The induction of DNA double-strand breaks (each resulting mainly from two interacting single-strand breaks after irradiation in solution) was measured by gel electrophoresis and correlated to the inactivation of a gene carried on the vector. The results suggested that double-strand breaks produced by X-rays or enzyme treatment are a major inactivating lesion.

(iii) Methods for the identification and cloning of DNA repair genes.

The use of high molecular weight human DNA transfers tagged with a selective marker gene into radiosensitive hamster mutants was explored particularly with the irs1 mutant.

Despite several attempts, in large experiments, to isolate transformants under conditions established with careful control experiments, no genuine transformants carrying human DNA were found.

The use of retroviral constructs for insertion mutagenesis was explored using standard endogenous genes as inactivation targets (tk and hprt) but it was found that the mutants detected did not mainly represent retroviral insertions. Thus the technique seems limited by the relative frequency of spontaneous to insertion mutations, and was not explored further for the isolation of radiosensitive mutants.

SV40 minichromosomes were isolated from unirradiated and UV-irradiated cells and their proteins analysed on gels. Two proteins of 220 and 230 kd were identified after UV irradiation which were not present in unirradiated samples. Such changes in protein profiles were not found after gamma irradiation.

3. Discussion

(i) New radiosensitive mutants: comparison to human A-T cells.

The isolation of 3 new mutants adds considerably to the bank of such radiosensitive mutants available for study, and these mutants are already being used widely in the scientific community to study various aspects of radiation sensitivity and repair. irs1 is particularly different from other known mutants with its enhanced sensitivity to MMC, reminiscent of certain bacterial repair mutants. It has no apparent DNA break repair defect when assessed by sucrose gradient sedimentation, but was found to have a lack of fidelity of rejoining endonuclease-induced breaks. irs2 is the mutant which most closely resembles the human A-T cells, particularly with respect to its overall pattern of sensitivity and the radioresistance of DNA synthesis. As such it may be a useful vehicle for cloning A-T like genes and for parallel sensitivity studies. All 3 mutants seem to fall into a class, with A-T, showing moderate levels of radiosensitivity and no break-repair defect measurable using standard biochemical

methods. This type of mutant may more closely represent those mutations which are a burden to man, because they are tolerated by human developmental processes while the highly sensitive types will be lethal *in utero*.

The classification of radiosensitive mutants into complementation (genetic) groups is of considerable importance to identify the number of genes which control radiosensitivity in mammals. At present it seems that at least 8 or 9 genes have been identified from this study, and the paucity of mutants in each genetic group suggests that many more remain to be found. The ultimate aim of such studies is to 'map out' the genetic relationships governing radiation damage repair as a necessary step towards understanding the underlying enzymic processes and predicting response in different cell types.

The complementation studies on different A-T cells are the first to attempt to study genetic relationships using standard clonogenic survival techniques (previous work was on single cells, either using DNA synthesis or chromosomal damage as endpoints). The development of transformed cell lines has made this possible, but has the inherent danger that these lines may not represent the original phenotype. While this was checked in every way possible in the present study, the results do not concur with previous studies. This could be because of different endpoints used (although the DNA synthesis response was also checked in bulk populations), or because the transformed lines lack the genes which can complement the A-T phenotype.

(ii) Rejoin fidelity of DNA breaks

It was found that DNA double-strand breaks correlate to inactivating lesions in a vector DNA system, but that the mutants analysed in these studies do not show overt DNA strand-break repair defects. Systems measuring the fidelity of break rejoining, both in cells and *in vitro*, were developed to asess the finer aspects of repair. Thus, it was found that an A-T cell line consistently has a rejoin fidelity defect, and this phenotype is also displayed by the *irs1* mutant (but

not other hamster mutants analysed). It is tempting to speculate on the possible importance of this type of defect, especially in view of the cancer-prone and immune-defective symptoms of the A-T disorder. However, lack of fidelity has only been clearly shown for one A-T line as yet and it remains to test other lines and fresh diploid cells with this assay before a correlation can be proved. While conclusions are tentative, the development of such molecular assays must be important for the final understanding of the processes of DNA repair and the radiosensitivity of individuals in the population.

(iii) Attempts to identify and clone DNA repair genes.

It is clear that some methods for cloning the genes involved in radiosensitivity are not workable with certain mutants because the selective efficiencies are too low for a chance of success. Other methods may be more workable, although with the irs1 mutant attempts to use cDNA libraries and MMC selection, for example, also met with a lack of success (N.J. Jones and L.H. Thompson, pers. comm.). The SV40 system for the detection of repair proteins showed promise, and should be tried further with ionising radiations. The cloning of genes is still clearly a very productive means of rapidly assessing the nature of repair processes for radiation damage, and despite the difficulties warrants further effort.

Overall these studies have identified new genetic groups affecting radiation sensitivity, as the start of a process mapping out the genetic and biochemical basis for human radiosensitivity. We have also put forward a new molecular explanation for the human disorder ataxia-telangiectasia, which otherwise still defies understanding. In addition a number of new methods have been developed which may be applied more widely to achieve molecular understanding of cellular repair processes, eventually leading to the prediction of human responses.

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

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V. Publications:

1. Major publications

Thacker, J., and A. Stretch (1985) Responses of 4 X-ray sensitive CHO cell mutants to different radiations and to irradiation conditions promoting cellular recovery. Mutation Res., 146, 99-108.

Herskind, C., J. Thacker and O. Westergaard (1985) Radiation-induced inactivation of single isolated genes mediated by secondary radicals. Radiat. Prot. Dosim., 13, 153-156.

Cox, R., P.G. Debenham, W.K. Masson and M.B.T. Webb (1986) Ataxia-telangiectasia: a human mutation giving high-frequency misrepair of DNA double-stranded scissions. Molec. Biol. Med., 3, 229-244.

Thacker, J. (1986) The use of recombinant DNA techniques to study radiation-induced damage, repair and genetic change in mammalian cells. Int. J. Radiat. Biol., 50, 1-30.

Jones, N.J., R. Cox & J. Thacker (1987) Isolation and cross-sensitivity of X-ray sensitive mutants of V79-4 hamster cells. Mutation Res., 183, 279-286.

Debenham, P.G., M.B.T. Webb, N.J. Jones and R. Cox (1987) Molecular studies on the nature of the repair defect in ataxia-telangiectasia and their implications for cellular radiobiology. J. Cell. Sci., Suppl.6, 177-189.

Jones, N.J., R. Cox & J. Thacker (1988) Six complementation groups for ionising-radiation sensitivity in Chinese hamster cells. Mutation Res., 193, 139-144.

Debenham, P.G., N.J. Jones and M.B.T. Webb (1988) Vector-mediated DNA double-strand break repair analysis in normal and radiation-sensitive Chinese hamster V79 cells. Mutation Res., 199, 1-9.

Herskind, C. & J. Thacker (1988) Inactivation of DNA-mediated transformation of hamster cells by gamma-rays and deoxyribonuclease I. Mutation Res., 198, 169-178.

Debenham, P.G., M.B.T. Webb, A. Stretch & J.Thacker (1988) Examination of vectors with two dominant selectable genes for DNA repair and mutation studies in mammalian cells. Mutation Res., 199, 145-158.

Thacker, J., and P.G. Debenham (1988) The molecular basis of radiosensitivity in the human disorder ataxia-telangiectasia. In Mechanisms and Consequences of DNA Damage Processing (Eds. E. Friedberg & P. Hanawalt), New York: Liss, pp. 361-369.

Thacker, J., M.B.T. Webb & P.G. Debenham (1988) Fingerprinting cell lines: use of human hypervariable DNA probes to characterize mammalian cell cultures. Somatic Cell Molec. Genetics, 14, 519-525.

Thacker, J. (1989) The use of integrating DNA vectors to analyse the molecular defects in ionising radiation-sensitive mutants of mammalian cells including ataxia-telangiectasia. Mutation Res., 220, 187-204.

Thacker, J., R.E. Wilkinson, A. Ganesh and P. North (1989) Mechanisms of resistance to ionising radiations: genetic and molecular studies on ataxia-telangiectasia and related radiation-sensitive mutants. In DNA Repair Mechanisms and their Biological Implications in Mammalian Cells., (Eds: M.W. Lambert et al.), New York: Plenum, in press.

Thacker, J. (1989) Use of recombinant DNA molecules to study the repair of DNA damage and radiation mutagenesis. In Somatic and Genetic Effects of Ionising Radiation. Scientific Communications Inc., Umea, Sweden, pp.43-50.

Thacker, J. (1989) Inherited sensitivity to X-rays in man. Bioessays, 11, 58-62.

Brown-Luedi, M.L., and T.C. Brown (1989) Two proteins of 220 and 230 kD bind to UV-damaged SV40 minichromosomes in irradiated monkey kidney cells. Mutation Res., 227, 227-231.

Thacker, J., and A.N. Ganesh (1989) DNA-break repair, radioresistance of DNA synthesis, and camptothecin sensitivity in the radiosensitive irs mutants: comparisons to ataxia-telangiectasia cells. Mutation Res. (in press).

2. Short Communications

Thacker, J., A. Stretch, A. Hamilton & N. Jones (1985) Cellular studies of repair and recombination in normal and radiosensitive hamster cells. Brit. J. Cancer, 51, 609.

Jones, N.J., P.G. Debenham and J. Thacker (1986) Characterization of novel X-ray sensitive mutants of cultured mammalian cells. Int. J. Radiat. Biol., 54, 349-350.

Jones, N.J., P.G. Debenham and J. Thacker (1986) New X-ray sensitive mutants of cultured hamster cells. Brit. J. Cancer, 54, 349-350.

Debenham, P.G. & J. Thacker (1987) The human genetic disorder ataxia-telangiectasia: new insights into the molecular basis of radiosensitivity. In Proc. 8th. Int. Congress Radiation Research 1987, (Eds. M. Fielden et al.), London: Taylor & Francis, Vol.2, pp. 437-442.

Thacker, J. (1987) Repair of X-ray induced DNA damage in mutant mammalian cells. Brit. J. Cancer, 56, 177.

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. J. Thacker

Scientific staff: Dr. P.G. Debenham Dr. F.E. Benson
Dr. J. Thacker

I. Objectives of the project:

To use knowledge of bacterial genes, which can be cloned and characterized with relative ease, to help understand the repair processes for ionising radiation damage in mammalian cells.

II. Objectives for the reporting period:

To isolate and characterize a mutant of bacterial cells showing a moderate level of radiation sensitivity, and to clone and describe the affected gene.

To transfer cloned genes into radiosensitive and normal mammalian cells to assess complementation or perturbation of endogenous repair pathways so as to functionally characterize their repair processes/defects.

III. Progress achieved.

1. Methodology/Results

A new bacterial gene (*rorB*) influencing sensitivity to ionising radiation (and to mitomycin C) was isolated and characterized. The gene was mapped by standard bacterial methods (conjugation followed by transduction mapping) to 84.5 min., and finely mapped using a lambda clone carrying the *ilvGEDAC* genomic region. The gene was cloned from an *E. coli* library onto a 10 kb fragment initially, and then subcloned to smaller fragments for analysis. It was found that the *rorB* gene only gave partial restoration of radioresistance, indicating that at least two genes were involved in the initial phenotype (this was subsequently shown to be correct by genetic analysis). The proteins produced from the *rorB* genetic region were analysed from maxicell extracts, and a protein of 15 kd was identified as unique to this region.

A region of about 1000 base pairs, encompassing the *rorB* function, was sequenced and two open reading frames (ORFs) identified. Searches of protein databases revealed that one of these genes is a member of the LysR group of bacterial activator proteins, with close homology to the IlvY product (this gene activates transcription of the *ilvC* gene). No homology has been found for the other ORF detected in this region. Further analysis, with particular use of insertions into the ORFs, has shown that the *rorB* function is encoded by the unknown gene. The 15 kd protein appears to be produced by this gene.

The *rorA* mutation (Glickman *et al.* 1971, Biochim. Biophys. Acta, **254**, 144-154) is one of the few other genetic changes in bacteria identified as conferring ionising radiation sensitivity without UV light sensitivity. The *rorA* gene was crudely mapped, by those who isolated it, into the genetic region carrying several *rec* (recombination-deficient) genes. We have transferred *rorA* into a well-characterized genetic background and tested for the ability of plasmids carrying *recB*, *recC* and *recD* to complement the mutation. We eliminated the possibility that *recC* is the gene responsible but have had

difficulty in distinguishing the other two genes because of plasmid copy-number effects on survival of wild type transformants after gamma-irradiation.

The recA gene was trimmed successively to obtain a functional construct for cloning into different mammalian expression vectors. Cells expressing the gene were not found after DNA transfer experiments with the original constructs (by antibody reaction), and other genes were tested in the vectors to show that these able to give expression in mammalian cells. It was surmised that expression was toxic to the mammalian cells, and the gene was further cloned into a commercial vector (pMSG) with an inducible promoter. After some difficulty in obtaining a construct with the gene in the correct orientation, this vector carrying recA was transferred into normal human cells and it was shown that the induced gene gave considerable reduction in growth potential compared to uninduced and control cells.

2. Discussion

Bacterial mutant gene systems still provide the most common models for the action of DNA repair processes in mammalian cells, because of the wealth of detailed knowledge available for processes such as excision repair of bulky lesions. However, very few mutant genes have been isolated with primary sensitivity to ionising radiation (as opposed to those also having sensitivity to UV light), and models of ionising radiation-specific processes have therefore not been forthcoming. It should be noted that the major ionising radiation-sensitivity syndrome in humans is ataxia-telangiectasia, which does not show sensitivity to UV light or other such DNA-damaging agents. The isolation and characterization of a new gene with this phenotype in bacterial cells is therefore of importance in its applicability to modelling the mammalian cell responses, especially since, like A-T, the rorB gene shows only a moderate sensitivity to ionising radiation. It is of interest that the rorB gene may represent a new class of bacterial

genes, since we have found no homology with other bacterial genes as yet. With recently-isolated human repair genes involved in excision repair, comparison of the encoded proteins to those known to be involved in similar functions in yeast and bacteria has shown homologies over some domains, suggesting functional conservation. Further use for genes such as rorB may therefore be in the search for similar domains of function in mammalian proteins, to identify those conserved on an evolutionary timescale for the repair of ionising radiation damage.

Study of rorA, as another mutant gene of the type described above (X-ray sensitive, not UV sensitive), was initiated to verify that it is an allele of one the genes in the recB,C,D group. If verified, this is a particularly interesting mutant since, at least for recB and C, other alleles show UV-sensitivity, and its analysis should reveal ionising radiation-specific aspects of the action of this group of genes. These genes are known to be involved in recombination repair of radiation damage, coding jointly for an exonuclease of complex activity.

The expression of recA in mammalian cells was conceived as a prototype experiment with which to carry out gene transfer and gene dose-response studies. It seems likely that the gene was expressed in our constructs and that it has some toxic effects on mammalian cells. This study therefore opens the way to further work on the transfer of genes which are potentially more relevant to ionising radiation damage (such as rorA and rorB), and the study of complementation of unknown mammalian genetic defects by characterized bacterial genes.

These projects largely represent initial attempts to pursue a much underrepresented area of study: the analysis of repair processes specific to ionising radiation damage. A major aim of such work is to derive models of radiation damage repair which can be used to assess similar processes in mammalian cells, a procedure which has been very successful in analysis of the effects of other DNA-damaging agents. It is evident

that until we understand such processes we will not be able to predict the effects of ionising radiation under conditions relevant to human population exposure with any confidence.

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

V. Publications:

1. Major publications

Debenham, P.G., and M.B.T. Webb (1988) The isolation and preliminary characterization of a novel Escherichia coli mutant rorB with enhanced sensitivity to ionising radiation. Molec. Gen. Genetics, 215, 161-164.

Debenham, P.G., M.B.T. Webb and J. Law (1988) The cloning of the rorB gene of Escherichia coli. Molec. Gen. Genet., 215, 156-160.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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Contract no.: BI6-E-331-GR

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Title of the research contract:

Construction and use of eucaryotic indicator cell lines for the assessment of radiation induced alterations leading into new defined phenotypes

List of projects:

1. Construction and use of eucaryotic indicator cell lines for the assessment of radiation induced alterations leading into new defined phenotypes

Title of the project no.:

Construction and the use of eukaryotic cell lines, for the assessment of radiation induced alterations leading into new defined phenotypes.

Head(s) of project:

Dr. Helen Thomou

Scientific staff:

Dr. P. Kitsiou, S. Papoutsi,
G. Spanakos and Dr. C. Sambani

I. Objectives of the project:

To develop in connection to passage of phenotypes via transfection indicator eukaryotic stably transformed cell lines that would respond to low levels of radiation, not affecting cell survival, by producing quantitative signals indicative of new defined phenotypes. Our approach will be the construction of stably transformed cell lines with the pBMT3X vector carrying the mouse promotor and human metalothionein genes. As it is known "foreign" genes integrated into the mammalian genome by transfection, are more mutagenic, confer higher frequencies of mutants per rad than "native" mammalian genes, thus improving the sensitivity range of bioresponse to radiation.

II. Objectives for the reporting period:

1. Construction of stably transformed cell lines with Bovine Papilloma Virus vectors carrying the mouse promotor of metallotheionein gene, and human metallotheionein gene, and in particular the pBMT3X vector.

2.a) Study of cell lines growing as monolayers that have been stably transformed with pBMT3X vector that carries the human and mouse metallotheionein genes and confers resistance to increasing cadmium concentration.
b) Preliminary results indicating that "foreign" genes were integrated as episomes into the mammalian genome by transfection, as these appear from results of recombinant DNA techniques.

III. Progress achieved

INTRODUCTION

Great advances have been made in the past few years in the development of molecular cloning vectors that allow the expression of cloned genes in bacteria. The bacterial synthesizing machine, however, imposes a number of constraints on the expression of many eucaryotic proteins.

Systems are therefore needed which allow the re-introduction of eucaryotic genes into eucaryotic cells and permit their expression in a controlled genetic environment. Most of the eucaryotic expression vectors already described are based on viral replicons. SV40 and adenovirus have been widly used for the propagation and expression of exogenous genes in cultured cells (Rigby 1982). These systems, however, present several limiations. Therefore, a vector is required that is able to replicate in a stable manner as a multicopy episome and that allows the continuous replication and expression of the cloned foreign gene, independently from chromosomal controls and without concomitant cell lysis. BVP is unusual among the DNA tumor viruses in that it is maintained in the nucleus of transformed cells as a free, autonomously replicating episome at 50-200 copies per cell. These episomes, which are packaged as chromatin (Green 1986), have no centromere and therefore are randomly distributed in the daughter cells. In the transformed cells, its genome persists exclusively as multicopy non-integrated DNA and this peculiarity has made it the choice vector for eucaryotic cells (Lancaster and Olsen 1982; Pfister 1984).

In the present study, in connection to passage of phenotypes via transfection we constructed indicator eucaryotic stably transformed cell lines that would respond to low levels of radiation, not affecting cell survival, by producing quantitative signals indicative of new defined phenotypes.

Methodology

Cells

A clone of the V-79 cell line, a semihaploid line of fibroblasts from Chenese hamster, a standard line in mutagenic work, was used as experimental material. The cells were cultured in plastic flasks with Eagle's minimal medium with Earl's salts supplemented with 10% fetus bovine serum. The cultures were incubated in the dark at 37°C in CO_2 incubator.

Transfection

The method of calcium phosphate trnsfection of cells with purified plasmid was employed (Segal S., 1986) with some modifications. Briefly, cells to be used in transfection were tested for sensitivity to glycerol. From 16 to 20 hr before transfection remove cells from adherent growth with trypsin, inactivate with trypsin inhibitor and plate in flasks or culture dishes at $3x10^5$ cells in complete medium. From 2 to 4 hr prior to transfection, remove medium from plates, add 5 ml complete growth medium, and incubate at 37°C.

$CaPO_4$/DNA was prepared and the mixture stood in a cell culture hood for 30 min. at room temperature. Mix precipitate by pipetting and add very slowly to plated cells. Incubate for 36-48 hr in a humidified CO_2 incubator. Plate cells in a selective medium after trypsinization if selection is necessary for stable transfections.

Contransformation

Contransfection of cells with a plasmid containing a selectable marker and an excess of a nonselected gene of interest permits the isolation of colonies which constitutively express the latter gene.

Growth of bacteria and amplification of the plasmid

Inoculate 500 ml of LB medium containing the appropriate antibiotic (ampicillin) with a single bacterial colony. Indubate at 37°C overnight with vigorous shaking until saturation. Harvest the bacterial cells by centrifugation and lysis by SDS was followed according to Maniatis protocol. Purification of the plasmid DNA was achieved by centrifugation to equilibrium in cesium chloride-ethidium bromide gradient.

Restriction of pBMT3X

Plasmid DNA was digested with BamHI for 1 h at 37°C, 4 ensyme units per µg of DNA. The fragments were resolved by electrophoresis through 0.8% agarose gels for 3 h at 50 V. The fragments revealed were~2.0 and~14 kb. The 2.0 kb fragments were extracted from the gels by cutting out the band and sink the gel slice in a dialysis bag containing 0.5xTBE. Pass electric current through the bag (usually 100V for 2-3 hours). During this time, the DNA is electroeluted out of the gel and onto the inner wall of the dialysis bag. Reverse the polarity of the current for 2 minutes to release the DNA from the wall of the bag. Open the bag and recover all the buffer surrounding the gel slice. Purification of the DNA was achieved by passage through DEAE-Sephacel according to Maniatis et al. Molecular Cloning, A Laboratory Manual (1982).

DNA hybridization: DNAs from transformed V-79 with plasmid pBMT3X and non transformed cells were purified and prepared for restriction endonuclease digestion. DEAE-Sephacel purified DNA containing the gene of human metallothionein was labeled with biotin-7-dATP, using a commercial kit (Blue GENE, GIBCO/BRL). Agarose gel electrophoresis and transfer of DNA to nylon (Hybond N Amersham), was carried as described (Southern, 1975). Hybridizations were carried at 65°C for 18 hrs in the presence of 5% sodium dodecyl sulfate (SDS), 0.5 M Na

phosphate buffer pH 7.2, 1% bovine serum albumin (fraction V) and 200 ng/ml probe. Filters were prehybridized in a buffer of the same composition (without probe) at the same temperature for 2 h; after hybridization they were washed twice in 5% SDS, 0.5 M Na phosphate buffer pH 7.2 at room temperature and once in 5% SDS, 40 mM Na phosphate pH 7.2, 1 mM ethylenediaminetetraacetate at 65°C.

Signal detection was by the nonradioactive enzymohistochemical protocol provided by the manufacturer (BRL).

Metaphase chromosomes

After colcemid treatment mitotic transformed and non transformed V-79 cells were harvested by selective detachment as we have previously described (Thomou et al. 1988). Chromosome preparations were made by swelling cells in a hypotonic solution of 0.075 M KCl for 10 min, fixing in two changes of methanol-acetic acid (3:1) and dropping onto glas microscope slides (Pantelias et al. 1986). Slides were stained with 5% Giemsa. Metaphase cells were analyzed by means of light microscopy.

Results and discussion

One way to approach the practical problems of human exposure to radiation, such as radiation protection or radiation therapy, is by extrapolating from molecular studies to the doses and dose-rates relevant in human exposure. More data using more advanced biological systems are needed towards this goal.

With this rational we have employed increasing amounts of $CdCl_2$ (5-20 μM) in V-79 cells, and cell survival response was followed for 6 days. The results obtained tend to support the aspect that even in the concentration of 20 μM $CdCl_2$, during

the 4 days of culture, V-79 cells have a significant percentage of survival ranging from 58-60% (see Table).

Days of culture in $CdCl_2$	Percentage of survivial in increased $CdCl_2$ concentration			
	5 µM	10 µM	15 µM	20 µM
1 d	100	87.96	80.61	62.10
2 d	90.5	83.33	78.57	62.10
3 d	81.13	75.92	67.28	58.94
4 d	75.47	68.51	64.34	60.00
5 d	79.24	70.37	59.18	58.94
6 d	73.58	69.44	57.14	52.57

So, having located the survival rates of V-79 we focused our attempts to the transfection and selection as well as preliminary characterization of stably transformed V-79 cells with pBMT3X plasmid.

The data obtained suggest that the inserted plasmid pBMT3X results in the appearance of numerous surviving colonies which stably express the new phenotype i.e. resistance to high concentration of $CdCl_2$ (40, 60 and 80 µM). (The maximum frequency of transformation observed was 8 colonies per 10^6 cells per 18 µg of DNA).

Controls of V-79 cells, subjected to the concentrations mentioned above did not survive as expected.

Fourteen clones were isolated from the transformed cells, after 8 days of culture in 40, 60 and 80 µM $CdCl_2$. These resistant clones were cultured for 35-40 days and then one part of the clones were analyzed cytogenetically and the rest were frozen. In all cases the transformed cells became resistant to cadmium toxicity due to the overproduction of metallotheionein mRNA and polypeptide (Hamer 1986).

Cytogenetic analysis:

Chromosomal analysis of transformed cells, cultured for 5 weeks after initial establishment, revealed a homogeneous cellular preparation. To characterize the chromosome content of the abundant cell in the population, we compared the karyotypes of V-79 and transformed cells. The analysis revealed that the V-79 cell line contains most frequently a total of 21 chromosomes and for the transformed cells the same chromosome number was found and moreover, traces of non-integrated DNA which is packaged as chromatin and is referred as a minichromosome without a centromere (Campo 1986). This minichromosome may be considered as the cytogenetic equivalent of the multiple episome presence.

Furthermore, in order to demonstrate that clones resistant to 40 and 60 μM $CdCl_2$ contain the plasmid pBMT3X, DNAs from resistant clones and from V-79 cells were analysed in agarose gel electrophoresis.

In the present sutdy we demonstrate the stable integration of the plasmid into the cellular DNA of the recipient host cell in several independently derived clones. The experimental approach we have adopted to examine the state of the transfected gene, involves restriction endonuclease treatment of cellular DNA followed by hybridization analysis of the DNA fragments which contain information homologous to the gene.

More specifically, in an attempt to unequivocally prove that the resistant clones do contain, the whole pBMT3X plasmid and consequently the gene for human metallotheionein and the promotor of the mouse metallotheionein gene (Pavlakis 1983) cellular DNA from clones resistant to 40 and 60 $CdCl_2$ and from V-79 cells were isolated. The DNAs were purified and prepared for restriction endonuclease digestion either with EcoRI or with Bam HI and hybridization analysis was performed using as probe the human gene of metallotheionein insert. Briefly, purified pBMT3X plasmid DNA was restricted a) with BamHI endonuclease that generates two fragments~14.5 and~2.0 kb. The small fragment corresponds to the intact human metallotheion-

ein gene (Schmidt 1985), and b) with EcoRI endonuclease that generates 3 fragments 8.0, 4.5 and 4.0 kb. The 8.0 kb fragment corresponds to the insert that contain the intact human metallotheionein. The fragment i.e. 2.0 was labeled with biotin-7-dATP as described under Methodology.

BamHI

This restriction endonuclease cleaves the 16.5 kb pBMT3X plasmid at two sites generating two fragments~14.5 and~2.0 kb. The second fragment corresponds to the part of the plasmid that contains the intact gene for human metallotheionein. The results of this experiment, in which the gene sequences are localized in a BamHI digest of cellular DNA are shown in Fig. 1. The control includes 30 µg of total V-79 digested DNA with EcoRI or BamHI. As expected it did not hybridize with human metallotheionein gene. In the transformed cellular DNA resistant to cadmium concentrations 40 and 60 µM, the two bands at 16.5 and~2.0 kb have identical pattern and intensity a fact that is concordant with the presence of pBMT3X plasmid and indicated that rearrangement is not involved during transformation.

It has been reported that some BPV have acquired foreign DNA sequences either from the host genome or from the carrier DNA and have sufferred rearrangements involving deletions and insertions (Stenlund 1983). The faint band at 16.5 kb in the transformed cellular DNA can possibly be attributed to partial digestion of pBMT3X plasmid. This band corresponds to uncut plasmid.

EcoRI

This restriction endonuclease cleaves the 16.5 kb pBMT3X plasmid at three sites generating 3 fragments~8.0, 4.5 and 4.0 kb. The first fragment corresponds to the part of the plasmid that contains the gene of human metallotheionein, the second fragment contains the ampicillin resistance gene of the

plasmid and the third fragment corresponds to the part of the plasmid that contains the promotor of the mouse metallotheionein gene. The location of the gene sequences in EcoRI digested transformed cell DNA is shown in Fig. 2. As it was expected only 8.0 kb fragment hybridizes with the DNAs of transformed cells. The identical pattern and intensity of both transformed V-79 indicated the lack of rearrangement.

Thus, the fact that plasmid DNA and DNA from transformed cells revealed the same pattern after digestion with Bam HI or EcoRI may support the aspect that plasmid DNA is able to replicate in a stable manner as a multicopy episome that allows the continuous replication and expression of the cloned foreign gene independently from chromosomal control.

In conclusion, Bovine Papilomavirus (BPV) DNA, establishes itself in transformed cells as multiple episomes, the number of which varies in different cell lines, approximately from 20 to 300 copies (Campo 1986). However, in a given cell line, their number remains constant over very long periods of time. As the BPV minichromosome does not contain a centromere, its segregation to the daughter cells is likely to be a random event, and the copy number is therefore an average. The factors, if any, that determine the number of episomes in a given cell, and that maintain a constant average number in a given cell line, are unknown. Nor is it known whether BPV DNA replicates at a particular stage of the cell cycle, or whether its synthesis can occur throughout.

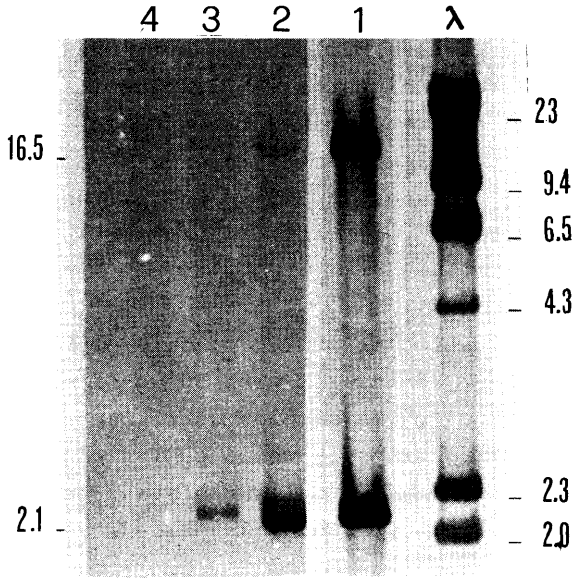

Fig. 1

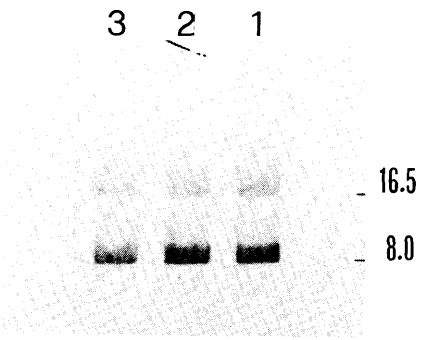

Fig. 2

Legends

Figure 1. Genomic DNA blot analysis: Thirty micrograms of each DNA digested with BamHI was electrophoresed on 0.8% agarose, blotted and hybridized to the insert of 2 kb of digested pBMT3X plasmid probe. DNA samples were prepared from the following: lane 1, pBMT3X whole plasmid; lane 2, transformed V-79 cells resistant to 40 μM $CdCl_2$; lane 3, transformed V-79 cells resistant to 60 μM $CdCl_2$ and lane 4, V-79 cells.

Figure 2. Hybridization of pBMT3X plasmid probe digested with BamHI (2 kb insert) with 30 μg of each DNA digested with EcoRI. DNA samples were prepared from the following: lane 1, whole pBMT3X plasmid; lane 2, transformed V-79 cells resistant to 40 μM $CdCl_2$; lane 3, transformed V-79 cells resistant to 60 μM $CdCl_2$.

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

V. Publications:

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Green, L., Schaffer, I., Wright, K., Moreno, M.L., Bernard, D., Hager, G., Stein, J. and Stein, G. (1986) Proc. Nat. Acad. Sci. USA, 83:2315.
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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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Contract no. BI6-E-167-NL

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

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Title of the research contract:

Processing of radiation induced and spontaneous genetic damage in prokaryotes and eukaryotes.

List of projects:

1. Processing of radiation induced and spontaneous genetic damage in prokaryotes and eukaryotes.

Title of the project no.:

Processing of radiation induced and spontaneous genetic damage in prokaryotes and eukaryotes.

Head(s) of project:

Prof.Dr. P. van de Putte
Dr. C. Backendorf

Scientific staff:

Dr. T. Kartasova
Drs. P. Belt
Drs. S. Gibbs
Mrs. W. Teubel

I. Objectives of the project:

Study of the influence of UV irradiation on cultured human keratinocytes. Identification of UV inducible genes. Analysis of the induction process. Relation to skin carcinogenesis.
Characterization of DNA repair systems in human cells. Development of a new vector system for the cloning and characterization of genes defective in xeroderma pigmentosum.

II. Objectives for the reporting period:

see objectives of the project (final report)

III. Progress achieved:

UV INDUCIBLE GENES IN HUMAN KERATINOCYTES:

Changes in gene expression might play an essential role during initial stages of carcinogenesis. During the contract period we have shown that unremoved DNA damage in UV irradiated human keratinocytes increases the synthesis of a number of proteins in a way similar to the bacterial SOS system. Several induced genes have been cloned and characterized. The regulatory elements responsible for the observed induction were isolated. Interestingly, we found that these genes are aswell regulated during normal development and differentiation. During the following years these findings will allow us to analyse in which way radiation interferes with normal gene regulation (at a molecular level) which again will allow us to detect those changes which might be crucial in early stages of carcinogenesis.

Methodology

Differential screening of cDNA libraries has been used to identify human genes whose expression is influenced by UV light in epidermal keratinocytes. The emphasis has been put on the study of those genes where the relative amount of specific mRNA increases after UV irradiation. In an initial screening more than 30 different cDNA clones were isolated. During the contract period most of our work has been focused on a family of related cDNA clones which code for small proline rich (spr) proteins. The expression of these genes has been monitored either by Northern blot analysis or by the use of specific antibodies in cultured keratinocytes or in skin sections. The regulatory elements of these genes have been cloned from genomic lambda EMBL3 libraries and fused to the CAT reporter gene. A new method for efficient transfection of primary human keratinocytes has been developed. A panel of human/mouse hybrid cell lines and in situ hybridisation to metaphase chromosomes were used to assess the chromosomal localization of the *spr* gene family.

Results

Structure of spr proteins: DNA sequencing of the different cDNA clones revealed an unusual protein structure in spr genes. Spr proteins are built up from repetitive units which are of two kinds: an N-terminal structure rich in proline and glutamine which is repeated twice in spr1 and spr3 but only present once in spr2 aswell as a middle repeat with the consensus sequence *K*PEP** present 6 times in spr1, 3 times in spr2 and 14 times in spr3. The spr1 and spr3 proteins have similar N- and C-terminal sequences but differ in the middle part of the proteins which made it possible to design specific probes for each of the two genes. Spr2 probes do not cross-hybridize with spr1 or spr3.

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 at short times after UV irradiation, this method is an easy way to monitor variations in cytoplasmic mRNA concentrations. Our analysis revealed that the different spr genes are not regulated in the same way : whereas spr2 and spr3 are induced after UV the amount of spr1 RNA remains constant. Total mRNA (polyA+) is decreased after UV irradiation.

Tumor promoters such as TPA are powerful modulators of gene expression in mammalian cells and have been shown to induce the expression of a number of target genes which can be expected to play an active role in early stages of carcinogenesis. Interestingly, *spr* genes turn out to be regulated aswell by tumor promoters. However in contrast to the results obtained with UV irradiation, TPA increases the cytoplasmic concentration of both spr1 and spr2 whereas spr3 is not influenced.

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. In a first instance the expression of the spr1 gene has been analysed. Specific an-

tibodies have been raised by using a synthetic peptide corresponding to the C-terminal part of the spr1 protein. This analysis shows that the amount of spr1 protein increases during terminal differentiation. These in vitro results have been confirmed by in vivo observations. An immunohistochemical analysis of human skin sections showed that spr1 expression is very weak in the proliferating basal cell layer but is clearly positive in the granular and spinal layer. No spr1 protein is detected in the upper cornified layer indicating that spr1 is transiently expressed during terminal differentiation. Recently we have aswell detected induction of spr2 transcription during terminal differentiation in cell culture.

Chromosomal location of spr genes: The spr genes constitute a multigene family where at least the spr2 gene is present in multiple copies in the human genome. In order to determine whether spr genes are localized on one or several human chromosomes, a mouse/human hybrid cell line panel was screened with specific probes for spr1, spr2 and spr3 in collaboration with Dr. A. Geurts van Kessel at the University of Rotterdam. From the results obtained it is clear that all spr genes are localized in the central part of human chromosome No. 1. This has been confirmed by in situ hybridisation to metaphase chromosomes (in coll. with Dr. T. Raap, University Leiden). From all spr genes genomic lambda clones have been obtained which are presently being analysed.

Isolation of the promoter of one member of the spr2 gene family:
Screening of the lambda EMBL3 human genomic library resulted in the isolation of several lambda clones which hybridize with the spr2 cDNA probe. Spr2-1 was analysed in detail. The gene is composed of two exons interrupted by an intervening sequence of approximately 800 basepairs. The whole spr2 coding sequence is localized on exon2. Exon1 is only 42 bp long and is preceeded by the spr2 promoter. Until now 600 base-pairs of the spr2 promoter have been sequenced and the following features have been observed: An RNA polymerase binding site (TATA box) is found at position -25. At position -193 the consensus sequence for a TPA responsive element (TRE) is found. This sequence which is the binding site for the fos and jun oncogenes has been shown to be involved in the regulation of other UV and TPA inducible genes isolated in other laboratories (Prof. P. Herrlich, University of Karlsruhe). Whether this sequence

is involved in the regulation of spr genes remains to be established. In any case promoter fusion to the cat reporter gene has shown that the cloned spr2 promoter harbours all the sequences necessary for regulated expression after UV irradiation, TPA treatment and induction of terminal differentiation.

Discussion

Recent work, in a number of laboratories, has shown that unrepaired DNA damage does not only induce mutations in living cells but also a temporary stress response reminescent of the bacterial SOS system. Since several years the department of Molecular Genetics is involved in studying these phenomena in cultured human keratinocytes. A number of UV inducible genes have been identified among which the spr genes have been studied in more detail. The spr gene family is composed of 3 subfamilies of genes which we have denominated spr1, spr2 and spr3. The exact number of copies per haploid genome of each of these genes is still under investigation and not exactly known. The spr2 subfamily is present in the human genome at approximately 8-10 copies. Genomic clones from 3 of these spr2 genes have been isolated. From one gene the promoter was identified and sequenced; this allows the study of the regulation of this gene on a molecular level (fusion to the cat reporter gene; deletion mapping). It will be interesting in the future to isolate promoter sequences from other members of the spr2 family in order to establish whether all spr2 genes are similarly regulated or whether different spr2 genes react differently to different stimuli. The same holds for promoter sequences from spr1 and spr3. .The isolation of promoter sequences will enable us to study and identify the transactivating proteins involved in the regulation of these genes. In particular it will be important to establish whether the AP-1 system (oncogenes jun and fos) which has been shown to be involved in the regulation of TPA responsive genes in human fibroblasts (and HeLa cells) is also implicated in the regulation of spr genes in primary keratinocytes. The presence of a TRE (=AP-1 binding site) in the promoter region of spr2 suggests that this might be the case. An analysis in primary keratinocytes is now possible as we developed a new method to transfect primary keratinocytes. Classical transfection methods do not work with these cells. We have shown that the different spr genes are are differentially regulated after UV or TPA treatment. Apparently different signal transduction mechanisms are involved in the regulation of the different members of the spr gene family. A molecular analysis of these processes is now possible.

EPISOMAL VECTORS FOR THE CLONING AND CHARACTERISATION OF DNA REPAIR GENES.

Human genes turn out to be very difficult to clone by direct transfection of mutant cell lines with DNA originating from normal cells. This is especially a shame in DNA repair research as a large number of mutant cell lines have been collected from patients with DNA damage syndromes (XP, BS, CS, AT, FA etc.). The difficulties encountered are essentially due to two intrinsic properties of human cells: 1) Uptake of only a few copies of foreign DNA (5-10 copies per cell). 2) Integration of mainly short fragments (up to 10 kb) of foreign DNA. Hence, it was important to develop a new transfection procedure which is able to cirucumvent these problems:
Use of cDNA expression vectors which can replicate autonomously in human cells: short DNA sequence; no integration problems; high copy number (50-100 copies/cell; direct selection for corrected phenotype in cell culture.

Methodology

1. Isolation of large amounts of polyA+ RNA from repair proficient cell lines and size fractionation on sucrose gradients.
2. Microinjection of size-fractionated mRNA samples into repair deficient cell lines.
3. cDNA synthesis from the correcting RNA fraction and cloning into lambda ZAP (lambda library).
4. Transfer of the cDNA insert from lambda into a newly designed episomal cDNA expression vector pECV25 (or related vectors) by using restriction enzymes which do poorly cut human DNA or the PCR technology (pECV library).
5. Transfection of the pECV library to mutant cell lines, selection for DNA uptake (hygromycine B) followed by selection for the mutant phenotype.
6. Isolation of transfected plasmids from corrected clones by using a low molecular weight DNA isolation procedure.
7. Characterization of cloned genes by antimessenger approach or overproduction of mutant proteins.

Results

Construction of episomal vectors and reconstruction experiments: A cDNA expression vector containing the element oriP and the sequence coding for the Epstein-Barr virus (EBV) nuclear antigen 1 (EBNA-1) as well as the hygromycin B resistance dominant marker gene (hph) has been constructed. Its characteristics have been compared to a similar vector lacking the EBV sequences: (a) The EBV^+ vector is maintained as an episome with a copy number of approximately 50 per cell, whereas the number of the integrated EBV^- copies is in general smaller than 10, when SV40-transformed xeroderma pigmentosum fibroblasts (XP20S-SV) constitute the recipient cell line. (b) The presence of the EBV sequences in the vector resulted in a 5-10-fold higher transfection efficiency with the calcium phosphate precipitation technique. (c) cDNA inserts in the EBV^+ vector are shown to be efficiently and properly expressed in the recipient cell. (d) If transfection is performed with a mixture of EBV^+ vectors with different inserts, transfectants are shown to harbour different plasmids within one cell. The ratio between these plasmids in one cell can be shifted in favour of a vector with a particular insert, when selection for this insert is performed. (f) Reconstruction experiments indicated that isolation of a low-abundance sequence from a mixture of vectors is at least 100-fold more efficient with the EBV^+ system, than with the EBV^- system. (g) Rescue of the episomal vector from transfected cells can be readily achieved.

Microinjection of purified mRNA fractions: As a first approach two different mutant cell lines derived from either a xeroderma pigmentosum (XP-A) or a Lesch-Nyhan (HPRT) patient were used. HPRT was included because the phenotype can be easily selected and the mRNA has a size which is comparable to XP-A (see below). Our analysis showed that a fraction of RNA (1200-1400 bp) was able to correct the XP-A defect. The same fraction corrected the Lesch-Nyhan defect as expected. Consequently a cDNA library constructed from this fraction can be used in transfection experiments with both cell lines.

Lambda libraries: Two different lambda libraries have been constructed: a) In lambda EH 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 ob-

tained. 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 sub-libraries 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 sub-library was positive for the HPRT gene (a rare mRNA species). b) In lambda ZAP the cDNA is inserted in both orientations into an EcoRI site, flanked by a NotI and a SalI site. This 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 two libraries with respectively 25.000 and 75.000 plaques have been obtained. The quality of the inserts was comparable to the EH library.

Construction of the pECV library: Both lambda ZAP libraries 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 in each case. 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 can be used for direct transfection of mutant cell lines.

Transfection of mutant HPRT cells: In a single transfection experiment (20 dishes with 1 million cells per dish) with the pECV library (original complexity 75.000) 4 independant HAT resistant clones were isolated. From 2 of these clones the correcting plasmids were rescued and shown to harbour the HPRT gene by DNA sequencing.

Transfection of XP-A cells: In 5 independent experiments with two different libraries (25.000 and 75.000 different cDNA inserts respectively) more than 200 dishes of XP_2OS-SV fibroblasts (10^6 cells/dish) were transfected and more than 10^5 hygromycin resistant transformants were obtained which were subsequently challenged with UV light. In all these experiments two independant UV resistant clone (denominated

6/4 and A24 respectively) were isolated.
a) Characterisation of clone 6/4: The use of a human polymorphic repetitive probe confirmed that this UV resistant clone was indeed derived from the UV sensitive XP_2OS-SV mother population. UV survival curves established that the 6/4 clone was about 70-80% resistant as compared to repair proficient fibroblasts or HeLa cells. A similar conclusion was reached after UDS measurements. In order to rescue a possible XP-A correcting EBV vector from the 6/4 clone low molecular weight DNA extractions were performed and transformed to E. coli. However, no ampicilline resistant bacterial colonies could be obtained. The reason for this failure turned out to be due to the fact that in 6/4 clones no episomal copies of the EBV vector were present anymore but rather that one copy of the vector had integrated into the host genome. Moreover the cDNA expression vector had been fractionated into at least several parts which will make the rescue of a correcting cDNA insert extremely difficult. Indeed the only vector sequences which can be detected are part of the ampicilline gene, the hygromycin selection marker and part of the RSV promoter. Unfortunately a break occurred between the RSV promoter and the cDNA insert.
b) Characterisation of clone A24: UV survival curves have indicated that the resistance of the A24 clone reaches nearly wild-type levels in contrast to clone 6/4. Furthermore episomal plasmid DNA could be isolated easily from this transfectant. Secondary transfection experiments are presently being performed to determine whether the acquired resistance is carried by the episomes.

Characterisation of cloned DNA repair genes: The episomal cDNA expression vectors can also been used for the characterisation of cloned repair genes. During this contract period experiments have been performed with the ERCC1 gene which has been cloned several years ago in the laboratory of Prof. Bootsma in Rotterdam by correction of a UV and mitomycin C sensitive CHO mutant. Although it had been univoqually established that the human gene can correct the rodent DNA repair defect, a direct involvement of the ERCC1 gene in human repair had not been shown. That this is indeed the case has now been shown with the use of the episomal vector. The ERCC1 gene has been cloned in an antisense orientation into pECV25 an this high copy vector has been introduced into repair proficient human cell lines. The increased sensitivity of the transfectants to mitomycin C indicates a direct involvement of the ERCC1

gene in human repair. The fact that sensitization to mitomycin C is greater than the one to UV suggests that ERCC1 is mainly involved in crosslink repair. An interesting result has aswell been obtained with a lab-made ERCC1 mutant which turned out to have a co-dominant phenotype.

Discussion

During the contract period a novel cDNA expression vector has been constructed. Reconstruction experiments have shown that this system is approximately 100 times more efficient than classical vector systems described until now. Although the final goal, the cloning of the XP-A gene, has not yet been reached the cloning of the HPRT gene from a cDNA library by correction of a human mutant cell line in a small scale experiment ($2x10^7$ cells transfected) shows that the episomal cDNA expression approach described here is by far the most efficient " cloning by phenotypic correction system" known today.

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

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V. Publications:

Backendorf, C. and Van de Putte, P. (1985) Expression of a bacterial repair gene in mammalian cells. Biochimie 67, 399-403.
Zwetsloot, J.C.M., Barbeiro, A.P., Vermeulen, W., Hoeijmakers, J.H.J. and Backendorf, C. (1986) Microinjection of Escherichia coli UvrA, B, C. and D proteins into fibroblasts of Xeroderma pigmentosum complementation groups A and C does not result in restoration of UV induced unscheduled DNA synthesis. Mutation Res. 166, 89-98.
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Kartasova, T., 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.

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Related work

Kaina, B., 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
Final Report

Contractor:

State University of Leiden
Stationsweg 46
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Contract no.: BI6-E-169-NL

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Telephone number: 071-276115

Title of the research contract:

The genetic and biochemical basis of radiation sensitivity in human and other mammalian cells in culture.

List of projects:

1. DNA repair and mutagenesis.

THE GENETIC AND BIOCHEMICAL BASIS OF RADIATION ACTIVITY IN HUMAN AND OTHER MAMMALIAN CELLS IN CULTURE

Project 1. DNA repair and mutagenesis
Contract no. BI6-E169-NL

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Introduction

Bacteria are known to adapt to various environmental stresses. such as DNA damaging agents, resulting in the transient activation of a number of phenomena, called SOS-functions. The induction of the SOS system is accompanied by a higher survival and higher mutagenesis of damaged phages infecting the treated bacteria, two responses that are known as Weigle reactivation (WR) and Weigle mutagenesis (WM), respectively.

The purpose of the present project is to investigate whether SOS-like responses can also be induced in mammalian cells by treatment with DNA damaging agents, using Herpes simplex virus type 1 (HSV-1) as a probe. Our working hypothesis is that such phenomena might be responsible for alterations in cells that can initiate certain steps in the proces of carcinogenesis. The studies are focussing on the possible relationships between SOS-functions, mutations and cancer and the identification of processes involved in the SOS-response.

Previous work in our laboratory had shown that the SOS-like phenomena Enhanced Reactivation (ER) and Enhanced Mutagenesis (EM) are induced after UV-treatment of normal human cells and certain XP cells (XP ER^+). 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 (XPER^-) could be correlated with the lack of tumors in the patients, even on sunlight-exposed skin aereas, whereas XP cells exhibiting the usual ER response (ER^+) were derived from patients that were cancer-prone. These results suggested that the ER response could somehow be involved in the process of oncogenic transformation.

In order to investigate this phenomenon further, we have also determined the time-course of induction of ER and EM in cells from repair deficient Trichothiodystrophy (TTD) patients, which in contrast to XP, show no increased cancer incidence. In addition, ER has been investigated in UV-treated skin fibroblasts derived from the following human heriditary cancer prone syndromes: Wilm's tumor (WT), Polyposis coli (PC), Von Recklingshausen's Neurofibromatosis (NF), Dysplastic Nevus syndrome (DNS), Von Hippel-Lindau syndrome (VHLS) and Bloom's syndrome (BS). To obtain further information on the putative relationship between

FR and radiation-induced carcinogenesis, we have started an investigation on the expression of UV-inducible genes in radiation-sensitive cells. The results on one of these studies, the UV-induced stabilization of the cellular tumor antigen p53, will be presented. The p53 tumor antigen is a protein which has an essential role in the control of cell proliferation (Caldbretta et al, 1986). The protein has a short half-life in most normal cells, but the stability, is greatly increased in certain tumor cells and virus-transformed cells (Sarnow et al., 1982). Recent data indicate that the p53 gene behaves as a tumor suppressor gene (Finlay et al., 1989).

1. Methodology

Cultures of normal human diploid skin fibroblasts and of skin fibroblasts from the following human cancer-prone syndromes: Wilm's tumor, Polyposis coli, von Recklingshausen's neurofibromatosis, Displastic naevus syndrome, Van Hippel-Lindau syndrome and Bloom's syndrome, were exposed to various UV-doses in order to induce SOS-like functions. Cultures were infected with unirradiated or UV-irradiated Herpes simplex virus (HSV-1) 24 hours after UV-treatment of the cells in order to quantitate the ER-response. In addition, the time-course of induction of FR and EM were studied after UV-exposure of Trichothiodystrophy (TTD cells). The procedures to measure ER and EM have been described by Abrahams et al. 1984.

The stabilization of the cellular p53 tumor antigen was also investigated after UV-treatment of normal human skin fibroblasts, ER^+ and ER^- XP cells and TTD cells. Shortly after UV-exposure, cultures were labeled with ^{35}S-methionine for a few hours, after which they were chased with non-radioactive medium for various periods of time. Using an anti-p53 monoclonal antibody, the p53 cellular tumor antigen was specifically immunoprecipiated and the protein complexes were analyzed by SDS-PAGE. In skin fibroblasts from Wilm's tumor patients, the kinetics of the removal of thymidine dimers were determined using the T4 UV-endonuclease method.

2. Results

a. Abnormal ER in certain XP cells and in cells from cancer-prone syndroms

We have previously shown that ER and EM are maximally expressed 24-48 hrs after UV-treatment of normal human skin fibroblasts and some XP cells ($XP\text{-}ER^+$) (Abrahams et al. 1984). However in other XP cells only induction of EM was observed, whereas ER was absent ($XP\text{-}ER^-$). It was then noticed that the $XP\text{-}ER^+$ cells were derived from XP patients which exhibited skin cancer as usual, whereas the $XP\text{-}ER^-$ cells were derived from patients that were reportedly free of skin tumors at the time they were described in the literature (Abrahams et al., 1988). This observation suggested that the ER response may possibly be related, directly or indirectly, to the proces of carcinogenesis.

In order to investigate this phenomenon further, we have determined the time-course of induction of ER and EM in cells derived from TTD patients which are characterized by high sensitivity of the skin to ultraviolet light, low survival of skin fibroblasts after UV-treatment but, in contrast to most XP patients, no increased cancer incidence. The survival of UV-irradiated HSV-1 in TTD cells was 2.5×10^{-3}, in XP-D cells 2.3×10^{-3}, and in normal cells 1.8×10^{-1} at a UV-dose of 40 $J.m^{-2}$. In time course experiments normal expression of EM was observed at 24 hrs after UV-treatment of the TTD cells, but ER could not be detected.

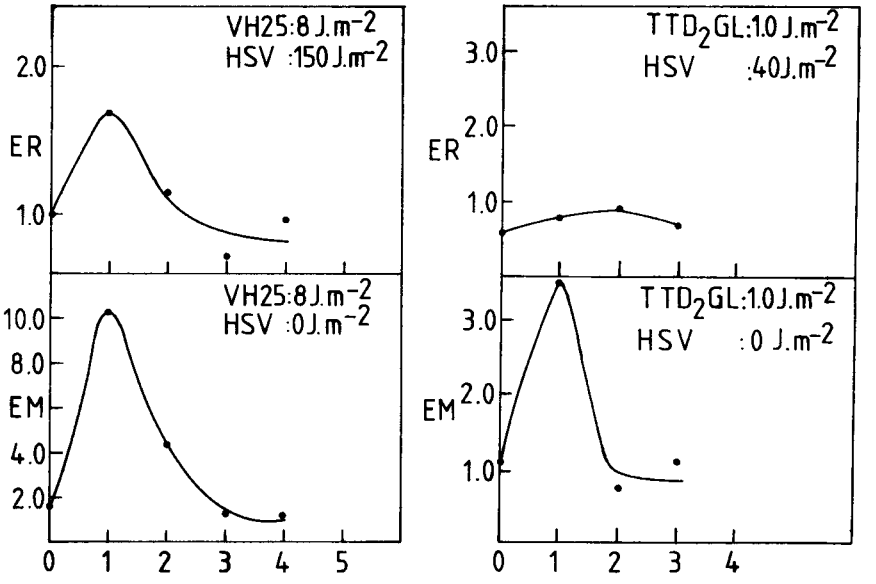

Fig.1. Time course of ER and EM of HSV in UV-irradiated normal cells (VH25) and Triochothiodystrophy cells (TTD_2GL) confluent cultures were kept under liquid holding conditions for 72h before irradiation. After irradiation the cells were maintained under liquid holding conditions until infection. The UV doses to the cells and to HSV are indicated. Irradiated cultures and unirradiated controls were infected at various times after irradiation of the cells for the determination of ER and were infected with unirradiated HSV for determination of EM.

This result supported our previous observation, that the ER response was somehow correlated with the induction of cancer. To study this correlation further, the ER response was determined in UV-irradiated skin fibroblasts from the following hereditary cancer-prone syndromes: Wilm's tumor (WT), Polyposis coli (PC), von Recklingshausen's neurofibromatosis (NF), Dysplastic Naevus syndrome (DNS) von Hippel-Lindau syndrome (VHLS) and Bloom's syndrome (BS). Interestingly, much higher levels of ER were observed in all these syndromes than in normal human skin fibroblasts. Examples of the dose reponse of ER in normal cells and in cells from the various cancer prone syndromes are shown in Table 1.
As can be seen, much higher levels of ER could be induced in all these syndromes than in normal skin fibroblasts. These results supported the notion that the ER response might somehow be related to the process of cancer induction, although the appearance of the same phenomenon in genetically different hereditary diseases is difficult to explain. Another unexpected result was obtained with cells from WT, DNS and VHLS patients. It was observed that the survival of UV-treated HSV-1 was much

Table I
ER values of HSV-1 in cell lines from normal humans and from various cancer rpone syndromes: Wilm's tumor (WT) Polyposis coli (PC), Von Recklingshausen's neurofibromatosis (NF), Dysplastic nevus syndrome (DNS) van Hippel Landau syndrome (VHLS), Blooms syndrome (BS)

UV dose to the cells	normal	WT	PC	NF	DNS	VHLS	BS
0 $J.m^{-2}$	1.0	1.0	1.0	1.0	1.0	1.0	1.0
10 "	1.8	4.7	2.7	5.6	2.4	5.4	4.0
15 "	2.2	8.1	4.6	10.3	7.5	10.2	5.2
20 "	2.8	12.6	7.2	16.5	23.7	13.6	7.0
25 "	2.3	18.4	11.3	16.9	29.2	21.0	4.8

Cultures of skin fibroblasts of normal cells and cells derived from various cancer prone syndromes were exposed to various UV doses and infected with UV-irradiated HSV-1 (150 $J.m^{-2}$) 24 hrs after UV exposure of the cells.

lower in skin fibroblasts from 3 out of 4 WT, 2 out of 10 DNS and 2 out of 5 VHLS patients, suggesting that the repair capacity of skin fibroblasts derived from various cancer prone syndromes is impaired. This was confirmed by an analysis of the rate of removal of thymidine dimers in various WT cells. Using the T4-UV endonuclease method, we observed in 2 WT cells a slower rate of dimer removal than in normal cells indicating that these WT cells might have a DNA repair deficiency (results not shown).

b. UV-induced stabilization of the cellular tumor antigen p53

In order to characterize the XP-ER^+ and XP-ER^-, TTD ER^- and normal wild type cells further we have also studied the UV-mediated stabilization of the p53 cellular tumor antigen in these cells. UV-irradiation and pulse-chase experiments were carried out as described in Methodology. In figure 2 the results of representative experiments are shown.
As can be seen a considerable stabilization of the p53 protein was observed in normal human cells after UV exposure. The half-life of p53 in untreated cells is about 1 hour, whereas the half-life in UV-exposed cells is 7 hours. (A) Similar pulse-chase experiments carried out with XP-ER^+ and XP-ER^- cells showed that only some of the XP cells exhibited stabilization of p53 protein (B), whereas most XP cells showed little if any stabilization (C). No clear correlation was observed between expression of the ER response and the stabilization of p53 protein in UV-treated XP cells. However, it was noticed that in untreated XP-ER^- cells the half-life of p53 was already somewhat higher ($t_{\frac{1}{2}}$ = 3 hrs) than in untreated normal cells ($t_{\frac{1}{2}}$ = 1 hrs), and in the $XPER^+$ cells ($t_{\frac{1}{2}}$ = 1.5 hrs) suggesting that the p53 is already somewhat stabilized in untreated $XPER^-$ cells. An abnormally long half life of p53 was observed in untreated TTD cells. In the 3 TTD cell lines studied, the following half-lifes were found: cell line 1: $t_{\frac{1}{2}}$ = 3.5 hrs; cell line 2: $t_{\frac{1}{2}}$ = 7.5 hrs; cell line 3: $t_{\frac{1}{2}}$ = 16 hrs. UV-treatment of the cells resulted in stabili-

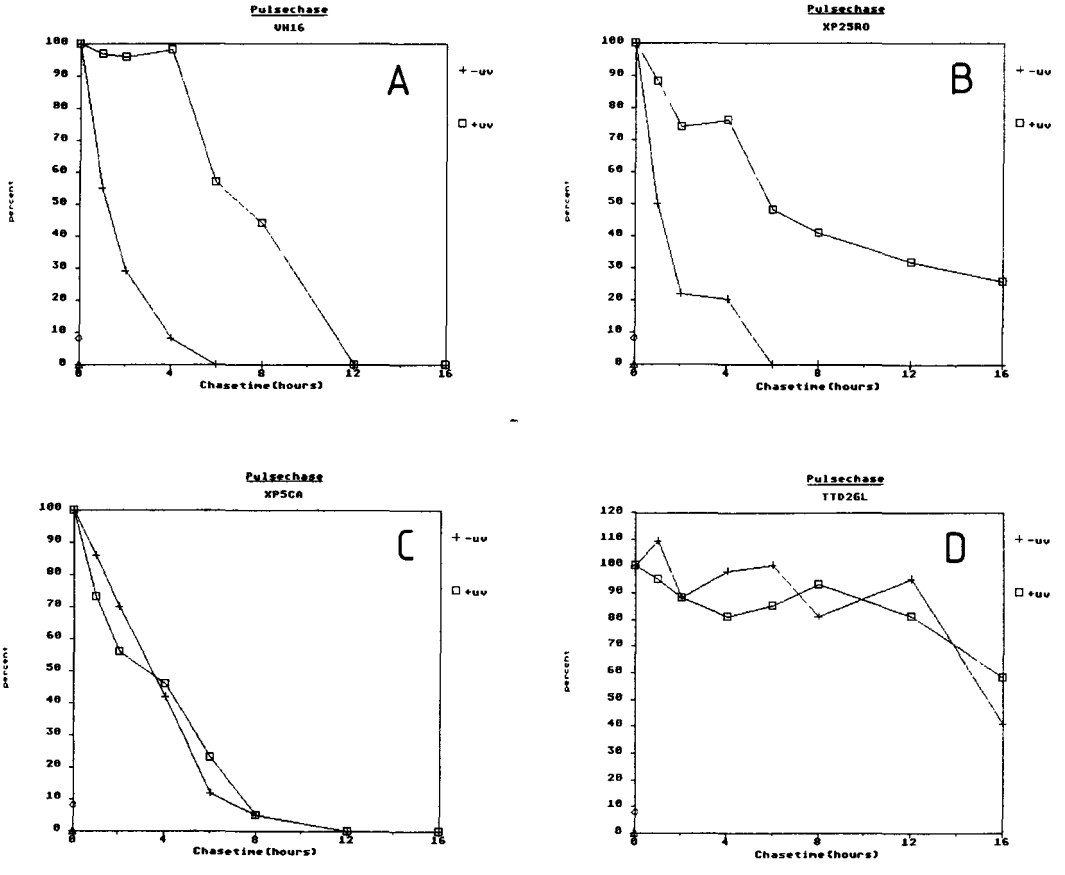

Fig.2. Metabolic stability of p53 in untreated and UV-treated VH16 (normal), XP25RO ($XPER^+$), XP5CA ($XPER^-$) and TTD2GL ($TTDER^-$) cells. Culture were exposed to UV-light (6.0 $J.m^{-2}$) and labeled with ^{35}S-methionine for 3 hrs. The procedures for chasing and immunoprecipitation of the p53 protein have been described in Methodology. The radioactivity associated with the p53 protein bands in the SDS-PAGE gels was quantitated by densitometry. The relative amount of p53 at each time point is shown graphically.

zation of p53 protein in cell line 2, whereas no effect was observed in cell line 1 and cell line 3. These results suggest that the p53 protein is already constitutively stabilized in untreaded TTD cells although the degree of stabilization varies from one strain to the other. In dose-response experiments, a dose dependent stabilization of p53 protein was observed in normal and $XPER^+$ cells, wherease this was not the case in $XPER^-$ and $TTDER^-$ cells.

3. Discussion

SOS-like responses have been studied in normal skin fibroblasts, in XP and TTD cells and in skin fibroblasts from various hereditary cancer-prone syndromes. In normal human and certain XP cells, derived from

patients which develop skin tumor, the ER and the EM responses are induced by UV-treatment. However, in XP cells, derived from patients exhibiting no skin cancer, only induction of EM could be detected, whereas ER was absent, suggesting that ER might somehow be related with the process of carcinogenesis. This observation was confirmed with cells derived from cancer-free TTD patients which show only induction of EM, whereas ER could not be detected. In addition, the ER response can be induced to very high levels in various hereditary cancer-prone syndromes. Why these genetically different diseases all show an abnormal ER response is unclear. Unexpectedly, some of the WT, DNS and VHLS cells exhibited a lower survival of UV-irradited HSV-1 than observed in normal cells, suggesting a deficiency in the DNA repair mechanism. Preliminary results indeed suggest that the rate of thymidine dimer removal is lower in the WT cells mentioned above, compared to the normal cells. The possible relationship between abnormal ER, cancerproness and defective DNA repair are still completely unclaer. We are presently attempting to detect molecular parameters that correlate with the abnormal ER responses. To that end the effect of UV-irradiation on the expression of various UV- and/or TPA-inducible genes are investigated by Northern-blotting, RNAse protection and by CAT-assays.

UV-treatment of normal human skin fibroblasts resulted in a transient stabilization of the p53 protein. However, in only some of the XP cells, the p53 protein was stabilized after UV-treatment, whereas in others little if any stabilization was observed. In the latter category the half-life of p53 appeared already constitutively increased in untreated $XPER^-$ cells compared to normal cells and $XPER^+$ cells. An even higher half-life of p53 was observed in some of the untreated TTD cells. The TTD syndrome is characterized by an XP-D-like UV-sensitivity but, in contrast to XP, no increased cancer incidence is observed.

Since recent studies in several laboratories have indicated that wild type p53 functions as a tumor suppressor protein we are investigating whether the stable p53 in ER^- cells is wild type or mutated. Preliminary data have shown that all p53 proteins in TTD cells which contain p53 with a constitutively increased half-life, is presumably not mutated but wild type. Whether this can explain the apparent resistance to cancer induction is unclear. Thus, together our results suggest that lack of cancer-proness, or "resistance" to sunlight-induced carcinogenesis in the skin correlates with an absence of the ER phenomenon. The same condition also correlates with a (partial) lack of UV-increased stabilization of the p53 cellular tumor antigen. In the latter cell strains, absence of UV-induced stabilization is accompanied with a more or less pronounced constitutive stabilization of p53 in the absence of UV-irradiation. The significance of the observed correlations, however, is far from clear, and will require much further work.

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Centre d'Etude de l'Energie Nucl.
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Contract no.: BI6-E-146-B

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Title of the research contract:

Radiation-induced structural chromosome aberrations in mammalian somatic cells.

List of projects:

1. Chromosome aberrations in peripheral blood lymphocytes of radiation therapy patients measured as biological indicators of genetic damage in man after partial body irradiation.
2. Accurate estimation of dose effect relationship for chromosome aberrations induced in human lymphocytes at low doses of X rays.

Title of the project no.: 1

Chromosome aberrations in peripheral blood lymphocytes of radiation therapy patients measured as biological indicators of genetic damage in man after partial body irradiation.

Head(s) of project:

Dr. A. Léonard

Scientific staff:

Gh. Deknudt
P. Jacquet

I. Objectives of the project:

Dose effect relationships for the induction of chromosome aberrations have been well established for irradiation in vitro but are still relatively scarce after in vivo inhomogeneous exposure such as expected after accidental irradiation. For that purpose information can be obtained from patients undergoing therapy under well controlled technical conditions. An advantage of this procedure is that the patients provide their own control and that chromosomal aberration yields can be followed in the same person after increasing dose levels. In this study cytogenetic observations were mainly made on patients receiving radiotherapy for malignant glioma, uterine- or prostatic cancer.

II. Objectives for the reporting period:

This report covers different cytogenetic analyses of patients before and after radiotherapy sessions. In interim reports each year's results have been summarized. This report is therefore only a brief summary of the studies performed earlier and contains additional results of work on patients investigated during the final year.

III. Progress achieved:

METHODOLOGY

1. IRRADIATION TECHNIQUE

A. Uterine cervix cancer

External irradiation, given with 18 MV X-rays from a Saturne linear accelerator, was delivered to the whole pelvis up to 50 Gy (2 Gy per fraction and five daily fractions per week), using the classical "box-technique".

According to the current protocol, total pelvis irradiation includes all the clinical apparent disease, the entire uterus, the paracervical, parametrial, the utero-sacral regions, as well as the iliac, hypogastric, and obturator lymph nodes. The boundaries of the target volume are typically the following :

- superior border : mid L5 ;
- inferior border : mid obturator foramen ;
- lateral border : at least 1cm beyond the lateral margin of the bony pelvis.

The sizes of the antero-posterior (AP) and postero-anterior fields are typically 18 cm in height and 15 cm in width.

External pelvic irradiation was followed by intracavitary therapy. When this was not possible, an additional external irradiation of 10 Gy (in five fractions) was delivered on a volume confined to the residual disease using AP and PA fields.

B. Prostatic adenocarcinoma

The whole pelvis was irradiated first up to 50 Gy in five weeks. The classical "box-technique" was used as for the cervix treatment : the target volume includes the prostate as well as the most frequent sites of regional nodal metastases (obturator, external iliac and hypogastric). The size of AP and PA fields was typically 14 cm in height and 14 cm in width. Then, a boost confined to the prostatic gland and any area of proven gross extraprostatic disease was given up to 66 Gy (additional dose of 16 Gy). The field size for the boost was typically 7 x 7 cm.

In order to get homogeneous data, the observations were limited to patients irradiated with doses up to 50 Gy.

C. Glioblastoma

The patients were treated with a ^{60}Co unit (Picker) according to the following protocol :

- a large homolateral field, to encompass the whole cerebral hemisphere corresponding to the tumor ;
- two opposed lateral fields, limited to the tumor volume ;
- a frontal or occipital field depending on the tumor localization.

Doses of 2.5 Gy were delivered four times a week. A total dose of 55 Gy was delivered to the tumour volume ; the opposite hemisphere received a third of that dose.

Blood samples were taken by venipuncture prior to the first radiotherapy session (i.e. control value, for each patient) and 24 h after 10, 20 and 30 Gy to the tumor volume.

2. BLOOD CULTURES

Blood samples were taken by venipuncture prior to the first radiotherapy session and 24 h after radiotherapy sessions, to allow the mixing of the irradiated lymphocytes in the circulating blood.
For chromosome studies, 0.5 ml heparinized blood was incubated at 37°C in 5ml Ham's F-10 medium supplemented with bovine serum, phytohaemmaglutinin, streptomycin and penicillin. After 45 h colchicine was added and the cells incubated for an additional period of 3 h. After hypotonic treatment with KCl the cells were fixed with methanol-acetic acid. From each culture and when possible 200 metaphase figures with at least 45 centromeres were analysed for the presence of dicentric and ring chromosomes.

RESULTS AND DISCUSSION

Results regarding the yield of chromosome aberrations in the blood samples from patients with pelvic tumors or glioblastoma are summarized in tables 1 and 2 respectively.
The yield of dicentric and ring chromosomes observed in the blood lymphocytes sampled prior to the first radiotherapy treatment was found to be much higher in the pelvic tumour group than usually reported in the controls (0.88% vs. 0.078%). This may be related to diagnostic exposures and/or unknown medical treatments received by some patients during the period preceding the detection of their cancer. However, the yield of dicentrics observed in the glioblastoma group prior to the first radiotherapy session was found to be in complete agreement with the values usually reported in the controls (Lloyd et al., 1980). Here, anomalies were unfrequent after irradiation too, probably because of the highly localized exposures as well as the low number of lymphocytes present in the irradiated regions.

On the basis of the maximum likelihood method a linear dose-response relationship was fitted through the experimental points for the pelvic tumours (figure 1) and a linear-quadratic dose-response relationship for the glioblastomas (figure 2). According to these relationships the dose D at the target volumes inducing 10 dicentrics and ring chromosomes per 100 cells can be estimated to 58 Gy for glioblastomas. This is considerably higher than those found for mammary carcinoma (15 Gy, Antoine et al., 1981), pelvic tumours (5.62 Gy) or after in vitro irradiation (1 Gy).

For a uniform irradiation, the distribution of aberrations among the cells is Poisson. For partial-body or non-uniform exposure, the aberrations are distributed among the cells with a variance greater than that predicted by Poisson distribution. By examining the magnitude of this overdispersion, it is theoretically possible to estimate approximatively the proportion of the body exposed and its average dose.

Our results indicate that in the case of fractionated exposures, confined to a small volume of the body, it is not possible to estimate the total dose administered and that the method only provides an estimate of the proportion of the lymphocytes irradiated. By comparison to the dose (1 Gy)

necessary to produce in blood samples irradiated in vitro 10 dicentrics or ring chromosomes one can estimate that this proportion is 17% for the pelvic tumours and about 10% after 4 x 2.5 Gy to about 20% after 12 x 2.5 Gy for glioblastomas.

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Table 1 : Yields and distribution of dicentrics and ring chromosomes.

Case	Dose (Gy)	Cells scored	Total dicentric rings	0	1	2	3	4	5	6	7	Mean value	X^2 value	P	Coeff. of dispersion
1	0	200	3	197	3							015	.07	78***	98
	10	200	49	163	28	7	1	1				.245	4.85	026	1.41
	17 5	200	103	128	46	23	1	2				.515	6 51	0105	1 22
	28	154	89	101	32	14	3	2	1		1	577	9.10	3×10^{-3}	1 90
	48	104	73	56	32	11	4				1	.702	.683	.586***	1.50
2	0	200	0	200											
	2	200	101	114	70	14	1					507	1.91	.164***	.83
	10	200	48	161	33	4	1	1				.240	.607	56***	1 30
	16	200	67	161	33	14	2					319	10.7	1.5×10^{-3}	1.27
	24	200	88	148	33	8	7	2	2			44	13 31	$5\,4\times 10^{-4}$	1 95
	44	64	64	18	30	15		1				1.0	2.58	.104***	.65
3	0	200	2	198	2							01	.12	732***	.99
	2*	200	65	140	55	5						325	1.91	163***	83
	7	200	35	168	30	1	1					.175	.034	.85***	1.05
	13	200	63	148	44	6	1	1				.315	.064	.795***	1.16
	21	200	62	154	35	7	3	1				.31	3 45	.06***	1.39
	41 75	200	167	105	52	25	12	3	1	2		.84	14.5	1×10^{-3}	1.59
4	0	200	0	200											
	10	200	51	158	34	7	1					.255	1.40	.23***	1.13
5	0	200	0	200											
	18	200	41	172	17	9	2					205	20.5	6×10^{-5}	1.53
	30	200	84	138	47	10	3	2				.420	1.6	.205***	1.32
6	0	200	0	200											
	2	100	58	52	38	10						.58	.99	.68***	.76
	16	200	56	155	36	7	2					.28	1.49	.22***	1.18
	24	200	90	134	47	15	3	1				.45	3.43	.061***	1.21
	36	200	60	150	40	10						.30	1.56	.21***	1.03
7	0	200	4	196	4							.02	.045	.82***	.98
	2*	200	85	132	54	12	1	1				.425	.027	.86***	1.07
	8	200	42	168	24	6	2					.21	5 91	.014	1.36
	16	200	97	136	44	11	7	1		1		.485	5.28	.020	1.61
8**	0	200	3	197	3							.15	.073	.784***	.985
	2*	200	71	140	52	6	1	1				.355	.25	.62***	1.06
	6	200	3	197	3							.015	.73	.784***	.985
	32	200	87	138	40	19	3					.435	8.9	3.10^{-3}	1.21
	40	100	35	80	9	7	4					.35	17 2	$1.5.10^{-4}$	1.73
	50	200	96	135	44	15	4	1		1		.48	5.48	.018	1.52
9**	0	200	4	196	4							.02	.046	.82***	.98
	2	200	67	139	55	6						.335	1.28	.26***	.84
	6	200	18	185	13	1	1					.09	.18	.67***	1.35
	14	200	75	138	52	7	3					.375	.013	.90	1.05
	32	200	150	104	63	16	15	1		1		.75	14.82	$9\,7.10^{-4}$	1.34

Table 2 : Patients irradiated for glioblastoma.

Dose (Gy)	Donor	Cell scored	Dicen-trics	Observed Dic/cell	Dicentric distribution 0	1	2	3
0	1	200	0	0				
	2	200	0	0				
	3	200	0	0				
	4	200	0	0				
	5	200	3	0.015	197	3		
	6	200	0	0				
	7	200	0	0				
	8	200	0	0				
	9	200	1	0.005	199	1		
	10	200	0	0				
10	1	77	0		77			
	2	200	2	0.010	198	2		
	3	200	0					
	4	200	0					
	5	200	1	0.005	199	1		
	6	200	5	0.025	195	5		
	7	200	2	0.010	198	2		
	8	200	3	0.015	197	3		
	9	200	1	0.015	199	1		
	10	200	4	0.015	197	2	1	
20	1	200	1	0.005	199	1		
	2	200	5	0.025	196	3	1	
	3	200	0					
	4	200	8	0.040	192	8		
	5	200	6	0.030	194	6		
	6	200	1	0.005	199	1		
	7	200	1	0.005	199	1		
	8	200	11	0.055	192	5	3	
	9	114	5	0.044	196	3	1	
	10	200	3	0.015	197	3		
30	1	200	0					
	2	200	3	0.015	197	3		
	3	200	8	0.040	194	4	2	
	4	200	15	0.075	185	15		
	5	no cells						
	6	200	3	0.015	198	1	1	
	7	200	7	0.035	195	3	2	
	8	200	11	0.055	191	7	2	
	9	200	13	0.065	190	8	1	1
	10	200	6	0.030	194	6		

Figure 1 : Dose-response relationship for the induction of dicentrics and rings during radiation therapy for pelvic tumour. The curve represents a fit to a linear function ($y = 1.77 \pm 0.0003 \times 10^{-2}D$). Background levels of chromosomal aberrations were substracted.

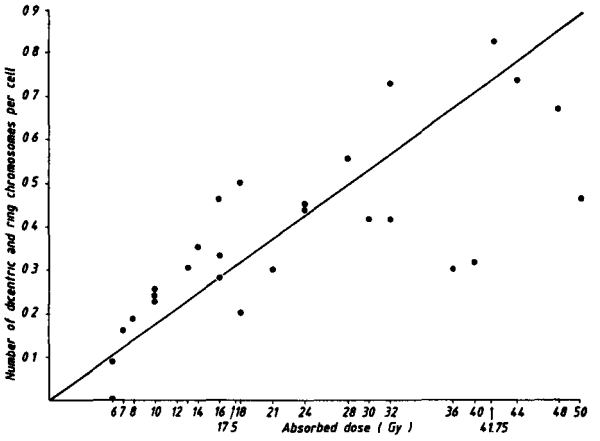
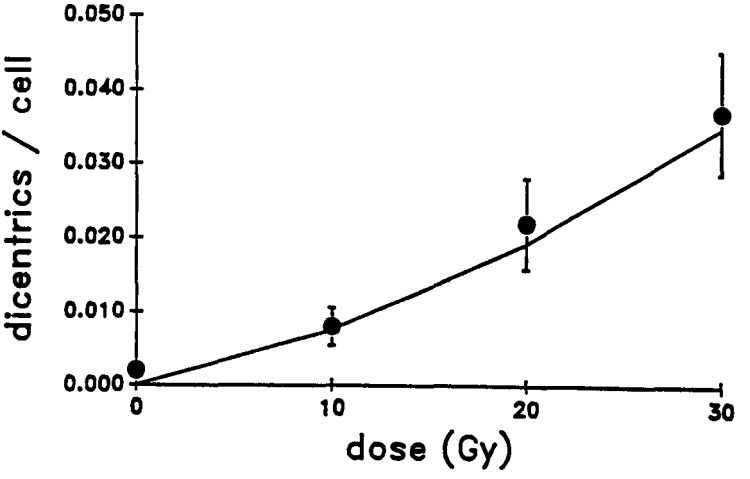


Figure 2 : Linear-quadratic dose-response relationship for the induction of dicentric chromosomes during radiation therapy for glioblastoma.



IV. 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

V. Publications:

- A. Léonard and G.B. Gerber (1985) Chromosome aberrations following partial body irradiation of man. In : Recent Trends in Medical Genetics, Pergamon Press, Oxford, pp. 187-206.
- L. Fabry, A. Léonard and A. Wambersie (1985) Induction of chromosome aberrations in G_0 human lymphocytes by low doses of ionizing radiation of different quality. Radiation Res. 103, 122-134.
- L. Fabry and C. Coton (1985) Study on the repair of the radioinduced lesions involved in the formation of chromosomal aberrations in G_0 human lymphocytes after exposure to gamma-rays and fast neutrons. Mutation Res. 149, 475-483.
- L. Fabry and M. Lemaire (1986) Dose response relationships for radiation- induced chromosome aberrations in human lymphocytes in vivo and in vitro. Strahlentherapie 162, 63-67.
- L. Fabry (1986) Cytogenetic damage induced in human lymphocytes by low dose of Co-60 gamma rays delivered at high and low dose rates. Acta Radiol. Oncol. 25, 143-146.
- L. Fabry (1986) Induction des anomalies chromosomiques dans les lymphocytes du lapin par des faibles doses de rayons X et de neutrons. C.R. Soc. Biol. 180, 212-217.
- A. Léonard, G. Decat, E.D. Léonard, A. Wambersie and J. Renard (1987) Chromosome aberrations in patients irradiated for pelvic tumours. Strahlentherapie und Onkologie 163, 795-799.
- L. Fabry, A. Léonard, G. Decat, Gh. Deknudt, P. Jacquet and E.D. Léonard (1988) Chromosome aberrations in mixed cultures of in vitro irradiated and unirradiated human lymphocytes. Strahlentherapie und Onkologie 164, 108-110.
- A. Léonard, Gh. Deknudt and E.D. Léonard (1988) Persistence of chromosome aberrations in an accidentally irradiated subject. Radiat. Prot. Dosim. 22, 55-57.
- A. Léonard, G. Decat, E.D. Léonard, M. Lemaire and L. Baugnet-Mahieu (1990) Chromosome aberrations in patients treated with telecobalt therapy for glioblastoma. 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:

Dr. L. Verschaeve
Dr. A. Léonard
Department of Radioprotection, SCK/CEN, Boeretang 200, B-2400 Mol

Scientific staff:

L. Verschaeve
Gh. 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 chromosomal aberrations. The primary objective was to verify the existence of any low dose plateau in the response over the range zero to a few tens of milligrays. Blood from a number of donors was irradiated and cultured in one laboratory and the microscope analysis was done in six collaborating laboratories. The extent of inter-donor and inter-laboratory variations in the data was also examined.

II. Objectives for the reporting period:

This report covers the whole study which has extended over 5 years. In interim reports each year's results have been summarized and during the final year the most recent work added data from blood of 20 donors irradiated to 5 and 300 mGy.

III. Progress achieved:

This study is a collaborative study involving 6 different European laboratories doing all the same cytogenetic analysis on coded microscope slides from the same origin. We therefore refer to the final report written by Dr. D.C. Lloyd who is the coordinator of the project.

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

Dr. D.C. Lloyd and A.A. Edwards - NRPB, Chilton, Didcot, U.K.
Dr. A. Natarajan - State University of Leiden, The Netherlands
Dr. G. Obe - University of Essen, Germany
Dr. F. Palitti - University of Rome, Italy
Dr. J. Tawn - BNF, Sellafield, U.K.

V. Publications:

D.C. Lloyd et al. (1988) Frequencies of chromosomal aberrations induced in human blood lymphocytes by low doses of X-rays. Int. J. Radiat. Biol. 53, 49-55.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-E-168-DK

Carlsberg Laboratory
Department of Physiology
10, Gamle Carlsberg Vej
DK-2500 Copenhagen Valby

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

Prof. D. von Wettstein
Department of Physiology
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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:

Maja Bojko, Joakim Glaman, Preben Bach Holm, Søren W. Rasmussen, Xingzhi Wang, Bente Wischmann

I. Objectives of the project:

The assessment of the effects of radiation and radiomimetic agents on human meiosis requires a detailed knowledge at the ultrastructural and molecular level of the normal course of meiosis. The work carried out during the previous program will be continued and extended to include the characterization of meiosis specific structures at the biochemical level, an analysis of the long and short term effects of radiation on meiosis, a reconstruction analysis of the meiotic prophase in the male mouse and the human female as well as an investigation of the effects of chromosome pairing and crossing over on regular disjunction in polyploid species.

II. Objectives for the reporting period:

The investigations comprise detailed studies of chromosome synapsis and crossing over in diploids and polyploid species aiming to gain further insight into the processes of the genetic regulation of chromosome pairing and crossing over, the extent of chromosome interlocking during synapsis and the short term effects of ionizing radiation on chromosomes in the meiotic prophase. The analysis of the biochemical composition of meiosis specific structures have been focussed on the possible role of DNA topoisomerase II and RecA-like proteins in meiosis.

III. Progress achieved:

1. The normal course of meiosis

1.1. Chromosome pairing in polyploids

The normal progression of chromosome pairing and crossing over has been studied in several situations selected to elucidate aspects of these processes which cannot normally be analysed in diploid species.

1.1.1. *Bombyx mori*

The silkworm, *Bombyx mori* comprises an ideal system for the study of chromosome pairing under a variety of conditions: The 56 chromosomes (2n) range in length from 5 to 11 μm at pachytene, a range comparable to that of human spermatocytes (5 to 20 μm). Pairing and synaptonemal complex (SC) formation start, as in human spermatocytes nearly always from the telomeres. Crossing over and chiasma formation is confined to the male sex which permits a comparison of chromosome pairing with and without a possible influence of crossovers. Finally, polyploidy is easily induced in both sexes, a situation rarely encountered among animals. It is therefore possible to study aspects of chromosome pairing not amenable to analysis in other animals, including man.

1.1.2. Correction of multivalents in autotetraploid *Bombyx*

Previous ultrastructural studies of synapsis and SC formation in achiasmatic autotetraploid (4n = 112) *Bombyx* oocytes revealed a strictly specific pairing of homologous chromosomes into bivalents and quadrivalents during zygotene. In pachytene nuclei nearly all quadrivalents had disappeared and the number of bivalents was close to the theoretical maximum of 56, i.e. a pairing pattern as normally observed in diploid organisms. Based on these observations it was concluded that the initial pairing with SC formation is succeeded by a correction process aiming to optimize regular bivalent formation at the expense of polyvalent chromosome associations. It can be inferred from these observations that the correction involves a turnover of the SC, whereby the central region of the SC is removed between "illegitimate" pairing partners followed by its reformation between chromosomes forming bivalents.

Crossing over and chiasma formation are absent in *Bombyx* females and the conversion of multivalents into bivalents can occur without being affected by crossing over between the homologous chromosomes of the multivalent associations.

In order to assess the effect of crossing over on the correction process, chromosome pairing and SC formation were analysed in recombination proficient autotetraploid *Bombyx* males. The number of bivalents and quadrivalents per nucleus was determined from spread and silver stained chromosome complements covering the period from zygotene to pachytene. At zygotene the nuclei contained between 7 and 18 quadrivalents, the remaining chromosomes forming bivalents (18-39 per nucleus) or trivalents-univalents (0-9 per nucleus). The mean number of quadrivalents decreased from a mean of 13.3 per nucleus at zygotene (66-95% pairing) through 11.1 at the zygotene-pachytene transition (95-99% pairing) to 8.7% at pachytene (99-100% pairing). A further reduction in the number of quadrivalents from 8.7 to 6.7 per nucleus occurs between pachytene and metaphase I (data from Kawaguchi, Cytologia (Tokyo) 9, 38-54, 1938). During the whole period, the disappearance of quadrivalents was matched by an increase in the number of bivalents.

The observation that the number of quadrivalents in many zygotene nuclei approached the number

expected upon random initiation of synapsis among the four homologues (67% or 18.8 quadrivalents per nucleus) and the fact that the number of univalents and trivalents was low (0-0.7 per nucleus) from mid zygotene onwards indicates that the chromosome movements and the recognition of homologous chromosome segments preceding the formation of the SC efficiently brings together all four homologous chromosomes. The subsequent reduction of the number of quadrivalents matched by an increase in the number of bivalents as pairing progresses shows that a correction of the initial pairing also takes place in *Bombyx* spermatocytes. Unlike in the oocytes, where virtually all multivalents were converted to bivalents, a mean of 8.7 quadrivalents per nucleus remained at pachytene in the male.

This is most likely the result of crossing over between the homologous chromosome held in polyvalent associations, the site where crossing over has occurred acting as a barrier to the turnover of the central region of the SC required for conversion of quadrivalents into bivalents. The further reduction in the number of quadrivalents between pachytene and metaphase I probably occurs when the SC resolve after pachytene and suitable chiasmata are absent to hold the four chromosomes together.

1.1.3. *Triticum aestivum*

Wheat, *Triticum aestivum* is an allohexaploid species combining the genomes of three closely related species. Despite the extensive homology between the three genomes (AA-BB-DD), crossing over and chiasma formation only occur between members of the same genome. This behavior is regulated by several genes on different chromosomes, the most important one being located on the long arm of chromosome 5B (5BL).

1.1.4. Genetic control of chromosome pairing in *Triticum*

The effect of 5BL on chromosome pairing was studied in plants containing 0, 1, 2, 4 or 6 copies of this chromosome arm. The chromosome complements of nuclei from the onset of chromosome pairing to the elimination of the synaptonemal complex (SC) were spread on membrane covered grids, silver stained and photographed in the electron microscope. Altogether 126 spread chromosome complements were fully analyzed. The extent of regular bivalent formation was assessed by determining the number of times a given chromosome formed a SC with more than one other chromosome giving rise to a polyvalent association rather than to a bivalent.

The analysis of this material at the high resolution provided by the electron microscope confirmed that the gene(s) on 5BL indeed play an important role in synapsis and SC formation: 1) In the absence of the entire chromosome 5B, SC formation ceased when 35-40% of the chromosome complement was paired. Among the partially paired chromosomes, switching of pairing partner gave rise to polyvalent chromosome associations affecting 56% of the complement. 2) SC formation increased to include 87% of the complement when one copy of chromosome 5B was present. This increase was accompanied by a reduction to 28% chromosomes involved in polyvalent associations. 3) In normal, euploid wheat with two copies of chromosome 5B, SC formation approached but never reached completion, the mean of 20 pachytene nuclei being 91% pairing. Multiple chromosome associations involved 15% of the complement at zygotene and 1.2% at pachytene. 4) A further increase in the number of long arms of chromosome 5B in the form of isochromosomes, composed of two fused long 5B arms, caused the SC formation to cease at 55% pairing with 4 copies of 5BL and at 25% pairing when 6 copies of 5BL were present. The number of polyvalent associations in the presence of 6 copies of 5BL was comparable to that observed in plants without 5BL whereas the percentage of chromosomes in polyvalent associations with 4 doses of 5BL was about the same as in zygotene nuclei of euploid plants.

A common feature of polyvalent chromosome associations in plants with more or less than two

copies of 5BL is that the polyvalent associations, once formed in zygotene persist until the SC is eliminated at diplotene. In euploid wheat on the other hand, there was a tenfold reduction of the frequency of polyvalent associations and a corresponding increase in regular bivalent formation between zygotene and pachytene, implying that pairing in the form of multivalents is somehow detected and corrected to optimize regular bivalent formation.

In euploid wheat, crossing over as identified by the number of chiasmata at metaphase I only occurs between homologous members of the same genome. This strict confinement of crossing over to homologous chromosomes is also observed in plants with one or four copies of 5BL despite the presence of a substantial number of polyvalent chromosome associations (which are expected to contain stretches of SC between perfectly homologous chromosome regions). This indicates that factors other than homologous pairing with SC formation are involved in regulating the occurrence and distribution of crossing over. In the absence of 5BL or when six copies of 5BL are present, the control breaks down and crossing over and chiasma formation occurs both between members of the same genome and between partly homologous chromosomes belonging to different genomes.

These results were confirmed by the study of the pairing behavior in wheat-rye hybrids containing 0, 1 or 2 copies of 5BL and in trihaploid wheat with or without 5BL, in both cases situations where only members of different, partly homologous genomes are available for pairing and SC formation. In all these genotypes, pairing and SC formation never reached completion at pachytene and always resulted in formation of polyvalent chromosome associations at zygotene, the highest frequency being observed when 5BL was missing. As in allohexaploid wheat there was a genotype dependent difference in the fate of the polyvalent associations between zygotene and pachytene (i.e. the stage of maximum pairing): Only when one copy of 5BL was present in polyhaploids and one or two copies in wheat-rye hybrids, was regular bivalent formation optimized by conversion of polyvalent associations into bivalents. In plants deficient of the 5BL, polyvalent associations were more frequent at zygotene and bivalent formation was not improved between zygotene and pachytene. With respect to crossing over and chiasma formation, the pattern was similar to that described for hexaploid wheat: The absence of 5BL removed the restraint on crossing over observed in haploids and hybrids causing an increase in chiasma frequency from virtually none in plants with 5BL to a mean of 7 per nucleus.

The study of chromosome pairing and chiasma formation in allopolyploid wheat thus showed that there are gene(s) located on the long arm of chromosome 5B which affect the initiation of synapsis and SC formation, the subsequent modification of the pairing pattern improving regular bivalent formation as well as the release of the strict confinement of crossing over and chiasma formation to members of the same genome.

1.3. Interlocking

A long standing enigma in chromosome pairing is why interlocked chromosomes are extremely rare at metaphase I considering the high probability with which interlocking (IL) is expected to occur during synapsis. This problem was solved when complete reconstructions of chromosome complements at zygotene became available. The first reconstruction study demonstrating ILs at zygotene was carried out on *Bombyx* oocytes where three of the four reconstructed zygotene nuclei contained one or more ILs. ILs were absent at pachytene and it was concluded that ILs were either rare or were normally resolved prior to pachytene. More extensive reconstruction analyses of human and *Bombyx* spermatocytes confirmed that chromosomes frequently interlock during synapsis and further showed that the interlocked chromosomes are released by breakage and rejoining of one or more of the involved chromosomes prior to the completion of pairing at pachytene. Subsequent studies by this and several other laboratories have confirmed these observations in a variety of organisms. An especially favorable situation exists in *Bombyx* spermatocytes in which cells in a particular stage of meiosis can be obtained and analysed in large

quantities by the spreading technique. This permitted a detailed study of the course of IL formation and resolution during zygotene and allowed quantitative estimates of the IL frequency to be made.

The analysis carried out under this contract, comprising 270 zygotene and 125 pachytene nuclei, showed that an average *Bombyx* spermatocyte nucleus will encounter a minimum of 4.2 interlockings during synapsis of its chromosome complement. The number of chromosomes affected by interlocking events (by interlocking other chromosomes, by being interlocked or by having their homologous pairing partner interlocked) was considerable, amounting to 60% of the complement in some nuclei. Free lateral component ends in putative interlocked chromosomes, indicating resolving ILs, were observed throughout zygotene at a frequency of 0.3 per nucleus. This implies that chromosome interlockings form and are resolved during the entire pairing process and that the frequency of ILs probably is underestimated.

Although untangling of intertwined chromosomes is theoretically possible without chromosome breakage, intertwining represents a similar restraint to chromosome movements as interlocking and may be detected and resolved by the same breakage-repair system which resolve true interlockings. The presence of a mean of 0.2 free lateral component ends per nucleus *not* associated with putative ILs supports this contention.

The observations in this as well as in a large number of earlier light and electron microscopical studies that interlockings and chromosome entanglements are virtually absent at pachytene or later stages of the meiotic prophase shows that the system which detects and resolves chromosome entanglements formed during synapsis at zygotene efficiently corrects this type of disorder and that chromosome breakage and reunion are regular and frequent events of chromosome pairing. In the assessment of the short term effects of radiation these observations are particularly relevant in the evaluation of the frequency of radiation induced chromosome breakage.

1.4. Conclusion

A meaningful assessment of radiation damage to meiotic chromosomes obviously depends upon a detailed knowledge of the normal progression of meiosis. The major aim of the research carried out under this and previous contracts has been to acquire this knowledge. The various events such as the formation of a chromosome bouquet as a prelude to synapsis, the recognition between homologous chromosomes or chromosome segments, the formation of the synaptonemal complex between the aligned homologues, the subsequent optimization of bivalent formation through turnover of the central region of the SC and the formation and resolution of interlockings all appear to be common features of meiosis in many organisms including man and all comprise possible targets for radiation induced malfunction.

2. Effects of ionizing radiation on meiotic chromosomes

2.1. Short term effects

The assessment of the short term effects of ionizing radiation was carried out on *Bombyx* spermatocytes irradiated at the beginning of the third larval instar. At this stage only premeiotic cells can be identified cytologically. The larvae were exposed to absorbed doses (^{60}Co source, 1.17 Me eV, 1.76 Gy/min, γ-radiation) of 0 (control group), 5 (group B), 10 (group C), 20 Gy (group D). About 10 animals were sacrificed at day 5, 7, 9, 11, 13 and 15 after radiation treatment and their chromosome complements spread, stained and examined in the electron microscope. The viability of the larvae during the period of analysis, even following a dose of 20 Gy, was normal. Degenerating nuclei were common in the preparations from group D but were seldom seen in spreads from groups B and C. The frequency of chromosomal aberrations in the control group (1 translocation and 6 tetraploid nuclei in a total of 1,570 nuclei) was constantly low from day 5 to day 15.

Among the irradiated nuclei, only 0.5% of the nuclei in group B and none in groups C and D were at zygotene at day 5 compared to 12% in the control group. At day 7 zygotene nuclei reappeared in the irradiated material, comprising 8% of the nuclei in group B, 11% in group C and 4% in group D. In the control group 9% were at zygotene. From day 7 onwards the frequency of zygotene nuclei varied between 15 and 24% in groups B and C and between 1 and 30% in

Table 1 The frequency (%) of pachytene cells with chromosomal aberrations after irradiation of the testes with absorbed doses of 0, 5, 10 and 20 Gy. (LC=lateral component of the synaptonemal complex; SC=Synaptonemal complex).

Day	Normal pachytene nuclei	Nuclei with rearrangements[a]	Tetraploid nuclei	Partial tetraploid nuclei	Nuclei with LC or SC breaks[b]	Number of nuclei
0 Gy						
	99.6	0.1	0.3	0.1	0.0	1,570
5 Gy						
5	63.3	20.6	5.9	3.4	6.9	996
7	62.2	19.6	10.4	7.8	0.0	878
9	85.3	10.4	3.0	1.4	0.0	569
11	94.7	5.0	0.0	0.4	0.0	666
13	97.2	2.0	0.5	0.3	0.0	776
15	99.2	0.7	0.2	0.0	0.0	721
10 Gy						
5	62.7	21.9	3.5	6.5	5.3	711
7	64.0	14.7	18.7	2.6	0.0	526
9	79.2	16.0	3.6	1.2	0.0	497
11	91.4	7.3	1.0	0.3	0.0	464
13	97.2	2.4	0.4	0.0	0.0	565
15	97.5	2.1	0.3	0.1	0.0	905
20 Gy						
5	49.4	28.0	9.7	7.7	5.2	731
7	53.1	20.4	22.8	3.8	0.0	455
9	64.8	30.7	3.4	1.1	0.0	101
11	85.4	13.5	1.0	0.0	0.0	110
13	93.1	6.9	0.0	0.0	0.0	32
15	87.5	12.5	0.0	0.0	0.0	46

[a] Simple reciprocal translocations, complex translocations involving more than two chromosomes, inversions and deletions.

[b] Does not include breaks in putative interlocked chromosomes, but possibly breaks generated by passage of intertwined chromosomes.

group D, values comparable or slightly in excess of the control. These observations suggest that the immediate response to radiation is a temporary arrest of meiosis. After a few days, progression of meiosis is resumed in the irradiated animals, repopulating the testis with a distribution of meiotic prophase cells at stages similar to that of the control group.

The frequency of chromosome interlockings was not determined quantitavely in the control group or in the irradiated material but appeared similar to that previously found in normal diploid nuclei.

Five days after irradiation, chromosome breaks and chromosomal rearrangements mainly in the form of simple reciprocal or complex multiple translocations were found in 27-33% of the spread complements of the three irradiated groups (Table 1). Unexpectedly, tetraploid nuclei as well as nuclei in which the lateral components (LC) were longitudinally split with a morphologically normal central region between the two half LCs ("partial" tetraploid nuclei in Table 1) were present at frequencies between 10 and 17%. In contrast to the tetraploid nuclei occasionally found in the control group (and in induced tetraploid larvae) the radiation induced 4n nuclei were devoid of quadrivalents. It is uncertain whether this radiation induced apparent chromosome doubling is accompanied by an additional round of premeiotic DNA replication or whether each chromatid forms its own LC following a single round of premeiotic DNA replication.

Between days 5 and 7, the most marked changes was the complete disappearance of LC breaks and a two to three fold increase in the frequency of tetraploid nuclei. The frequency of chromosomal rearrangements on the other hand remained constant in group B and decreased in the C and D groups. From day 7 onwards, the frequencies of aberrations declined in groups B and C, 99.2% of all complements being morphologically normal at day 15 in the B group and 97.5% in the C group. In group D, the total number of cells decreased from day 9 onwards and the reduction in the frequency of chromosomal rearrangements reached at day 15 was considerably less than in groups B and C.

These data show that even after irradiation with absorbed doses of up to 10 Gy, the gonads have the capacity of restoring a morphologically almost normal meiotic prophase within 13-15 days after the exposure to ionizing radiation. The primary lesion introduced by the radiation seems to include both the induction of transient chromosome breaks detectable either as discontinuities of the LCs or by the formation of chromosomal rearrangements and the introduction of a more general disturbance of the premeiotic DNA synthesis (or of the assembly/organization of the lateral components) resulting in apparent or true tetraploidy.

The correlation between the radiation dose and the observed frequency of induced chromosomal aberrations as measured by aberrant pairing behavior during SC formation depends upon the assumption that SC formation at pachytene faithfully mirrors the homologies of the chromosomes involved in the aberrant associations. This assumption is only to some extent correct. As described above the initial chromosome pairing and SC formation in polyploid species (and most likely also in normal diploids) is followed by a correction phase in which regular bivalent formation is optimized by conversion of polyvalent associations into two-by-two associations. This process probably also occurs in the irradiated nuclei thereby reducing the number of detectable chromosomal rearrangements. Additional correction of pairing and SC formation occur at the *intra*bivalent level ("synaptic adjustment", Moses, Chromosomes Today vol. 6, 71-82, 1977) which replace loops reflecting heterozygosity for inversions, duplications and deletions with nonhomologously paired straight stretches of apparently normal SC. Synaptic adjustment may at least in part explain the rare occurrence of these types of aberrations in the irradiated material (24 deletions and 4 inversions in 1,138 nuclei containing one or more chromosomal rearrangement). The frequency of radiation induced chromosomal rearrangements determined in the present study is thus almost certainly underestimated whereas the frequency of unrepaired breaks is possibly overestimated.

2.2. Long term effects

A study of the long term effects of radiation on meiosis was carried out on four patients having received radiotherapy for testicular cancer. The effect of gonadal radiation with absorbed doses

ranging from 0.35 to 1.08 Gy were analysed on testicular biopsies 27 to 40 months after radiation treatment. Pachytene nuclei were serially sectioned and the chromosome complements reconstructed from serial sections photographed in the electron microscope. In three of the four patients chromosome pairing and SC formation were normal in both autosomes and the sex chromosomes. Also the number and distribution of structures related to crossing over (recombination nodules and bars) were normal indicating that meiosis is fully restored. A biopsy taken from the fourth patient prior to radiotherapy showed carcinoma in situ changes in three quarters of the seminiferous tubules and the spermatogenic arrest observed 36 months after radiation evidently originated prior to radiotherapy.

3. Biochemical characterization of meiosis

3.1. RecA-like proteins

Specific pairing of homologous chromosomes prior to or during formation of the synaptonemal complex depends upon a recognition mechanism permitting a test of homology presumably at the DNA level. The simplest way to envisage such a test is by temporary DNA-DNA hybridization between DNA sequences of the involved chromosome regions (which may be homologous or not). Successful recognition would then depend upon the stability, i.e. the degree of DNA homology between the segments of the two chromosomes. It is well known from a number of studies that RecA and RecA-like proteins catalyse the transfer of single-stranded DNA to a homologous double-stranded molecule by hybridization with the complementary DNA strand. The reaction is, however, only completed by heteroduplex formation if the participating molecules are homologous. In cases of limited or no homology, the transient three-stranded complex is resolved.

In order to determine whether proteins sharing homology with the bacterial RecA or carrying out the DNA transfer reaction described above are present in protein extracts from meiotic nuclei, a sensitive strand transfer assay was developed based on the recent report of McCarthy et al. (PNAS USA, 85, 5854-5858, 1989). The RecA protein was furthermore isolated from *E. coli* carrying the cloned RecA gene and used to elicit polyclonal rabbit antibodies. The polyclonal antibodies gave a single band when tested against crude protein extracts from *E. coli* in an immunoblot assay but failed to identify any polypeptides in crude and partly fractionated extracts from somatic *Drosophila* cells and meiotic *Bombyx* cells.

Strand transfer activity was, however, detected in extracts both from somatic *Drosophila* and meiotic *Bombyx* cells. It was noted that extraction of proteins under conditions (80 mM DTT) where both the chromosome scaffold and the synaptonemal complex disintegrate improved the yield of the strand transfer activity.

3.2 DNA topoisomerase II

The observation that the resolution of chromosome interlockings and possibly of other entanglements as well require transient breakage and reunion of both sister chromatids implies a controlled process in which the DNA of the passing chromosome and its lateral components are held in register during the passage. This requirement inspired the proposal that DNA topoisomerase II which catalyses breakage, passage and rejoining of double stranded DNA molecules is somehow involved in the passage of one chromosome through another chromosome or bivalent. To test this hypothesis, DNA topoisomerase II was isolated from *Drosophila* Kc cells grown in continuous suspension cultures. The purified enzyme (166,00 kDa) was used to generate monospecific polyclonal rabbit antibodies. The polyclonal antibodies recognized in an immunoblot assay the DNA topoisomerase II in crude extracts prepared from somatic *Drosophila* cells and was able to inhibit the enzyme activity when assayed in a strand passage assay (unknotting of knotted,

circular P4 DNA). Both the enzyme activity and the signal in immunoblot assays of the protein extracts were stable for several months at 4°C (although proteolytic breakdown of the protein was evident).The antibodies furthermore recognized DNA topoisomerase II isolated from calf thymus (a generous gift from Anni H. Andersen, Dept. Mol. Biol. & Plant Physiol. University of Aarhus).

In crude protein extracts and partly purified DNA topoisomerase II prepared from *Bombyx* testes in which the majority of the cells are in various stages of meiosis, the strand passage activity of topoisomerase II was readily detected in a P4 assay, the estimated activity per cell being similar to that obtained with the same isolation procedure from somatic *Drosophila* cells. Unexpectedly, the protein extracts prepared from *Bombyx* testes exhibited a barely detectable cross reaction in an immunoblot assay when probed with the polyclonal antibodies raised against somatic *Drosophila* topoisomerase II. The weak cross reaction was only detectable in freshly prepared protein extracts from meiotic *Bombyx* nuclei whereas the enzyme activity was stable for several weeks at 4°C. The topoisomerase activity from meiotic *Bombyx* cells was inhibited by the polyclonal antibody against the *Drosophila* enzyme but required a tenfold excess per enzyme unit for complete inhibition.

It has previously been reported that polyclonal antibodies raised against mammalian DNA topoisomerases crossreact with cell lysates from a number of other species (e.g. antiserum raised against topoisomerase II isolated from calf thymus recognizes a single polypeptide of 180,000 kDa in immunoblots of whole cell lysates from calf, human, monkey, hamster and chicken. Earnshaw et al. J. Cell Biol. 100, 1706-1715, 1985). With this in mind, the extremely weak cross reaction between the anti *Drosophila* topoisomerase II antibody and the corresponding protein from meiotic *Bombyx* cells is unexpected. Whether this signifies the existence of a unique, meiosis specific enzyme which partly or entirely accounts for the enzyme activity of crude protein extracts and partly purified topoisomerase II from *Bombyx* or whether only one enzyme species exists in *Bombyx* which is immunologically very different from the *Drosophila* enzyme is being investigated.

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

V. Publications:

1. Bojko, M.: Human meiosis IX. Crossing over and chiasma formation in oocytes. Carlsberg Res. Commun. **50**, *43-72*, 1985
2. Glamann, J.: Crossing over in the male mouse as analysed by recombination nodules and bars. Carlsberg Res. Commun. **51**, *143-162*, 1986
3. Holm, P.B.: Ultrastructural characterization of meiosis. Biol. Skr. Dan. Vid. Selsk. **25**, 39-90, 1985
4. Holm, P.B.: Chromosome pairing and chiasma formation in allohexaploid wheat, Triticum aestivum analyzed by spreading of meiotic nuclei. Carlsberg Res. Commun. **51**, *239-294*, 1986
5. Holm, P.B.: Ultrastructural analysis of meiotic recombination and chiasma formation. Tokai J. Exp. Clin. Med. **11**, *416-436*, 1986
6. Holm, P.B.: Chromosome pairing and synaptonemal complex formation in hexaploid wheat, monosomic for chromosome 5B. Carlsberg Res. Commun. **53**, *57-89*, 1988
7. Holm, P.B.: Chromosome pairing and synaptonemal complex formation in hexaploid wheat, nullisomic for chromosome 5B. Carlsberg Res. Commun. **53**, *91-110*, 1988
8. 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**, *111-133*, 1988
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10. Holm, P.B., Wang, X. and Wischmann, B.: An ultrastructural analysis of the effect of chromosome 5B on chromosome pairing in allohexaploid wheat. Kew Chromosome Conference III. Ed. P.E. Brandham, *p281-291*, 1988
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12. Rasmussen, S.W.: Initiation of synapsis and interlocking of chromosomes during zygotene in Bombyx spermatocytes. Carlsberg Res. Commun. **51**, *401-432*, 1986
13. Rasmussen, S.W.: Chromosome pairing in autotetraploid Bombyx males. Inhibition of multivalent correction by crossing over. Carlsberg Res. Commun. **52**, *211-242* 1987
14. Wang, W. and Holm, P.B.: Chromosome pairing and synaptonemal complex formation in wheat-rye hybrids. Carlsberg Res. Commun. **53**, *167-190*, 1988
15. Wang, X.: Chromosome pairing analysis in haploid wheat by spreading of meiotic nuclei. Carlsberg Res. Commun. **53**, *135-166*, 1988
16. Wischmann, B.: Chromosome pairing and chiasma formation in wheat plants triisosomic for the long arm of chromosome 5B. Carlsberg Res. Commun. **51**, *1-25*, 1986

Abstracts and theses

1. Berthelsen, J.G.: Andrological aspects of testicular cancer. D.Sci. Thesis. Scriptor, Copenhagen, 1984
2. Bojko, M.: Ultrastructural investigations of meiosis as a tool in assessing radiation damage in man. Ph.D. Thesis. University of Copenhagen, 1985
3. Glamann, J.: Recombination nodules and bars in mouse spermatocytes. 9th Int. Chr. Conf. Marseille, 1985
4. Holm, P.B.: Meiotic recombination and chiasma formation as assessed by ultrastructural analysis. Tokay Univ. Europ. Symp. on Meiosis, Copenhagen, 1986
5. Holm P.B.: An ultrastructural analysis of the effect of chromosome 5B on chromosome pairing in allohexaploid wheat. Kew Chromosome Conf. III, 1987
6. Rasmussen, S.W.: Bivalent formation - A prerequisite for regular disjunction at meiosis. Tokay Univ. Europ. Symp. on Meiosis, Copenhagen, 1986
7. Wang, X.: Chromosome pairing analysis in haploid wheat by spreading of meiotic nuclei. Kew Chromosome Conf. III, 1987
8. Wang, X.: An ultrastructural investigation of the influence of chromosome 5B on chromosome pairing in allopolyploid wheat. Ph.D. Thesis, University of Copenhagen, 1988
9. Wischmann, B.: Dosage effect of the long arm of chromosome 5B. Kew Chromosome Conf. III, 1987

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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Contract no.: BI6-E-170-DK

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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.: 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.

Head(s) of project: **Ole Frederik Nielsen and Ole Westergaard**

Scientific staff: **J. Alsner, A.H. Andersen, B.J. Bonven, K. Christiansen, C. Herskind, E. Kjeldsen, P.S. Jensen, S.M. Kristensen, K. Lund, U.H. Mortensen, T.V. Stevnsner, J.Q. Svejstrup, B.S. Sørensen and B. Thomsen.**

I. Objectives of the project:

The aim of the project has been to investigate the effect of ionizing radiation and carcinogens in specific regions of the eukaryotic genome with special reference to the humane genome, and to clarify the molecular mechanisms by which DNA damages are repaired.

II. Objectives for the reporting period:

Our investigations have been concentrated on the study of mechanisms leading to DNA damage when chromatin is exposed to ionizing radiation and the effect of scavengers on the processes. Furthermore, we have studied enzymes involved in repair and recombinational processes with special attention to the function of the sequence specific enzymes, type I and type II topoisomerases.

III. Progress achieved:

The eukaryotic genome exists as chromatin the structure of which reflects the functional status of the enclosed genes. Thus inactive genes are packed into a dense chromatin structure, while avtive genes exist as more open chromatin. We have for our studies of radiation induced DNA damage and repair used as a model a specific gene coding for ribosomal RNA (1-4). This transcriptionally active gene can be studied both in vivo and in vitro as it can be isolated as transcriptionally active chromatin, r-chromatin. We have studied the structure of this chromatin and found that the spacer regions flanking the coding region have a well defined phased nucleosome pattern, in contrast to the open chromatin structure of the coding region (1).

Radiation induced DNA damage in a specific eukaryotic gene isolated as transcriptionally active chromatin

This biologically active eukaryotic chromatin system has been applied for radiobiological in vitro studies. The endogenous transcriptional activity was used for the study of inactivation of the gene by ionizing radiation, amd the number of critical lesions in DNA, which block transcription, was estimated from the obtained inactivation curves. The biologically important lesions detected in this system represent initial DNA damage as cellular DNA repair does not occur.

The sensitivity of the r-chromatin when irradiated as purified nucleoli in dilute phosphate buffer in the absence of added compounds showed that direct ionization of DNA only contributes to a neglegible fraction of the inactivation (3). This was confirmed by a radiation-chemical analyses which showed that OH-radicals are the major inactivation species. Under these conditions a rather small protective effect of oxygen was observed which could be ascribed to scavenging of the H-radicals. The inactivation by hydrated electrons, e_{aq}, appeared to be insignificant. In the solution of purified nucleoli the radiosensitivity of the gene was nearly the same in the presence of oxygen as under anoxia conditions.

However, a strong, apparently protective effect of oxygen could be produced by irradiation in the presence of simple organic OH-scavengers, including

t-butanol, due to less protection by the scavenger under nitrogen than under oxygen (9). This effect was ascribed to inactivation by secondary radicals of the scavengers under anoxia.

Sulfhydryl compounds were found to restore most of the protective effect of t-butanol under nitrogen (10). The observed protection may be explained essentially in terms of (i) OH-scavenging, (ii) "repair" of DNA radicals by H-atom transfer from the sulfhydryl group under nitrogen in competition with fixation of damage under oxygen, 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 sulfhydryl compounds is reduced by t-butanol and may even be reversed to produce an apparently protective effect.

It appears from the studies that secondary radicals of low molecular weight compounds may react with DNA and its constituents. The experiments show that inactivation of transcription by indirect effects may be explained by a hierachy of primary and secondary radicals. Radical-scavengers reacting with the most important inactivating species may form secondary radicals which contribute to inactivation in the next place. In addition, species which give a small contribution to inactivation in the absence of scavengers may become relatively more important when the scavengers remove the major inactivating species.

Studies on inactivation of transcription of the gene when isolated nuclei were irradiated gave oxygen enhancement ratios about three without addition of sulfhydryl compounds. This indicates the presence in nuclei of compounds that permit the same type of reactions as for isolated chromatin in the presence of sulfhydryl compounds leading to higher protection under anoxia. The observation may be of importance for development of hypoxic radiosensitizers to be used in therapeutic irradiation of tumors containing hypoxic cells.

Interaction of topoisomerases with chromatin; its implication for DNA repair and recombination.

It has already for a couple of years been known that preferential repair of transcriptionally active genes occurs in the eukaryotic genome suggesting that specific chromatin or DNA structures might dictate the DNA-repair pattern. The DNA repair mechanisms operate through a set of enzymes which constantly survey the DNA for damage by restoring the original nucleotide sequence. DNA topoisomerases play a crucial role in regulation of essential cellular processes such as replication, transcription and recombination, and experiments suggest that the enzymes might be required for the control of repair as well. Thus, abnormal expression of DNA topoisomerase I and II has been implicated in modifying the sensitivity of several eukaryotic cells to DNA damaging agents and radiation.

Fundamental to all the biological functions of topoisomerases including their function in recombination and repair is their ability to cleave and rejoin the sugar-phosphate backbone of DNA. During this process an intermediate is formed, where the enzyme is covalently bound to the ends of the cleaved nucleic-acid molecule (1,16). This intermediate can be trapped by the addition of a rapidly acting protein denaturant (1,2). Because of its likely relevance to the physiological function of the enzymes, the cleavage reaction has been the subject to extensive studies, and it has been utilized to define the sites of interaction of the enzymes on DNA *in vivo* (1) and *in vitro* (2,6,7). We have mapped the occurrence of type I and type II topoisomerases in our model system, and found that the enzymes are present in the coding regions as well as in the spacer regions (14).

Taken together, our investigations of topoisomerase I demonstrate that at least two classes of potential eukaryotic cleavage sequences can be destinguished (14). The first class, the socalled master sites, conforming to a highly conserved hexadecameric sequence motif (1,2,6,7) acts as an unusually efficient substrate for catalytic activity of the enzyme (8). The second class, the socalled slave sites, occurs for every 2-3 helical turn. The sites of this class lack sequence homology, but conformational energy calculations reveal that all the slave sites have common structural features (14). A detailed knowledge on the anatomy of the enzyme-DNA complex

formed at the master sites have been performed by footprint and modification interference analyses (18,22,24). More information about the structural features of the master sites is now derived from scanning tunnelling microscopy. The capability of the scannning tunnelling microscope to produce atomic resolution images has been used for high resolution topographic

images of linear DNA fragments. The structure has a periodicity of 38 ± 6 Å and a width of 28 ± 4 Å, consistent with the molecular dimensions of the helical repeat of double stranded DNA (19). The supreme resolving power of the microscope permits direct visualization of the major and minor groves in the DNA helix. This microscope is now being used for visualization of unusual bended structures found at master sites, and for visualization of the formation of cleavable complexes at the different classes of sites.

The fact that the hexadecameric sequence motif appears at open regulatory regions on the chromatin (1,14), and that the preference for the recognition motif has been found to be an intrinsic property of all eukaryotic type I topoisomerases (6) implies that the interaction at the master sites might be of importance in fundamental regulatory processes. In contrast, the slave sites have a more general function as they may facilitate such a process as transcription.

Similar considerations holds for topoisomerase II, as we recently have demonstrated that also the recognition sites for topoisomerase II can be divided into two classes. The enzyme-mediated cleavage sites belonging to the first class, the master sites, appear as efficient substrates for both cleavage and catalytic activity, and by use of these sites it has been possible to demonstrate DNA strand specificity of the topoisomerase II-mediated double-stranded DNA cleavage reaction (16,17). Also in this case the preference for the master sites is an intrinsic property of all eukaryotic type II topoisomerases (17).

Recently, we have initiated studies on the physiological relevans of master sites and have monitored the molecular interactions between enzyme and DNA by novel techniques. These techniques, which completely avoid the use of denaturants, have enabled us to prove the existence of a cleaved intermediate in the catalytic cycle (23). We have also developed molecular systems

which allow us to determine the minimal DNA requirement for both topoisomerase I (23) and II (20), and thereby to monitor minor changes in the conformation of the enzymes. The development of these socalled "suicide" substrates, where the enzyme can cleave but is unable to religate the DNA until a proper DNA substrate is added, have enabled us to study the individual processes in the catalytic cycle separately and thereby to get insight

into fundamental processes in repair and exchange of DNA during recombination.

The importance of topoisomerase-DNA interactions is underscored by the fact that a large number of clinically important antineoplastic agents exert their physiological effect by interfering with the activities of DNA topoisomerases (7,13,14). Besides their effect in cancer chemotherapy these agents contribute to a great extent in the delineation of the mechanism of action of these enzymes as well as the biological function of these. The efficacies of a considerable number of these agents correlate with their abilities to stabilize the enzyme-DNA cleavage intermediates. There is little information in mammalian cells regarding the mechanism by which the cleavable complexes result in cell death. The DNA breaks are in general rapidly reversed following drug removal, but it would appear that the repair is not complete or that unknown events consequent to the breaks result in more lasting forms of DNA damage. Cell lines that are resistent to antineoplastic agents due to modified enzymes have recently been isolated (11,12,15). The topoisomerases from one of these cell lines are now studied extensively with respect to possible changes in the involvement of these enzymes in biological processes as repair and with respect to the enzymes mechanism of action.

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

V. Publications:

1. Bonven, B.J., Gocke, E. and Westergaard, O. Sequence specific interaction of topoisomerase I with DNA underlying nucleosome free regions in the spacers flanking rRNA genes in Tetrahymena. Cell 41, 542 (1986).

2. Andersen, A.H., Bonven, B.J., Gocke, E., Nielsen, O.F. and Westergaard, O. Properties of sequence specific topoisomerase I associated with DNase I hypersensitive sites. Nucleic Acids Res. 13, 1543 (1985).

3. Herskind, C. and Westergaard, O. Inactivation of a single eukaryotic gene irradiated in vitro in transcriptionally active chromatin form. Radiat. Res. 106, 331 (1986).

4. Herskind, C., Thacker, J. and Westergaard, O. Radiation-induced inactivation of single isolated genes irradiated by secondary radicals. In Radiation Protection Dosimetry 13, 153, Nuclear Technology Publishing, 1986.

5. 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).

6. 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).

7. 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).

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11. Kjeldsen, E., Bonven, B.J., Andoh, T., Ishii, K., Okada, K., Bolund, L. and Westergaard, O. Characterization of a camptothecin resistent human DNA topoisomerase I. J. Biol. Chem. 263, 3912 (1988).

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15. Andoh, T., Kjeldsen, E., Bonven, B.J., Westergaard, O., Ishii, K. and Okada, K. Camptothecin-resistent DNA topoisomerase I. In DNA Topoisomerases and Cancer (Kohn K.W. and Potmesil, M. eds.), Oxford Press, England (1989).

16. Zechiedrich, E.L., Christiansen, K., Andersen, A.H., Westergaard, O. and Osheroff, N. The double-stranded DNA cleavage/religation reaction of eukaryotic topoisomerase II: evidence for a nicked DNA cleavage intermediate. Biochemistry 28, 6229 (1989).

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21. Tamura, H-O, Kohchi, C., Yamada, R., Ikeda, T., Keene, J.D., Okada, K., Kjeldsen, E., Westergaard,O. and Andoh, T. Molecular cloning of a cDNA of camptothecin-resistent human DNA topoisomerase I: identification of mutation sites responsible for the resistance. Submitted for publication (1989).

22. Mortensen, U.H., Stevnsner, T., Krogh, S., Olesen, K., Westergaard, O. and Bonven, B.J. Distamycin inhibition of topoisomerase I-DNA interaction. A mechanistic analysis. Submitted for publication. (1989).

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24. Bendixen, C., Thomsen, B., Alsner, J. and Westergaard, O. Camptothecin-stabilized topoisomerase I-DNA adducts cause premature termination of transcription. Submitted for publication (1989).

Theses:

Bonven, B.J. Sequence specific association of topoisomerase I with exposed regions in eukaryotic regions in eukaryotic chromatin. Ph.D. thesis, University of Aarhus, 1985.

Juul-Madsen, H.R. A search for specific binding of topoisomerase I to regulatory regions of the eukaryotic rRNA genes. Thesis, University of Aarhus, 1985.

Andersen, A.H. Topoisomerase I has a strong binding preference for a conserved tetradecameric sequence located at DNaseI-hypersensitive regions flanking the ribosomal RNA genes of Tetrahymena. Thesis, University of Aarhus, 1985.

Christiansen, K. Topoisomerase I er sekvensspecifikt associeret med DNaseI-hypersensitive områder i Tetrahymena r-kromatin. Thesis, University of Aarhus, 1986.

Thomsen, B. Analysis of the sequence specificity og eukaryotic topoisomerase I by site directed mutagenesis of a hexadecameric recognition motiv. Thesis, University of Aarhus, 1987.

Busk, H. Preferential base-specific relaxation of plasmids containing a conserved hexadecameric sequence from the intragenic spacer of rDNA from Tetrahymena recognized by topoisomerase I in vitro. Thesis, University of Aarhus, 1987.

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, 1987.

Kristensen, S.M. Purification of topoisomerase I from Tetrahymena, human cells and wheat germ and studies on their DNA sequence specificity in the absence and presence of camptothecin. Thesis, University of Aarhus, 1988.

Sørensen, B.S. Eukaryotic topoisomerase II is associated with specific sites in the rDNA spacer of Tetrahymena thermophila. Thesis, University of Aarhus, 1988.

Jensen, P.S. Topoisomerase II: purification and characterization of the type II topoisomerase from calf thymus, Drosophila melanogaster, human tissue culture cells and Tetrahymena thermophila. Thesis, University of Aarhus, 1988.

Hindkjær, J.J. Production of monoclonal antibodies directed against human topoisomerase I. Thesis, University of Aarhus, 1988.

Stevnsner, T.V. Interaction between DNA topoisomerase I and a specific binding sequence. Thesis, University of Aarhus, 1988.
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Svejstrup, J.Q. DNA topoisomerase I and a specific binding sequence. Thesis, University of Aarhus, 1989.

RADIATION PROTECTION PROGRAMME

Progress Report

1989

Contractor:

Contract no.: BI6-E-206-GR

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Nuclear Research Center
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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, Ph.D.

I. Objectives of the project:

1. To develop 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 elucidate the mechanisms of radiation action at the molecular, chromosomal and cellular levels by the study of the:
   a) effects of DNA repair inhibitors on the repair of radiation damage,
   b) effects of BrdUrd incorporation on radiation damage,
   c) effects of hyperthermia on the induction and repair of radiation-induced damage, and
   d) induction and repair of radiation damage in an X-ray sensitive CHO mutant cell line.

II. Objectives for the reporting period:

MECHANISMS OF RADIATION ACTION

To study the effects of hyperthermia on the induction and repair of radiation-induced chromosome damage as visualized by the PCC technique.

To study induction and repair of chromosome damage in an X-ray sensitive CHO mutant cell line.

III. Progress achieved:

1. BIOLOGICAL DOSIMETRY

Methodology

Cytogenetic dosimetry following ionizing radiation usually involves the analysis of metaphase chromosomes from mitogen stimulated peripheral blood lymphocytes. This project aimed at the development of a new cytogenetic approach for biological dosimetry which would allow the early assessment of radiation injury and the establishment of absorbed dose estimates in accidental overexposures. This new approach to biological dosimetry is based on the analysis of radiation-induced chromosomal aberrations scored in C-banded peripheral blood lymphocyte prematurely condensed chromosomes (PCCs). Lymphocytes are separated from irradiated whole blood by Ficoll-Paque sedimentation and fused with mitotic Chinese Hamster Ovary (CHO) cells for PCC induction[1]. Radiation-induced chromosomal fragments increase the number of PCCs beyond the diploid chromosome number of 46. The yield of fragments, however, decreases with time after irradation due to repair processes and formation of exchanges such as dicentrics and rings. Since conventional Giemsa staining of PCCs does not allow visualization of chromosome centromeric regions, a C-banding procedure has been developed to identify centric rings and dicentric PCCs. Air dried chromosome preparations are placed in 0.2M HCl 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 immersed in 0.2M HCl, rinsed in Sorensen's buffer (pH 6.8), and placed in hot Sorensen's buffer (60 ^{o}C) for one hour. They are then stained in 7% Giemsa for 7-10 min.

Results and Discussion

The cytogenetic approach used in this project, which allows the direct observation of chromosome damage without having lymphocytes proceed to mitosis, can be very useful for biological dosimetry purposes. Ring and dicentric analysis in PCCs provides not only confirmatory evidence of an exposure, especially when blood samples are not available soon after irradiation, but also quantitative estimates in terms of equivalent whole-body doses. Figures 1 and 2 show dicentrics (dic) and a centric ring (cr) in peripheral blood lymphocyte PCCs obtained 6h after exposure to 5 Gy X-rays.

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1. Pantelias, G.E., and H.D. Maillie (1983). A simple method for premature chromosome condensation induction in primary human and rodent cells using polyethylene glycol, Somat.Cell Genet,. 9, 533-547.

Pantelias, G.E. and H.D. Maillie (1984). The use of peripheral blood mononuclear cell prematurely condensed chromosomes for biological dosimetry. Radiation Res., 99, 140-150.

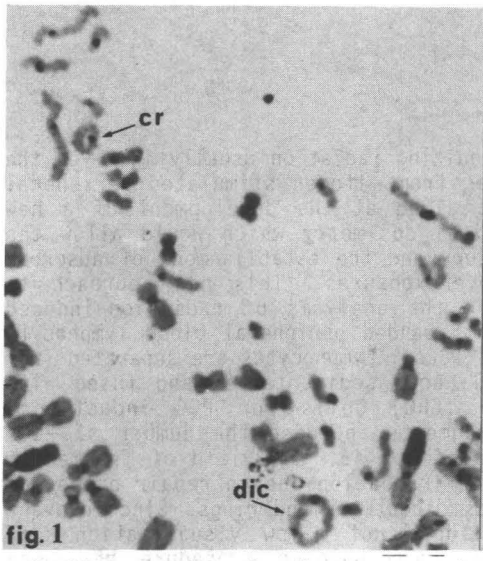

fig. 1

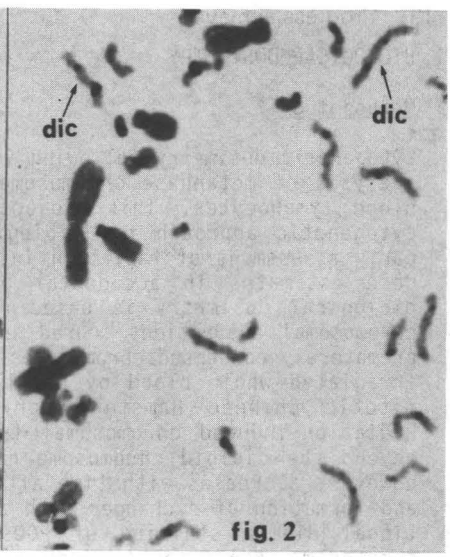

fig. 2

Preliminary data for X-rays suggest that the aberration yields observed in PCC preparations are similar to those in metaphase spreads. However, further experiments are required in order to establish dose response curves for the most common radiation sources, dose rates and qualities used, and compare them with those established using the conventional metaphase chromosome analysis.

2. MECHANISMS OF RADIATION ACTION

A. EFFECTS OF DNA REPAIR INHIBITORS ON REPAIR OF RADIATION DAMAGE

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 study the ability of compounds acting via inhibition of DNA polymerases such as araA, araC and aphidicolin, to inhibit repair of radiation-induced damage at the DNA, chromosomal and cellular levels in plateau phase CHO cells. For the experiments at the chromosomal level, the PCC technique was used. Repair of total DNA breaks was measured by the unwinding technique and repair of DNA double strand breaks by the neutral filter elution technique. The results obtained at these end points were compared with results of experiments measuring fixation of radiation-induced PLD by these compounds. When the potentiation of killing of a given araA concentration was studied, araA was added to the cells one hour before irradiation. Plateau phase cells were chilled on ice before irradiation to prevent repair. They were then irradiated on ice using a Siemens theurapeutic X-ray machine operated at 250kV, 15 mA with 2mm Al filter. After treatment, cells were analyzed either immediately or after a 24-48h incubation in araA-free condi-

tioned medium. Conditioned medium was obtained from plateau phase cultures and used after filtration to remove floating cells and debris.

Results

In agreement with the hypothesis that DNA polymerase activity is involved in cellular repair reactions, araA and aphidicolin strongly inhibited repair of radiation induced damage at the DNA and the chromosomal level. Also, araC inhibited repair at these endpoints but only to a very limited extent. The relative inhibition of repair by these compounds was similar at the various end points studied. At the survival level araA effectively fixed radiation induced PLD resulting in survival levels corresponding to an exponential survival curve. On the other hand, araC and aphidicolin had only a small effect on cell survival. DNA replication was effectively inhibited by aphidicolin, moderately by araC, and even less by araA.

- araA 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.

- 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.

- 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 after 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. Furthermore, the results obtained demonstrate that the efficacy of a polymerase inhibitor to inhibit DNA and chromosome repair does not always coincide with its ability to fix radiation induced PLD, or with its ability to inhibit DNA replication. This finding indicated that partly different biochemical pahtways may underlie PLD fixation, DNA repair inhibition and DNA replication inhibition.

B. EFFECT OF BrdUrd INCORPORATION ON RADIATION DAMAGE

Methodology

In order to characterize the relative contribution of increased DNA damage induction and increased PLD fixation in the halogenated-pyrimidine-induced radiosensitization, experiments were carried out at the DNA level, using non-unwinding DNA filter elution, at the chromosomal level, using the PCC technique, and at the cellular level. Exponentially growing and plateau phase cultures of CHO cells, and a radiation sensitive mutant of CHO, the xrs-5 cell line, were used as biological systems in order to enable parallel experiments at DNA, chromosomal and cellular levels.

Results and Discussion

Incorporation of BrdUrd into DNA, in the place of thymidine, sensitizes exponentially growing and plateau phase CHO cells to a subsequent exposure to low LET radiation. An increase in the amount of DNA and chromosome damage induced per unit radiation dose was observed with increasing incorporation of BrdUrd into DNA that was quantitatively similar to the increase observed in the survival curve slope. Although sensitization was observed both in cells irradiated in the exponential as well as in cells irradiated in the plateau phase of growth, the degree of sensitization was significantly larger in exponentially growing cells for the same degree of thymidine replacement by BrdUrd in the DNA. It is hypothesized that this result indicates the possible importance of chromatin structure at the time of irradiation and/or the importance of chromatin conformation changes after irradiation in the expression of radiation induced potentially lethal damage in BrdUrd containing cells. BrdUrd incorporation affected both the slope and the shoulder width of the survival curve, and increased the induction of DNA and chromosome

damage per unit absorbed dose. The increase observed in the survival curve slope was quantitatively similar to the increase observed in damage induction at the DNA and the chromosomal level, suggesting a cause-effect relationship between these phenomena. Reduction in the shoulder width did not correlate with the increse in DNA and chromosome damage induction suggesting that different phenomena, probably related with enhanced fixation of radiation induced PLD in BrdUrd containing cells, underlie its modulation.
xrs-5 cells are known to be deficient in DNA double strand break repair. Compared to similar results obtained in similar stages of growth with repair proficient CHO cells, a reduction was observed with xrs-5 cells in BrdUrd-induced radiosensitization for similar levels of incorporation. BrdUrd incorporation did not affect the induction of DNA double strand breaks as measured by the non-unwinding filter elution technique, a result that contradicts findings with CHO cells. At the chromosomal level, an increase was observed in the induction of breaks in the BrdUrd containing cells, as measured in non-cycling G_1 cells using the PCC technique. This increase in chromosome breakage was comparable to that observed in similar experiments with CHO cells. These results indicate similarities in the effect of BrdUrd at the chromosomal level in irradiated CHO and xrs-5 cells, suggesting that DNA dsb repair proficiency is a prerequisite for halogenated pyrimidine-induced radiosensitization.

C. EFFECT OF HYPERTHERMIA ON INDUCTION AND REPAIR OF RADIATION DAMAGE

1. Hyperthermia

Methodology

In order to study the effect of hyperthermia on the induction and repair of radiation induced chromosome damage, as a first stage, experiments were designed to study the effects of heat (43 and 45.5 C) on chromatin morphology and nuclear organization, as visualized by PCC, in exponentially growing and plateau CHO cells. Experiments were also carried out with exponentially growing HeLa cells.

Results and Discussion

The results obtained indicate that exposure to heat drastically reduces the ability of interphase chromatin to condense and the ability of the nucleolar organizing region to disintegrate under the influence of factors provided by mitotic cells when fused to interphase cells. The fraction of cells with non-disintegrated nucleoli increased with increasing exposure time at 45.5 C and reached a plateau at almost 100% after about 20 min. Exponentially growing and plateau phase cells showed similar response. Recovery from the effects of heat on chromatin condensation and disintegration of the nucleolar organizing region depended upon the duration of the heat treatment. For exposures up to 15 min at 45.5 C, a gradual reduction in the fraction of cells with non-disintegrated nucleoli was observed when cells were allowed for repair at 37 C. However, only a very limited amount of repair was observed after a 30 min exposure to 45.5 C. The repair times observed at the chromosome level were similar to those reported for the removal of excess protein accumulating in chromatin or the nuclear matrix, suggesting a causal

relationship between the two phenomna. It is proposed that nuclear protein accumulation on chromatin or in the nuclear matrix reduces the accessibility of chromatin to enzymes responsible for the phosphorylation reactions necessary for chromatin condensation and disintegration of the nucleolus.

2. Effect of hyperthermia on the induction and repair of radiation-induced chromosome damage

The effect of pre-exposure to heat on the induction and repair of chromosome damage was measured in plateau phase CHO cells usipg the PCC technique. Plateau phase cultures were obtained by growing 10^5 cells in T25 tissue culture flasks for 4 days without refeeding. Cells were exposed to heat (45.5 C) for 8 or 15 min in fresh growth medium without serum, were irradiated, either immediately or at various times thereafte (up to 16h), and were returned to the incubator at 37 C. At various time intervals after irradiation (up to 24h) flasks were trypsinized and an aliquot containing 10^6 cells were mixed with an equal amount of cells selected at mitosis using nocodazole. The cell mixture was treated with PEG to effect fusion and PCC induction. Exposure to heat prevented chromatin from fully condensing and nucleoli from disintegrating. Therefore, measurements of residual chromosome fragments were carried out after reversion of these effects. Despite these inherent difficulties in measuring chromosome breaks in interphase cells after exposure to heat, the results obtained clearly indicated a significant reduction in the ability of heated cells to repair radiation-induced chromosome damage. The experiments also indicated a larger induction by radiation of chromosome damage in heated cells.

D. INDUCTION AND REPAIR OF CHROMOSOME DAMAGE IN AN X-RAY SENSITIVE CHO MUTANT CELL LINE

Induction and repair of chromosome damage were studied in interphase xrs-5 cells by means of the PCC technique. The results obtained were compared to those previously reported for CHO cells. Induction of chromosome damage per unit of absorbed radiation dose was in xrs-5 cells larger by a factor of 2.6 than in CHO cells. Changes in chromatin structure, associated with the radiation sensitive phenotype of xrs-5 cells, that increase the probability of conversion of a DNA dsb to a chromosome break are invoqued to explain this effect. Repair of chromosome breaks as measured in plateau-phase G_1 cells was deficient in xrs-5 cells, and the number of residual chromosome breaks was practically identical to the number of lethal lesions calculated from survival data. This observation suggests than non-repaired chromosome breaks are likely to be manifestations of lethal events in the cell. The yield of ring chromosomes scored after a few hours of repair was higher by a factor of 3 in xrs-5 compared to CHO cells. This increase in ring formation suggests an increase in the probability of misrepair of chromosome damage that may stem either from the reduced ability of xrs-5 cells to repair DNA dsb, or from the higher production of chromosome fragments observed per cell and per Gy.

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

Most of the experimental work involved in the achievement of the objectives of this project was carried out in collaboration with Professor Dr.G.Iliakis in the Laboratory of Experimental Radiation Oncology, Thomas Jefferson University Hospital, Department of Radiation Oncology and Nuclear Medicine, Philadelphia, PA 19107, USA.

V. Publications:

1. PUBLICATIONS IN SCIENTIFIC JOURNALS

Iliakis, G., G.E.Pantelias, R.Okayasu, and R.Seaner (1987). 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.

Iliakis, G., G.E.Pantelias, and R.Seaner (1988). 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, 114, 361-378.

Pantelias G.E., G. Iliakis, R. Okayasu and R. Seaner. The use of premature chromosome condensation technique in the study of the mechanisms of radiation-induced chromosome breakage and rearrangement. 37th Annual Meeting of Radiation Research Society and 9th Annual Meeting of North American Hyperthermia Group, Seattle, March 1989.

Iliakis, G., G.E. Pantelias, R.Seaner and R.Okayasu (1989). Comparative studies on repair inhibition by araA, araC and aphidicolin of radiation-induced DNA and chromosome damage in rodent cells: Comparison with fixation of PLD. International Journal of Radiation Oncology, Biology and Physics, 16, 1261-1265.

Pantelias, G.E., G. Iliakis and R. Seaner. Effect of hyperthermia on the induction and repair of radiation induced chromosome damage as visualized by the technique of premature chromosome condensation. 37th Annual Meeting of Radiation Research Society and 9th Annual Meeting of North American Hyperthermia Group. Seattle, March, 1989.

Iliakis G. and G.E.Pantelias (1989). Effect of hyperthermia on chromatin condensation and nucleoli disintegration as visualized by induction of premature chromosome condensation in interphase mammalian cells. Cancer Research, 49, 1254-1260.

Iliakis G., S.Kurtzman, G.E.Pantelias and R.Okayasu (1989). Mechanism of radiosensitization by halogenated pyrimidines: Effect of BrdUrd on radiation-induced DNA and chromosome damage and its correlation with cell killing, Radiation Research, 119, 286-304.

Pantelias, G.E. and G. Iliakis (1989). Production and repair of chromosome damage in an X-ray sensitive CHO mutant visualized and analyzed in interphase using the technique of premature chromosome condensation, International Journal of Radiation Biology, Submitted.

Pantelias, G.E. and G.Iliakis. Chromosome aberration formation in cells exposed to heat in various phases of the cell cycle measured in interphase by the technique of premature chromosome condensation. 38th Annual Meeting of Radiation Research Society and 10th Annual Meeting of North American Hyperthermia Group, New Orleans, April 1990.

Pantelias, G.E. and G.Iliakis. Production and repair of chromosome damage in an X-ray sensitive CHO mutant visualized and analyzed in interphase using the technique of premature chromosome condensation. 38th Annual Meeting of Radiation Research Society and 10th Annual Meeting of North American Hyperthermia Group, New Orleans, April 1990.

2. THESES

Sambani, K.D. (1989). Premature chromosome condensation induction in peripheral blood cells from normal individuals and AML and CML patients: Comparative kinetic studies in G_1 phase. NRCPS "Democritos" and Medical Shool of Medicine, University of Athens.

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
Final Report

Contractor:

Universidad Politecnica de Madrid
Departamento de Tecnologia Nuclear
C/José Gutierrez Abascal, 2
E-28006 Madrid

Contract no : BI6-F-227-E

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

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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:

The main objective of the research project was the development of a computer model for the probabilistic assessment of the off-site economic risk derived from nuclear accidents, taking into consideration the direct costs caused by the different countermeasures adopted following an accident to prevent both the early and chronic exposure of the population to the radionuclides released, as well as the direct costs derived from health damage to the affected population.

A second, but not less important, objective was the development of a detailed socio-economic data base to be managed by the new model, containing distributions of population, farmland use, crops and livestock, as well as of employment, salaries and added value for different economic sectors.

II. Objectives for the reporting period: (July 1987 - December 1989)

- Development of the new economic consequence assessment model.
- Coupling of the model to the Accident Consequence Assessment code MACCS (developed by Sandia National Laboratories for the U.S. NRC).
- Development of a detailed socio-economic data base, with a structure compatible with the existing European Grid, to allow site-specific analyses.
- Collection of available Spanish data, taken from official statistics.
- Achievement of a first demonstration exercise of the model system, including uncertainty and sensitivity analysis of the new model, using the Latin Hypercube Sampling (LHS) method.

III. Progress achieved:

The main objective of the research project was the development of a computer model for the probabilistic assessment of the off-site economic risk derived from nuclear accidents, taking into consideration the direct costs caused by the different countermeasures adopted, following an accident, to prevent both the early and chronic exposure of the population to the radionuclides released, as well as the direct costs derived from health damage to the affected population. The computer code is called MECA (Model for Economic Consequence Assessment), and it is written in FORTRAN 77, in order to facilitate its portability.

THE MODEL FOR ECONOMIC CONSEQUENCE ASSESSMENT, MECA.

The submodels included in MECA and the different economic items considered are the following:

* Population evacuation and/or temporary relocation costs, including transportation, housing and feeding, organization and monitoring, salary and/or added value losses.

* Food ban costs of directly contaminated agricultural and livestock products, evaluated at the prices perceived by farmers.

* Decontamination costs, considering up to eight possible decontamination levels and different types of urban and rural surfaces. Production losses during decontamination are included as temporary relocation costs.

* Temporary interdiction costs, evaluated as the loss of production for rural areas, and as the loss of wealth for urban property due to depreciation, during the interdiction period.

* Permanent interdiction costs, sum of the land values for farmland and of the per person values of property for urban areas.

* Population relocation costs, including production (added value) losses during a transition period.

* Health effect costs, including reparations and medical care.

INFORMATION REQUIRED BY THE MODEL.

Three main sources of data are needed by MECA:

(1) A general Accident Consequence Assessment code.

(2) A site-specific description data base.

(3) A data set for the definition of the economic model.

(1) The actual version of MECA is based on MACCS 1.4[1] (the MELCOR Accident Consequence Code System, developed by Sandia National Laboratories for the U. S. Nuclear Regulatory Commission), which will provide the accident specific inputs, such as probability, countermeasures needed at each element of the calculational grid (evacuation, relocation, food disposal, decontamination or interdiction), and the expected number of health effects, for each meteorological trial analysed. An outline of the joint structure of both codes is shown in Figure 1,

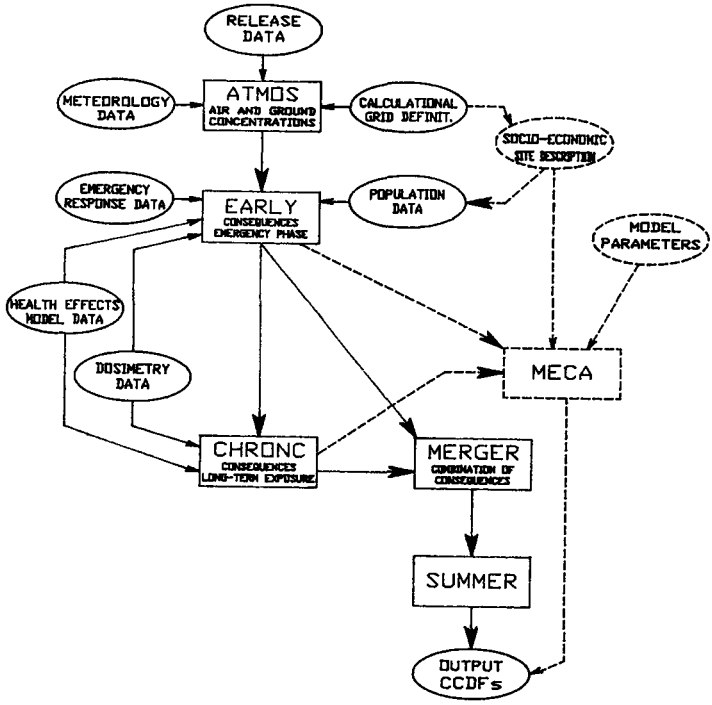

Figure 1.- Outline of the joint structure of the MECA-MACCS codes.

(1) Ritchie L.T., Chanin D.I., Sprung J.L., MELCOR Accident Consequence Code System (MACCS Version 1.4). (Draft). U.S. NRC Report NUREG/CR-4691. (SAND86-1562). Sandia National Laboratories (1987).

where solid lines represent the structure of MACCS 1.4, which remains unchanged with respect to its original aspect. Three interface files have been created to transfer the information from a MACCS run to MECA.

Two aditional modifications have been made in MACCS 1.4. The first one is related with the meteorological sampling scheme, which has been made more flexible. The spatial intervals used to classify rain and slowdowns in wind speed observations can now be adapted more adequately to the geographical distribution of towns or industrial areas around the nuclear site. The effect of this change on risk estimations is generally a sligth increase of the mean values of accident consequences, resulting from a more realistic consideration of those meteorological sequences that are risk dominant.

Additionaly, another important feature that has been implemented in MACCS 1.4 is the possibility of considering up to eight decontamination levels, instead of the original three. This allows a more accurate representation of decontamination activities after an accident, and a more precise economic evaluation of them.

(2) Site-specific data are organized in the **socio-economic data base**, including detailed distributions of population, agricultural production and farmland use, livestock census, as well as of employment, salaries and added value for different economic sectors. This data base, to be managed by the MECA code, has been completed for Spain, based on available official statistics, with partial support of the Spanish Consejo de Seguridad Nuclear. A distribution of the Spanish population and farming census into the European Grid has also been performed.

The data have been collected at the finest available level and different transformation programmes distribute them into the calculational grids (circles and sectors, site dependent), or into the European Grid (quasi-square elements of the same area, limited by meridians and parallels, and site independent). The overall structure of the data base is represented in Figure 2.

The population data for all the municipalities of Spain (more than 8000), based on the 1986 census, have been obtained from the Instituto Geográfico Nacional of Spain.

The data about total and paid employment fractions (per inhabitant), Gross Added Value at factors cost (per inhabitant), and salary costs (per paid employee), for each basic economic sector (agriculture, industry and services), were not available in Spain at a very detailed level, but are collected for each province (51 administrative divisions) in the Regional Accounting of Spain. As a distribution into

the calculational grid around a nuclear site is needed, several transformation programs have been developed to obtain a distribution proportional to the population of each province living inside each element of the grid.

The farming data used by MECA for each element of the grid include the average crop production (up to 41 different products), the farmland use area (up to 15 types of farmland), and the livestock census (up to 15 species). The basic sources of data have been the Agricultural and Livestock census of Spain, 1982, whose municipal results have been obtained in a magnetic tape from the Instituto Nacional de Estadística and the average yields of each crop, for each province, elaborated from the Yearbooks on Farming Statistics, published by the Spanish Ministerio de Agricultura, Pesca y Alimentación.

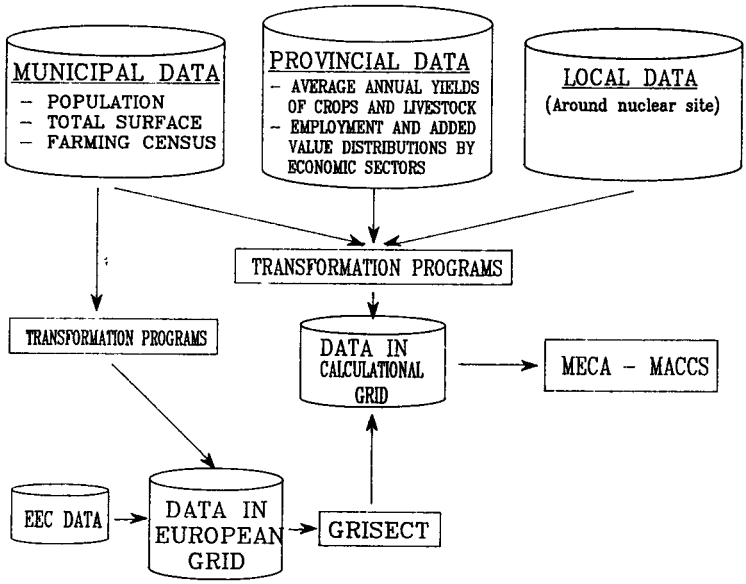

Figure 2.- Structure of the socio-economic data base used by MECA.

(3) A user input file with the parameters needed by the submodels should be supplied to the code. It contains data needed to evaluate the costs of evacuation, decontamination, interdiction, farming products disposal and health effects. It also contains controlling switches for the output to be produced by MECA.

The output of MECA is in the form of CCDF (Complementary Cumulative Distribution Functions) of the different economic consequences, together with the mean and peak values, and some selected quantiles of the distribution functions.

The new code MECA, coupled to a general probabilistic accident consequence code, such as the MACCS 1.4 in this report, provides capability to complete probabilistic risk assessments from the point of view of the off-site economic consequences, and also to perform cost-effectiveness analysis of the different countermeasures that could be needed in the field of emergency preparedness.

RESULTS. EXAMPLES ANALYSED.

Two hypothetical examples considering different source term magnitudes have been analysed to demonstrate the functioning and capabilities of the new model. The radionuclide inventory considered is equivalent to an end of equilibrium cycle, for a 3412 thermal Mw Pressurized Water Reactor. The source term characteristics (release fractions, heat contents, timing) have been taken equal to the CLUSTER10, and CLUSTER19 for Surry 1 NPP, of the first version of the Reactor Risk Reference Document [2].

The results obtained (see Figures 3 and 4) show an important dependence of the distribution functions of the economic consequences on the source term and, for results related with the urban environment, on the population distribution around the reactor site, causing the largest costs for meteorological sequences having a low probability but allowing the contaminants to reach large and distant cities. For results related with the rural environment, a more continuous response in the distribution functions of the costs is obtained, due to the generally more uniform distributions of crops and livestock around the reactor site. The costs referred to health effects show a greater importance for sequences with high probability, always dominating the costs associated to latent health effects, and with the early health effect cost having the greatest sensitivity to source term variations.

It is important to remark that no specific conclusions about economic risks on nuclear power plants should be extracted from the present analysis. First of all, because the data sets used for this example are hypothetical: meteorological data have been taken from one site, socio-economic data from another one, and source terms are generic. Second, because the values of many parameters have been simply assumed (the uncertainty analysis included in the following section tries to quantify the importance of such assumptions). Finally, another important reason to avoid deriving specific conclusions, is that MACCS 1.4 will be soon substituted by

(2) United States Nuclear Regulatory Commission, Reactor Risk Reference Document. (Draft for comment), U.S. NRC Report NUREG-1150, U.S. NRC. (1988).

a more reliable version of the code, the 1.5, whose development is including an extensive quality assurance programme[3]. Therefore, the inputs that MACCS 1.4 provides to MECA should also be considered as hypothetical. However, any of the former reasons removes the validity of this analysis as illustrative example of the functioning of the code system and, in particular, of MECA as a probabilistic tool for the study of site-specific economic risks derived from nuclear accidents.

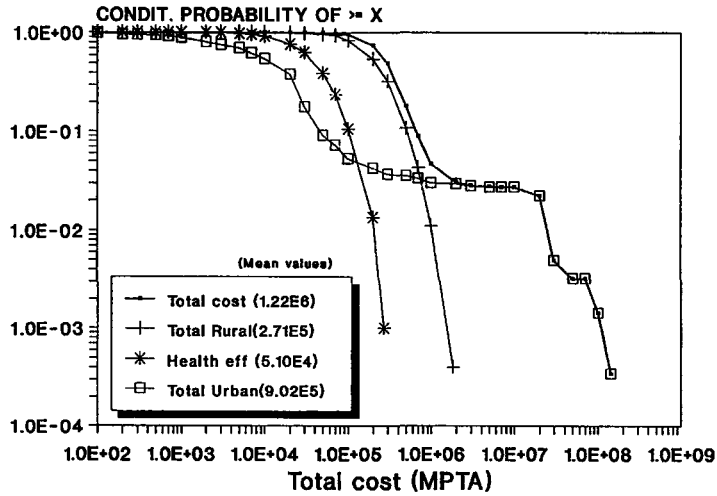

Figure 3.- Total costs for the hypothetical case using Source Term 1.

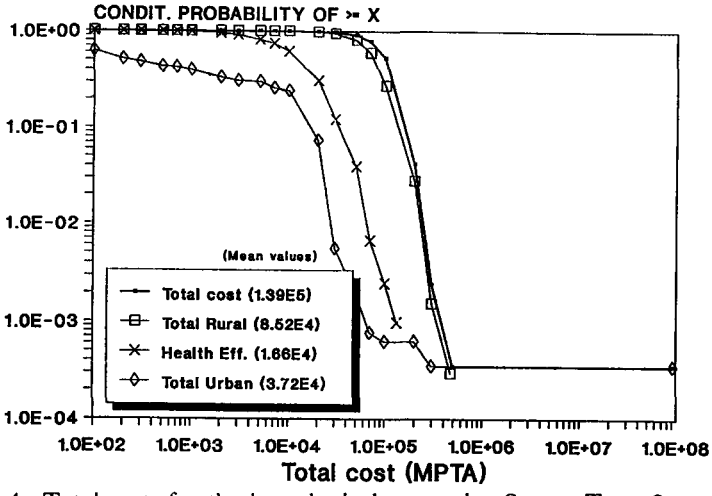

Figure 4.- Total costs for the hypothetical case using Source Term 2.

(3) Acharya S., A brief report on the U.S. NRC activities in the area of offsite consequence analysis since the last GRECA meeting. OECD/NEA, Task Group on Environmental Consequences of Accidents, SINDOC(89)14. (1989).

UNCERTAINTY AND SENSITIVITY ANALYSIS.

A limited uncertainty and sensitivity analysis of the results obtained with Source Term 2 has been performed, putting a greater emphasis in the urban and health effect cost models, in which the larger number of assumed parameters may influence. In the assessment, the accident sequences analysed by MACCS are taken as a fixed input. Therefore, the uncertainties that we could observe are only due to the variations on the response of MECA to the changes in its own input data.

The uncertainty and sensitivity analysis has been performed using the very well known LHS code[4] for the generation of samples of input data, and the PCCSRC code[5] for the calculation of partial correlation and standarized regression coefficients between the input and the output provided by MECA. Both codes have been kindly supplied by Sandia National Laboratories (U.S.A.).

A total of 16 parameters have been selected for the limited uncertainty / sensitivity analysis. They include the costs of transportation, housing and feeding, affecting the evacuation and relocation cost models; the number of full-time decontamination workers considered for the urban areas, that will influence on the duration of decontamination and thus on temporal relocation; the areas of each type of urban surfaces per inhabitant (roofs, exterior walls, asphalt, concrete and lawns, all having different decontamination costs and rates); the time needed to provide new dwellings to relocated people, multipliers of the costs assumed for new employments and of the transition periods up to the set up of new employments, and the sum of all the urban property values, affecting the permanent relocation costs. Finally, the parameters regarding the health effect cost models are multipliers affecting the costs of medical care, the compensations for early health effects, the compensations for latent health effects, and the annual societal discount rate.

With these 16 parameters, a set of 40 samples (2.5 times the number of parameters) was generated using the LHS code. The results of running MECA 40 times are displayed in the Figures 5 and 6, with reference to urban and health effect costs respectively. The figures summarize the 40 CCDFs obtained. For each probability level appearing on the y axis, the extreme values (dotted lines) represent

(4) Iman R.L. and Shortencarier M.J., A FORTRAN-77 Program and User's Guide for the Generation of Latin Hypercube and Random Samples for Use With Computer Models. U.S. NRC Report NUREG/CR-3624, Sandia National Laboratories. (1984).

(5) Iman R.L., Shortencarier M.J. and Johnson J.D., A FORTRAN-77 Program and User's Guide for the Calculation of the Partial Correlation and Standardized Regression Coefficients. U.S. NRC Report NUREG/CR-4122, Sandia National Laboratories. (1985).

the 5% and 95% of the 40 values provided by MECA; that is, the 90% of the runs resulted in values inside the uncertainty bands shown in the figures, that could be accepted as subjetive confidence intervals, conditional on the assumptions made previously. The figures also display the mean and median values corresponding to each probability level on the y axis, giving some idea about the shape of the distribution functions obtained after the uncertainty analysis.

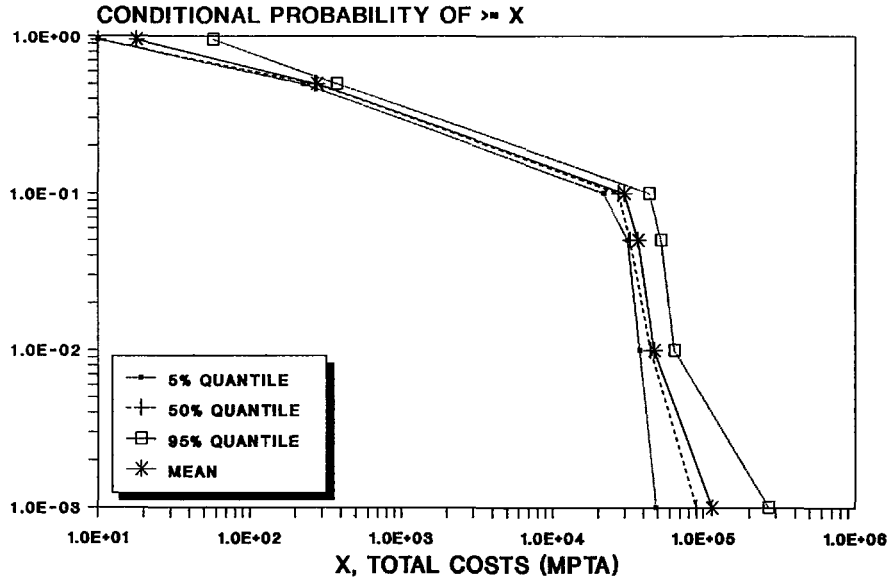

Figure 5.- Total urban costs (Source Term 2), 5%, median, mean and 95% CCDF's.

With regard to the urban costs, the greatest uncertainty is obtained for extreme probability values of the CCDFs, ranging from approximately between 1/2.4 and 2.4 times the mean value for the low probability and large consequences zone, and between 0 cost and 3.2 times the mean value, for sequences with high probability and low consequences. For all the ranges observed, the mean is greater than the median, indicating than more runs gave results lying in the lower part of the uncertainty band. The main contributors to that uncertainty are those affecting the duration of temporary relocation during decontamination, such as the number of decontamination workers and the assumed areas of roofs, walls and lawns per inhabitant, mainly due to the high decontamination times needed for these types of

surfaces. Directly related with the cost of that temporary relocation is the cost of housing and feeding the relocated people, that also shows a significant importance. The 0.99 quantile of the urban cost is also sensitive to the parameters referred to permanent interdiction and relocation of people.

In the case of health effect costs, the variations range between about 1/3 and 2 times their mean value, with the cost of medical care to the damageded persons being the most influencing parameter. The cost of reparations has a different importance in the case of early and latent health effects, being higher for the last. The societal discount rate does not seem to be a very sensitive parameter within the range of variation considered.

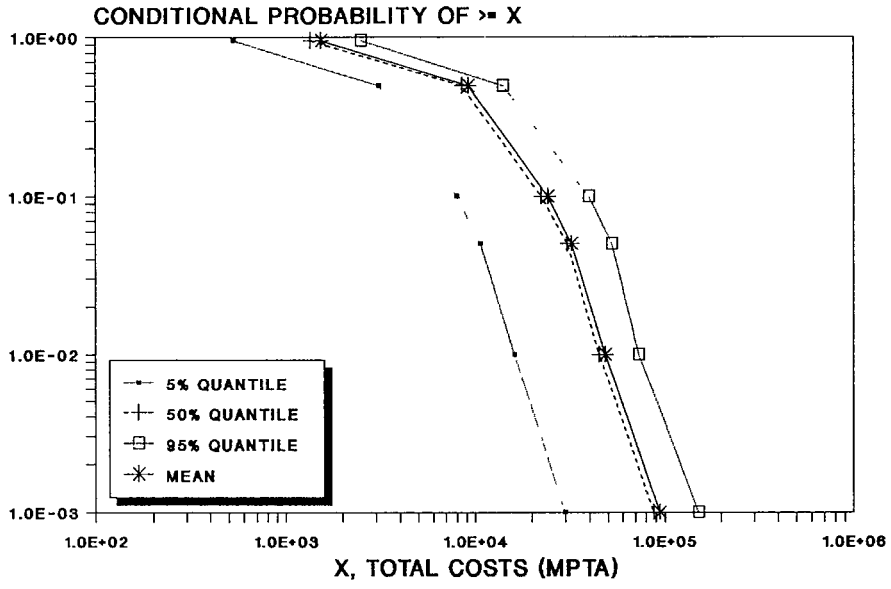

Figure 6.- Health effect costs (Source Term 2). 5%, median, mean and 95% CCDF's.

The influence in the mean value of total cost varies by a factor between 0.8 to 1.5 times around its central tendency (the median or the mean of the distribution function). If we substract the mean rural cost, always considered constant, from the mean total, this variation ranges between a factor 0.5 to 2.2, more in accordance with the variations observed previosly for the urban and health effect costs.

CONCLUSIONS.

The research project has reasonably reached all the proposed objectives, with regard to the development of a new model for the probabilistic evaluation of the off-site economic risk derived from nuclear accidents, including the necessary detailed socio-economic description of the sites being analysed and the capability to easily perform uncertainty/sensitivity analysis of the results obtained.

The main conclusions are summarized in the following points:

(1) The Model for Economic Consequence Assessment (MECA), coupled to the MACCS code, provides a complete and detailed output for the probabilistic evaluation of the off-site economic risk derived from nuclear accidents, covering a wide spectrum of items involved in the economic impact of countermeasures, as well as of health effects.

(2) The cost assessment for the rural environment seems to be approximate enough, since it is based on real data about distributions of crops and livestock census, production yields and prices for each individual product.

(3) The impact of the uncertainties in the parameters referred to urban and health effect cost models on the total cost of the accident could range from 0.5 to 2.2 times the mean value, depending strongly on the source terms considered and on the characteristics of the site being analysed.

(4) The examples analysed with the new model show a correct functioning of the code system MACCS-MECA, allowing the necessary uncertainty and sensitivity analyses for specific accident scenarios, combining different source terms and detailed site characteristic description.

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

V. Publications:

- Alonso, A. and 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). Radiation Protection Dosimetry, 21, No. 1-3, pp. 151-158 (1987).

- Alonso, A., Gallego, E. and Martín, J.E., Una herramienta para el diseño de planes de emergencia. Los modelos para análisis probabilista de las consecuencias de los accidentes. Revista de la Sociedad Nuclear Española, No.77, pp. 35-40 (1989).

- Alonso, A. and Gallego, E., Experience on the Evaluation of the Off-Site Costs of Recator Accidents in Spain. CEC Workshop on Radiological Consequences of Chernobyl. (Brussels, Belgium, 3-5th, February, 1897).

- Alonso, A. and Gallego, E., A Model for the Calculation of the Off-Site Economic Consequences of Nuclear Recator Accidents. In Proc. Joint CEC/OECD(NEA) Workshop on Recent Advances in Reactor Accident Consequence Assessment, (Rome, Italy, 25-29th January, 1988). Report EUR 11408 EN.

- Gallego, E. and Martín, J.E., Aplicación del código MACCS al análisis de planes de emergencia. In Proc. XIV Annual Meeting of the Spanish Nuclear Society, (Marbella, Spain, 26-28th October, 1988).

- Gallego, E., Evaluación de las consecuencias económicas externas de los accidentes nucleares. Experiencia y perspectivas. In Proc. XIV Annual Meeting of the Spanish Nuclear Society, (Marbella, Spain, 26-28th October, 1988).

- Gallego, E. and Martín, J.E., Modelos y bases de datos para la evaluación del riesgo económico al exterior en caso de accidente nuclear. In Proc. XV Annual Meeting of the Spanish Nuclear Society, (Alicante, Spain, 23-25th October, 1989).

- Gallego, E., Un modelo para estimación de las consecuencias económicas exteriores de los accidentes nucleares. In Proc. III Congreso Nacional de Protección Radiológica, (Valencia, Spain, 29th November-1st December, 1989).

- Gallego, E. and Martín, J.E., Modifications to MACCS 1.4 (Revision 1 for MECA-MACCS). Internal Report CTN-72/87. (Madrid, December 1987).

- Gallego, E. and Martín, J.E., Distribution of the Spanish Population in the European Grid. Internal Report CTN-87/88. (Madrid, November 1987).

- Gallego, E., MECA, A Model for Economic Consequence Assessment. User's Guide. Internal Report CTN-58/89. (Madrid, July 1989).

- Alonso, A.,Gallego, E. and Martín, J.E., Off-Site Economic Consequences of Nuclear Reactor Accidents (Final Report, Draft). Internal Report CTN-80/89. (Madrid, September 1989).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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Division de Medicina
Avenida Complutense, 22
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Contract no.. BI6-F-229-E

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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.: BI 6-0229-E

EPIDEMIOLOGICAL STUDY OF THE HEALTH EFFECTS DERIVED FROM CHRONIC LOW LEVEL IONIZING RADIATION EXPOSURE AMONG
WORKERS OF THE SPANISH NUCLEAR COUNCIL (JEN)

Head(s) of the project.:

FERNANDO RODRIGUEZ ARTALEJO, BENJAMIN SANCHEZ F. MURIAS

Scientific staff:

MAGDALENA RODRIGUEZ COMA, FRANCISCO MINGOT BUADES, RAFAEL SAENZ GANCEDO, CARMEN VAZQUEZ LOPEZ, MILAGROS AMARO JIMENO, ANTONIO REBOLLAR RIVAS, SANTIAGO CASTAÑO LARA.

I. Objectives of the project:

The specific objectives of this study are:

-To compare the mortality of JEN workers with the national rate.

-If there were any evidence that suggested that the mortality at JEN is greater than that in Spain, to establish whether this difference is related to exposure to ionizing radiation.

-To suggest priorities for epidemiological research in the near future about the health of JEN workers.

II. Objectives for the reporting period:

III. Progress achieved:

MATERIAL AND METHODS

III.1. Study population

A retrospective study was conducted on a cohort of 5.303 JEN workers - 4,553 men and 770 women - who had rendered their services, in a direct employment relationship, for a period of time greater than six months between January 1, 1954 and December 31, 1986. This cohort constitutes 85% of the 6,224 workers who have made up the work force at JEN since its installation. The workers not included in the study, 6,224 - 5,303 = 921, were excluded because they did not satisfy the defining criteria established for the cohort.

III.2. Data Collection

The following information was collected for each member of the study population:

-Administrative and clinical data.

-Exposure data (dosimetries)

-General (allcauses) and cause specific mortality data.

III.2.1. Administrative and clinical data

a) Administrative data

All data needed for identification, characterization and monitoring of the workers were collected.

The personnel department's records were used as a primary source of information about the cohort; when some essential information was not contained in these records an exhaustive search was conducted

using other sources such as the entity's clinical history records or its General Registry.

The variables collected were the following:

National Identity Document number, Social Security number, sex, work in the mining sector, total time of employment at JEN, total time of employment in the mining sector, date of entry into the study follow-up and age of first exposure to ionizing radiation.

Clinical data

The information was obtained from the clinical records filled by the JEN Medical Service. The variables collected were the following:

Tobacco use, alcohol consumption, obesity, hypertension, hypercholesterolemia and hiperglycemia.

III.2.2. Exposure data (dosimetries)

Information was obtained from the JEN Dosymetry Service records. In particular accumulated whole body exposure was measured through external film badge dosimeters that were carried by all workers supposedly exposed in JEN. Total body dose was measured in milliSieverts (mSv).

III.2.3 General and cause specific mortality data

The vital status of employees who were working as of December 31, 1986 was taken as known directly. For former employees the investigation was initiated by consulting the Social Security's Computer Department's data base. In those cases in which this approach did not permit a determination of the vital status, direct contact was made with the person or with his family, by using the last address which appeared either in the personnel departments records or in the worker's clinical record if the latter were more current.

The vital status of 3,381 workers (63.8%) were ascertained, with mean period of follow-up of 21.4 years (ranging from 1 - 33.0). The high rate of losts to follow-up (36.2%) can be explained by the large geographic dispersion of work centers, fundamentally mining sectors, and the great mobility of personnel between them.

289 deaths occurred (8.5% of the workers whose vital status was known as of December 31, 1986) during this monitoring period. The causes of death, obtained from death certificates, clinical records, hospital registries, civil registries and cemetary registry books, were examined and codified according the four digit tabular list of subcategories contained in the International Classification of Diseases (ICD) ninth edition.

III.3 Validation of the information

The information was introduced into a file system created for such purpose in a IBM/3090 computer; a series of programs designed to systematically detect the most obvious errors (numeric characters in alphabetic fields, and viceversa, dates outside the permitted range, non-existent codes, etc.) was used. In order to reduce transcription errors during the recording of the data, all essential variables were introduced twice, with the files resulting therefrom being scrupulously compared with the original sources to confirm conflicting information.

III.4 Statistical analysis

Three types of analysis were performed:

III.4.1. Description of the study population

Administrative, employment and medical characterestics of the study population were considered, with special emphasis on the distribution of ionizing radiation exposure and the distribution of the main mortality predictors.

III.4.2. External comparison of the mortality of the JEN cohort with the mortality of the spanish population.

Mortality Ratios (SMR) adjusting for sex, age and calendar time were calculated. These measures compare deaths observed at JEN, for total deaths and for specific causes, with those which would be "expected" if the study population behaviour with the mortality rates of the spanish population. An SMR greater or less than 100 suggests that there are more or less deaths at JEN than would be expected if the members of the JEN cohort were otherwise similar to those of the nation's people as a whole.

Expected deaths are calculated multiplying the time of observation (person - years) of the JEN personnel, desaggregated into two sex groups, sixteen 5-year age groups from 20 to 85 years of age and into seven 5-year calendar time groups, from 1954 to 1986, by the death rates for the spanish population for the same groups.

Calculation of the collective person-years of observation was conducted according to Monson[1]. Spanish mortality rates were calculated using the number of deaths for the numerator from the "Movimiento Natural de la Población" and population estimates for the denominator from intercensus projections. Finally, confidence intervals (95%) were calculated for the SMRs.

Obviously this analysis was restricted to the sample cohort formed by the 3,381 workers whose vital status was known.

III.4.3. Internal comparison of the mortality according to the level of exposure to ionizing radiation.

This analysis has been restricted to the study of cancer mortality among JEN workers exposed to diffferent levels of radiation. Two methods were used. The first is a straight linear model that considers mortality as a function of radiation exposure.

The form of the model was $y = a+3bx$ where y represents the mortality rate for cancer, x represents the radiation dose and 3 is a

factor that corrects for the fact that in underground uranium mining (the main activity of the study cohort) the total exposure dose is three times that which is measured by the dosimeter.

The second model is a logistic function that used percent mortality rate due to cancer as dependent variable and the accumulated whole body dose (DOSI) as independent regressor. The variable DOSI has been categorized into three groups (low: <5mSv, Medium 5-25mSv, high >25 mSv). The effect of extraneous variables as tobacco has been controlled in this analysis, considering three categories: smokers, no smokers and persons for whom such information was not available.

This analysis was performed with the statistics program SAS 5.16 in the VSE environment of a IBM-4361 system and obviously was restricted to those subjects whose vital status , exposure status and tobacco consumption were known.

RESULTS AND DISCUSSION

III.5. Description of the study population

The study population consisted of 5,303 workers at JEN for the period from January 1, 1954 to December 31, 1986. A total of 4,533 men and 770 women were studied (table 1). 2,220 (41.9%) of them workerd in the mining sectors of JEN.

Overall the cohort was young at the time of beginning service at JEN (x= 29.6 years); members' exposure to ionizing radiation began in the fourth decade of life, and their employment experience was slightly greater than ten years.

Dosimetric registry is available for 3,297 people (62.2%), with doses greater than the threshold level for 2,905 of them (88.1% of the persons with dosimetric registry).

The population (5,303 persons) was followed for an average of 15.7 years, and was exposed to radiation (3,297 persons)for an average of 11.3 years. This provides 83,257 person years of observation and 37,256 person - years of exposure to ionizing radiation.

At the time of publication of this report the vital status of 3,381 JEN workers is known (table 1), 289 of whom had dead (8.6% of the cohort of persons whose vital status in known).

Table 2 presents the study population distribution by sex, dose measurement records and vital status. There are not, for either sex, any relationship between vital status and the presence of dose measurement records.

Table 3 shows the distribution of the study population by radiation exposure. The collective external exposure of the JEN personnel was 39,069 mSv-person, which represents a median and mean accumulated exposure in the persons with dose reading of 4.27 and 11.85 mSv respectively. These exposure measurements were lower for women.

If the average exposure of 11.3 years is taken into account, an individual annual average value of approximately 1 mSv-person is obtained. Finally it is important to point out that the real doses of this cohort, miners who worked in underground mines, must have been three times greater than doses that were registered as external dosimetry. As could be expected, the distribution of the exposure is asymetric, with the maximum being in doses near the natural background. Only 937 (28.4%) of the workers exceed an accumulated external dose of 10 mSv throughout their professional lifes, and only 46 (1.4%) of them exceed and accumulated dose of 100 mSv.

Lastly, table 4 shows the distribution of the study population by sex and the principal indicators of risk of death registered by the JEN Medical Service. The high presence of tobacco and alcohol use, especially among the men in the study cohort, deserves special mention. We cannot know the extent to which the noteworthy absence of information produces biased results. Finally, and although these data correspond to a long

period of follow-up of the study cohort, we have the impression, in some cases documented [2-10] that they are not very different from those of the spanish population of the same age and sex.

III.6. External comparison of the mortality of the JEN cohort with the mortality of the spanish population.

Table 5 shows the Standardized Mortality Ratios (SMR) for the principal causes of death for the period 1954 - 1986. The total SMR is 0.88 (CI 95%= 0.78 - 0.99). This means that fewer deaths were observed than would have been expected in light of national mortality rates. This is due fundamentally to the low mortality for Cardiovascular (ICD 9, 390 - 459), Nervous System (ICD 9, 320 - 389), Digestive Tract (ICD, 520 - 579) and natural infectious and parasitic diseases (ICD 9, 1 - 139). These results are consistent whith the well known "healthy worker effect" (48-51).

However, an excess number of deaths is observed for traumatisms and poisoning (ICD 9, 800 - 999) and for diseases of the respiratory system. (ICD 9, 460 - 519). This mortality is mainly related to mining accicents and pneumoconiosis, cammon in this labour activity. Lastly, the excess number of deaths (SMR = 1.29, CI 95% = 1.04 - 1.59) due to malignant tumors (9, 104 - 208) is specially noteworthy.

When the specific SMRs for tumors are examined (table 6), it is observed that the excess mortality is fundamentally attributable to lung cancer (ICD 9, 162 - 163), cancer of the nervous system (ICD 9, 191 - 192) and cancer of the bones (ICD 9, 170). The rest of the localizations (although in some cases have a strong influence on the overall SMR for malignant tumors) have such a small observed frequency that they are statistically unstable and difficult to interpret.

III.7- Internal comparison of the mortality according to the level of exposure to ionizing radition.

III.7.1. Internaal comparison using a proportionality effect-dose function

Figure 2 shows the relationship between dose exposure and risk

of cancer death. Though it has not achieved statistical significance due to small figures, the data suggest a positive relation whose regression coefficient takes a value of 4'8 % excess risk Sv^{-1}. This value is consistent with those accepted in the international literature.

III.7.2. Internal comparison using a logistic regression model

The model presents the best fitness for the stratum of tobacco status nuknown (LR= 0'66) and the worst for the non smokers (LR=0'31, Table 7). This is consistent with the greater number of cases in the stratum with unknown tobacco status and its smaller residual values (table,7).

The values for the DOSI coefficient are not significant (p>0,05) in any of the strata. However it is specially noteworthy its value among nonsmokers (p=0'07), suggesting that among this stratum exposure to ionizing radiation at the doses present at JEN may increase the proportion of cancer deaths.

On the other hand, the small number of deaths due to specific tumor locations made it unfeasible the study of its relation to ionizing radiation using this methodology.

III.8 DISCUSSION

We will present a brief summary of the main factors that may influence the validity and precision of the results.

Factors that influence validity and precision of the results

DESCRIPTION OF STUDY POPULATION

- REGISTRY OF THE EXPOSURE

(Only external dosimetry is considered, methods of dosimetry

have changed since 1954, presence of lower and upper detection limits, angular dependence of the registry, response to unwanted radiations, etc....)

- VITAL STATUS ASCERTAINMENT

(40% of losts to follow-up, possibility of cause of death misclassification, defficient characterization of the mortality (8'5% deaths among the population with known vital status)

- REGISTRY OF CLINICAL DATA

(it is a noteworthy aspect of this study, including information predictors of death not usually gathered in other studies; however there is a lack of information on these variables for 40% of the study cohort).

EXTERNAL COMPARISON OF THE MORTALITY OF THE JEN COHORT TO THE MORTALITY OF THE SPANISH POPULATION

-FACTORS WHICH AFFECT THE VALIDITY OF THE SMRs

("healthy worker effect", losts to follow-up, small duration of the follow-up, latency period not taken into account; misclassification errors in the cause of death, etc).

-FACTOR WHICK AFFECT THE PRECISION OF THE SMRs

(small sample of the study cohort, losts to follow-up, small duration of the follow-up, etc.)

INTERNAL COMPARISOW OF THE MORTALITY ACCORDING TO THE LEVEL OF EXPOSURE TO IONIZING RADIATION.

(Similar to the aboved mentioned except the "healthy worker effect", misespecification of the model).

FIGURE 1. EXTERNAL COMPARISON OF THE MORTALITY OF JEN WITH THE MORTALITY OF THE SPANISH POPULATION.

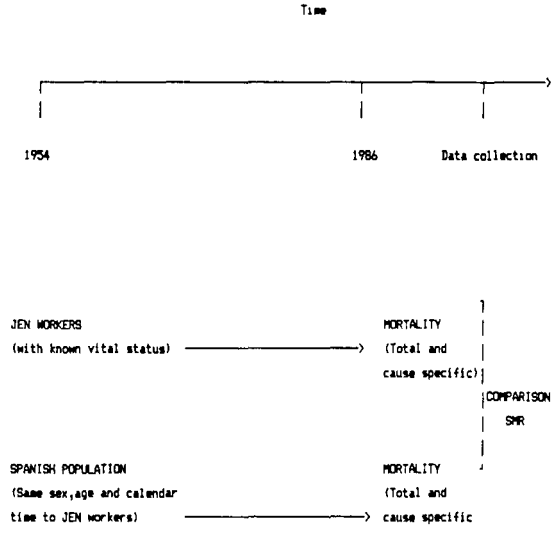
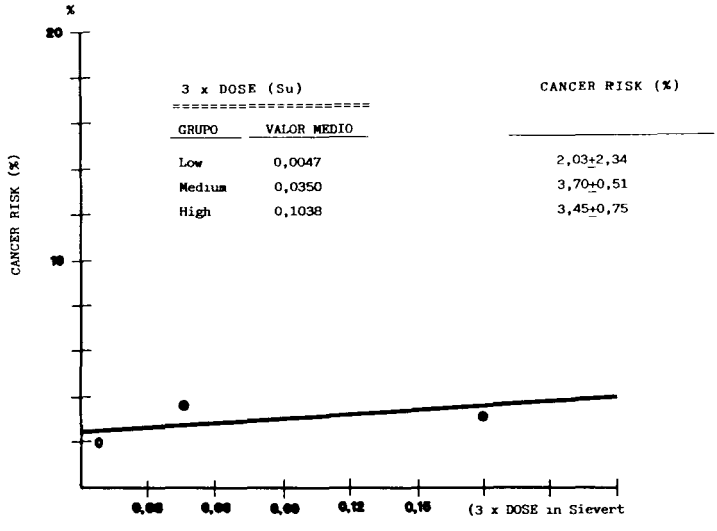


FIGURA 2 — STRAIGHT LINEAR REGRESSION MODEL

Y = a x 3 x dose + b



3 x DOSE (Su)		CANCER RISK (%)
GRUPO	VALOR MEDIO	
Low	0,0047	2,03±2,34
Medium	0,0350	3,70±0,51
High	0,1038	3,45±0,75

TABLE 1. Administrative, exposure and vital status data by sex in the study population.

	TOTAL	MEN	WOMEN
A) ADMINISTRATIVE DATA			
-Study population	5303	4533	770
-Study population working in mines	2220(41,9)	2138(47,2)	82(10,7)
-Mean age at entry in JEN (years)	29,6±9,2	30,0±9,1	26,9±9,2
-Mean time working at JEN (years)	12,3±10,4	12,4±10,5	11,5±9,4
-Mean time working in mines	11,4±9,7	11,4±9,8	10,9±8,4
B) EXPOSURE DATA			
-Population with dosimetric record	3297(62,2)	2760(60,9)	537(69,7)
-Population with dosimetric record and exposure over 0 mSv	2905(54,8)	2498(55,1)	407(52,9)
-Mean age at first exposure (years)	30,3±9,2	30,6±9,1	28,1±9,7
-Average duration of exposure(years)	11,3±9,3	11,7±9,5	8,8± 7,6
C) VITAL STATUS DATA			
-Population with known vital status	3381(63,8)	2794(61,4)	587(76,2)
-Deaths in the study population	289	278	11
-Average duration of follow-up(years)	15,7±11,3	15,9±11,5	14,4±10,2
-Population with dosimetric record and Known vital status	2469(46,6)	2043(45,1)	426(55,3)

Percentages on the study population are presented between brackets. Mean ± standard deviation are presented for continous variables .

TABLE 2.- STUDY POPULATION BY SEX; DOSIMETRIC RECORD AND VITAL STATUS

	MEN		WOMEN			
VITAL STATUS	With Dosimetric Record	Without Dosime Tric Record	With Dosimetric Record	Without Dosi-metric(record)	Total	(%)
Live	1865	651	417	159	3092	58,3
Dead	178	100	9	2	289	5,5
Unknown	717	1022	111	72	1922	36,2
TOTAL	2760	1773	537	233	5303	100,0

TABLA 3 .- DISTRIBUTION OF STUDY POPULATION WITH DOSIMETRIC RECORD BY SEX AND MAGNITUDE OF RADIATION EXPOSURE (Accumulated whole body exposure, mSv)

	MAGNITUDE OF RADIATION EXPOSURE (mSv)									
Sex	0	> 0 ≤	5-	10-	20-	50-	100-	Median exposure (mSv)	Mean exposure (mSv)	Mean annual exposure
MEN	262	1108	527	383	329	109	42	5,10	12,81	1,13
WOMEN	130	268	66	36	25	8	4	1,76	6,96	0,59
TOTAL	392(11,9)	1376(41,7)	593(18,0)	419(12,7)	354(10,7)	117(3,6)	46(1,4)	4,27	11,85	

Percentages over the study population with dosimetric record are presented between brackets

TABLA 4 DISTRIBUTION OF STUDY POPULATION BY SEX AND THE PRINCIPAL PREDICTORS OF DEATH

	MEN			WOMEN			TOTAL		
	n	N	(%)	n	N	(%)	n	N	(%)
Tobacco	1303	2420	(53,8)	169	497	(34,0)	1472	2917	(50,5)
Excesive alcohol	1066	2445	(43,6)	72	517	(13,9)	1138	2962	(38,4)
Obesity	282	2102	(13,4)	42	441	(9,5)	324	2543	(12,7)
Hypertension	257	2259	(11,4)	17	505	(3,4)	274	2764	(9,9)
Hypercholesterolemia	224	2141	(10,5)	43	463	(9,30)	267	2604	(10,3)
Hypergycemia	111	2259	(4,9)	4	500	(0,8)	115	2759	(4,2)

TABLE 5. STANDARDIZED MORTALITY RATIOS (SMR) FOR THE PRINCIPAL CAUSES OF DEATH. 1954 - 86

CAUSES OF DEATH (ICD - 9)*	Observed deaths	SMR	Confidence Limits 95%
All causes	289	0,88	0,78 - 0,99
Infectious and parasitic	5	0,85	0,28 - 1,95
Malignant tumors	89	1,29	1,04 - 1,59
Nervous System	3	0,65	0,13 - 1,84
Circulatory System	74	0,52	0,40 - 0,64
Respiratory	54	1,22	0,92 - 1,59
Digestive	19	0,66	0,40 - 1,03
Violence	38	1,46	1,03 - 2,05

* ICD - 9 CODE BETWEEN BRACKETS.

TABLE 6. STANDARDIZED MORTALITY RATIOS (SMR) FOR THE PRINCIPAL TUMORAL LOCATIONS. 1954 - 86

CAUSE OF DEATH (ICD - 9) *	Observed deaths	SMR	Confidence Limits 95%
All tumors	89	1,29	1,04 - 1,59
Buccal cavity and pharynx	6	3,13	1,15 - 6,72
Stomach	12	1,15	0,60 - 2,00
Large intestine	4	0,85	0,23 - 2,13
Liver and liver ducts	9	1,16	0,53 - 2,19
Trachea, bronchus, lung	20	1,37	0,84 - 2,12
Bone	5	5,15	1,67 - 11,83
Prostate	3	0,44	0,08 - 1,25
Kidney	3	2,11	0,42 - 5,99
Nervous system	10	4,15	1,99 - 7,59
Hodgkin disease	2	3,64	0,42 - 12,49
Myeloma and other lymphatic	2	0,82	0,09 - 2,82
Leukemia	2	0,52	0,06 - 1,79

* ICD-9 CODE BETWEEN BRACKETS

TABLE 7.- LOGISTIC FUNCTION RELATING RADIATION EXPOSURE AND PERCENT CAN CONTROLLING FOR TOBACCO: A) TOBACCO UNKNOWN B) SMOKERS C) NON - SMOKERS

ANALYSIS OF INDIVIDUAL PARAMETERS

A)

EFFECT	PARAMETER	ESTIMATE	STANDARD ERROR	CHI-SQUARE	PROB
INTERCEPT	1	-.780405	0.51294	2.31	0.1282
DOSI	2	-.011201	0.275858	0.00	0.9676

	DF	CHI-SQUARE	PROB
LIKELIHOOD RATIO	1	0.19	0.6655

ANALYSIS OF INDIVIDUAL PARAMETERS

B)

EFFECT	PARAMETER	ESTIMATE	STANDARD ERROR	CHI-SQUARE	PROB
INTERCEPT	1	-.635284	0.933641	0.46	0.4962
DOSI	2	0.320737	0.481615	0.44	0.5054

	DF	CHI-SQUARE	PROB
LIKELIHOOD RATIO	1	0.50	0.4781

ANALYSIS OF INDIVIDUAL PARAMETERS

C)

EFFECT	PARAMETER	ESTIMATE	STANDARD ERROR	CHI-SQUARE	PROB
INTERCEPT	1	-2.77977	1.60128	3.01	0.0826
DOSI	2	1.60897	0.861214	3.49	0.0617

	DF	CHI-SQUARE	PROB
LIKELIHOOD RATIO	1	0.99	0.3187

				OBSERVED		PREDICTED		
SAMPLE	DOSI	MORTA	FUNCTION NUMBER	FUNCTION	STANDARD ERROR	FUNCTION	STANDARD ERROR	RESIDUAL
1	BAJA		1	-0.838329	0.299211	-0.791606	0.276437	-0.04672
		M_TUMORES	P1	0.301887	0.063059	0.311824	0.0593206	-.0099370
		M_OTRAS CAUSAS	P2	0.698113	0.063059	0.688176	0.0593206	0.0099370
2	MEDIA		1	-0.719123	0.279772	-0.802807	0.20475	0.083684
		M_TUMORES	P1	0.327586	0.0616264	0.309425	0.0437512	0.018160
		M_OTRAS CAUSAS	P2	0.672414	0.0616264	0.690575	0.0437512	-0.018160
3	ALTA		1	-0.955511	0.526235	-0.814008	0.399528	-0.14150
		M_TUMORES	P1	0.277778	0.105572	0.307037	0.0850056	-0.029259
		M_OTRAS CAUSAS	P2	0.722222	0.105572	0.692963	0.0850056	0.029259
1	BAJA		1	-0.154151	0.556349	-0.314546	0.512556	0.16039
		M_TUMORES	P1	0.461538	0.138264	0.422005	0.125021	0.03953
		M_OTRAS CAUSAS	P2	0.538462	0.138264	0.577995	0.125021	-0.03953
2	MEDIA		1	-0.223144	0.474342	0.0061909	0.342998	-0.22933
		M_TUMORES	P1	0.444444	0.117121	0.501548	0.0857488	-0.057103
B)		M_OTRAS CAUSAS	P2	0.555556	0.117121	0.498452	0.0857488	0.057103
3	ALTA		1	0.693147	0.866025	0.326928	0.660674	0.36621
		M_TUMORES	P1	0.666667	0.19245	0.581012	0.160832	0.085654
		M_OTRAS CAUSAS	P2	0.333333	0.19245	0.418988	0.160832	-0.085654
1	**BAJA**		**1**	**-0.916291**	**0.83666**	**-1.1708**	**0.824113**	**0.254508**
		M_TUMORES	**P1**	**0.285714**	**0.170747**	**0.236711**	**0.1489**	**0.0490037**
		M_OTRAS CAUSAS	**P2**	**0.714286**	**0.170747**	**0.763289**	**0.1489**	**-0.0490037**
2	**MEDIA**		**1**	**0.182322**	**0.60553**	**0.43817**	**0.526895**	**-0.255848**
		M_TUMORES	**P1**	**0.545455**	**0.150131**	**0.607823**	**0.125598**	**-0.0623683**
		M_OTRAS CAUSAS	**P2**	**0.454545**	**0.150131**	**0.392177**	**0.125598**	**0.0623683**
3	**ALTA**		**1**	**1.79176**	**1.52753**	**2.04714**	**1.16596**	**-0.255379**
		M_TUMORES	**P1**	**1**	**0**	**0.885658**	**0.118074**	**0.114342**
		M_OTRAS CAUSAS	**P2**	**0**	**0**	**0.114342**	**0.118074**	**-0.114342**

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no : BI6-F-125-D

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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
Statistician	M. Kloos

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:

- Completion of the main-frame computer version of each of the packages indicated under III.1, previous report, with the exception of DVA;
- Completion of the documentation of ICD, SAR, UST;
- Preliminary documentation of the package RES which is still to be developed.

Methodology for Probabilistic Uncertainty Analysis of Computational Assessments

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Abstract

For introduction this report provides several arguments in favour of an uncertainty analysis and explains necessary distinctions. Subsequently, it describes the objectives of the project for the time period 1985 - 1989. The results obtained are presented in form of the GRS programme package for uncertainty and sensitivity analysis. Their importance for the Radiation Protection Progamme becomes immediately evident from the arguments given in section 1.1.

The features of the package, the correspondence of the programmes in the package with the main steps of a parameter uncertainty analysis and the main characteristics of each individual programme are discussed. A separate chapter is devoted to practical applications as well as limitations. Finally, conclusions are drawn from the applications and an outlook on the topics of ongoing work is given.

1. Introduction

The estimation of uncertainty in measured, computationally assessed or predicted quantities is fundamental to most scientific activities. However, the current situation appears paradox. In earlier days it was a good tradition to perform an error analysis of computed results. Mainly in physics and engineering the "propagation of error" formulae were well known and widely used. This, although most computations showed only moderate complexity, the fuctional relationships were usually well established and the "errors" in parameter values generally originated from small fluctuations about some nominal values.

In present days large complex computations are being performed. The functional relationships are not seldom scarcely more than working hypotheses and parameter values are not only subject to small fluctuations but frequently to considerable lack of knowledge of even the nominal values. Under these circumstances one would expect "error analysis", or more appropriately "uncertainty analysis", to receive ever so much attention. However, the percentage of computed results that include a quantitative assessment of the combined impact of uncertainties is alarmlingly small.

1.1 The need for uncertainty analysis

The situation is upsetting as more and more important decisions are being based on results from computer models. Decisions require the comparison of alternatives like action or no action, alternative sites, designs, strategies etc. Crucial differences between alternatives may, however, quite frequently be seen not so much from the computed values but from their uncertainties. For one alternative the relevant parameter values may be well-known, the functional relationships reasonably well established and the combined impact of their uncertainties on the computed value may be small. For another alternative little may be known about some of the parameter values and some of the functional relationships may not be well understood or may be very sensitive to even small uncertainties in parameter values. An uncertainty analysis provides quantitative information about the combined impact of the individual uncertainties on the computed results. This quantitative information may indicate that more data collection, testing, experiments and research etc. is needed and where it is particularly needed.

Also, alternatives are not seldom favoured by opposing groups. If the analyst does not care for the impact of uncertainties on the result computed for a particular alternative those in favour of any of the other alternatives may. Quite frequently they will compute a result based on a so-called worst case combination of modeling assumptions and parameter values.

The only apt scientific answer to a worst case consideration is an uncertainty analysis. It does not exclude any of the so-called worst case combinations but puts their results into perspective to the set of results obtained with the totality of combinations of alternative modeling assumptions and parameter values. Additionally, it permits the analyst to process any preferences for particular modeling assumptions and subranges of parameter values which may be suggested by his state of knowledge.

If an uncertainty analysis is not performed some may feel they have to guess what the combined effect of the most obvious uncertainties is. It will generally be very difficult to argue on the basis of guessed overall uncertainties. This is particularly so if several disciplines contribute to the computed result and none of the individuals has the expertise to make overall judgements.

In safety related issues it has been common practice to account for uncertainties by so-called "conservatisms" at various instances of the computation. If the computed result is to serve a decision between several alternatives it will be difficult to allocate conservatisms in each of the corresponding computations in a fair manner. Also, even for moderately complex computations it will often be challenging to judge how "conservative" the final result is after all. If an uncertainty analysis is performed each of the computations may proceed with so-called "best estimates". The decision may then be based on the computed "best estimate" results and the quantitative uncertainty statements obtained from the analysis.

In technical safety and environmental health related issues computed values are quite frequently to be compared to limiting values (safety or regulatory standards) and a decision is to be made as to whether or not there is compliance. While the point values ("best estimate" results, for instance) to all alternative designs or strategies or sites etc. may comply with the limiting value their uncertainties may indicate that there is considerable degree of belief for some to substantially violate the limit.

With little additional effort the information obtained from an uncertainty

analysis can be used to identify those modeling assumptions and parameter values that contribute most to the uncertainty in the computed result (sensitivity information). This provides guidance for data collection and future research efforts and is therefore valuable input to the decision making process.

1.2 Some necessary distinctions

[1] discusses various distinctions that are required in order to obtain meaningful results from an uncertainty analysis. These distinctions involve: the type of answer required to an assessment question; the type of uncertainty; the probability concept to be used; the type of prediction to be made; and the type of model needed to obtain the prediction.

Typically, model predictions are used to answer questions like: "How many units of a particular quantity of interest will there be per reference unit?"

Two basic types of assessment questions are considered: those that have a deterministic answer and those that have a probabilistic answer. Because of this distinction it is essential to distinguish between two fundamentally different types of uncertainty, subsequently referred to as Type A and Type B.

Type A uncertainty is due to stochastic variability with respect to the reference unit of the assessment question.

Type B uncertainty is due to lack of knowledge about items that are invariant with respect to the reference unit of the assessment question.

The presence of Type A uncertainty in the quantity of interest requires a probabilistic answer to the question. A probabilistic answer represents the stochastic variability of the quantity of interest in the form of a distribution.

In practice, deterministic as well as probabilistic answers can be obtained only imprecisely because of Type B uncertainties. Type B un-

certainties suggest a range, not of variability but of alternative, possibly true deterministic or probabilistic answers to the assessment question.

"Probability can be thought of as the mathematical language of uncertainty" [2]. The practical interpretation of the term probability is, however, different for Type A and Type B uncertainties. For Type A uncertainty, probability is interpreted as the limiting value of relative frequencies (i.e. the frequentistic interpretation). For Type B, probability is interpreted as the degree of belief that, for instance, a determined but vaguely or imprecisely known value is within a specified interval (i.e. the subjectivistic interpretation).

If probaility is used in the classical or frequentistic interpretation, it simply is called "probability". If it is used in the Bayesian or subjectivistic interpretation, it is called "subjective probability".

Modeling Type B uncertainty via subjective probabilities has received increased attention in the past one or two decades. The reason for this is the increasing need to predict the behaviour of complex systems under conditions in which a substantial lack of knowledge prevails. In this situation, the stochastic variability of a quantity about its mean value is quite often negligible compared to the lack of knowledge about the mean value itself.

2. Objectives of the Project

It is assumed that either a deterministic model is adequate to answer the assessment question or that the model is probabilistic, expressing by probability distributions all non-negligible Type A uncertainties. The prediction will then be a single value or a single ccdf (complementary cumulative distribution function). Consequently, all that is left for the uncertainty analysis is to assess Type B uncertainty.

The programmes developed under this project primarily address Type B uncertainties. However, since they are based on methods from probability calculus and on tools from statistics they are equally well suited for

Type A. Sole condition being that the necessary distinction between the two types is strictly observed in probabilistic modeling, propagation and presentation of uncertainties.

The objectives of the project were to

- review the spectrum of methods
- provide an application-oriented judgement of their relative merits and drawbacks
- enhance their range of applicability
- identify unresolved issues.

Finally there should be a set of programmes, supporting the main steps of an uncertainty analysis, together with adequate documentation. The documentation is to address the objectives mentioned above with respect to the corresponding step of the analysis and is to serve as a user's guide.

3. The GRS Programme package for Uncertainty and Sensitivity Analysis

A set of five programmes [3,4,5,6,7] has been developed suitable for use on mainframe computers. Each programme is accompanied by a report providing a detailed discussion of the methods implemented in the respective programme and simultaneously serving as a user's guide.

3.1 General features of the programme package

The package consists of so-called "general purpose" programmes. That means the programmes may be used for the analysis of an application of any computer code. They are not specifically taylored to the uncertainty analysis of a particular computer model like, for instance, a fault tree analysis code, thereby exploiting specific features of the code.

The implemented methods, measures and approaches are primarily based on random sampling and on tools from statistics. It is assumed that modeling uncertainties are represented by additional uncertain parameters. Our experience indicates that this can be achieved in many in-

stances through either the selection of the preferred modeling assumption and the introduction of so-called correction factors or additive terms which are then uncertain parameters or if there is a set of alternative possibly correct modeling assumptions that is thought to be complete and the index of the correct assumption is introduced as an uncertain parameter. Therefore the implemented procedures primarily address uncertainties in model predictions due to those in model parameters. The set of programmes for uncertainty and sensitivity analysis is structured according to the main steps of a parameter uncertainty analysis. Necessary prerequisite for their application is that all potentially important uncertain parameters are readily accessible in the computer model.

3.2 Programmes of the package and main steps of a parameter uncertainty analysis

S T E P	Supporting Program
1 List all of the parameters, phenomena and modeling assumptions that are potentially important contributors to model output uncertainty (prepare for <u>parameter</u> uncertainty analysis)	
2 Specify the maximum conceivable range of possibly applicable alternative parameter values	
3 Specify a subjective probability distribution to represent the state of knowledge.	DIVIS
4 Account for dependences among uncertain model parameters	DIVIS/ MEDUSA
5 Propagate the joint subjective PDF[1)] through the model to obtain the subjective PDF of the model output	MEDUSA

[1)] probability density function

6	Derive quantitative statements about the uncertainty in the model output that is due to the combined impact of the parameter uncertainties	EQUUS
7	Rank the parameters with respect to their contribution to model output uncertainty	SAMOS
8	Present and interpret the results of the analysis	TUSSIS

3.3 Main features of the individual programmes

- DIVIS [3]

  Purpose: Support the expert in the specification of subjective probability distributions as well as measures of association for uncertain parameters.

  Scope: DIVIS offers a wide range of distribution types and several measures of association. It accepts fractiles (or quantiles), parameters, moments and truncations as input to quantify the expert's state of knowledge. On output it provides density function, cumulative (or complementary cumulative) distribution functions of the selected distribution type and according to the specified characteristics. Scatter plots illustrate the effect of the specified measures of association between pairs of uncertain parameters. The output may be viewed on the monitor and possibly adjusted in an interactive manner or obtained as hardcopy. DIVIS is panel driven and prompts the user for the required input.

- MEDUSA [4]

  Purpose: Generation of random experimental designs according to the subjective probability distributions and measures of association specified by the experts.

Scope: MEDUSA supports all distribution types and measures of association offered by DIVIS. It permits a choice between the population or sample interpretation of the measures of association. Both interpretations are possible for simple random sampling as well as Latin Hypercube sampling. The MEDUSA publication [12] discusses the principal properties of the various design options offered.

- EQUUS [5]

Purpose: Derivation of empirical uncertainty statements from the sample of size n of model output values obtained with the sample of parameter vectors selected by MEDUSA.

Scope: EQUUS derives distribution-free fractile estimates and statistical tolerance limits of the unknown subjective probability distribution of the model output. Additionally, it performs tests for and the fitting of distribution types with subsequent derivation of fractile estimates and possibly statistical tolerance limits from the fitted distributions. If the model output is not of scalar type, measures of association may be determined between selected components.

- SAMOS [6]

Purpose: Derivation and graphical presentation of empirical sensitivity measures for the model output with respect to the parameter uncertainties.

Scope: SAMOS derives correlation coefficients, partial correlation coefficients, standardized partial regression coefficients with and without rank transformation of the sample data (log. transformation may also be selected). Additionally, sensitivity measures from two by two contingency tables, the quadrant measure, stepwise (rank) regression and correlation ratios are offered.

- TUSSIS [7]

  Purpose: Derivation and graphical presentation of empirical uncertainty statements and sensitivity measures, if the model output is a function of time, space or any other independent variable.

  Scope: TUSSIS presents the alternative model results for the sample of size n of parameter vectors. It prepares the set of alternative model results with respect to range and discrete points of the independent variable selected for graphical presentation. Subsequently, it initiates the derivation and graphical presentation of uncertainty statements, R^2-values as well as sensitivity measures and parameter rankings for all parameters (or for a user-specified subset) at the specified discrete points. These local analysis results are connected through linear interpolation over the independent variable.

3.4 Applications

First versions of MEDUSA and SAMOS were used to perform uncertainty and sensitivity analyses of applications of the atmospheric dispersion models in two different accident consequence codes as well as of a food-chain model. Statistical tolerance limits and parameter rankings were obtained for various model output quantities like

- averaged centreline concentrations
- ccdf of the centreline concetration

at selected distances from the site, for I-131 and Cs-137 and with respect to plume, ground surface and air at 1 m above ground surface

- the ccdfs of
  - crop area lost
  - milk volume lost
  - meat production lost
  - collective dose after bans.

The results of these applications are documented in /8, 9, 10/.

In addition, the final versions of the programmes in the package have been used for uncertainty and sensitivity analyses of applications of computer models for

- severe (in-vessel) accident analysis
- Markovian reliability analysis
- simulation of the condensation of vapour on liquid surfaces
- boiling liquid expanding vapour explosions
- oil and cable fire simulation.

3.5 Limitations

There is still room for improvements. The affordable number of model runs is still a severe limitation in a considerable fraction of practical uncertainty and sensitivity analyses. Particularly, of course, in uncertainty analyses of real-time applications of accident consequence codes in an emergency response context and of model applications where extreme fractiles of the subjective probability distribution of the model output are of interest (for instance, if the extremely low subjective probability for violation of a threshold value is to be determined). Also, correlation/regression based sensitivity measures, while judged adequate generally, provide a questionable ranking in all those situations where they refere to only a relatively small fraction of the model output uncertainty (R^2 less than 50 %). How to satisfactorily remedy this shortcoming in a general purpose sensitivity analysis procedure is still to be resolved. The set of generally applied sensitivity measures is not yet considered as being complete. Alternative or supplementary measures are under investigation.

Irrespective of whether an assessment model has been subjected to the process of validation, there will be uncertainty associated with all relevant model applications. Consequently, assessments must be accompanied by an uncertainty analysis. It is expected that uncertainty in

assessments will be smaller for those applications that are closely related to situations in which the model has been validated than for applications where validation for related situations was either impossible or impracticable. It is, however, not quite clear how the information from a validation process could best be used in the uncertainty analysis of a particular application of the assessment model.

4. Conclusions and Outlook

In conclusion, the outcome of the project is a package of mainframe programmes suitable for the uncertainty and sensitivity analysis of applications of a wide range of computer models. The computational effort of the implemented methods for uncertainty analysis is independent of the number of uncertain parameters. Therefore they permit the treatment of as many uncertain parameters as may be judged to be potentially important. For the different analysis steps the package offers a spectrum of methods and measures based on random sampling and on tools from statistics as well as various options for the graphical presentation of analysis results. Detailed discussion of the implemented methods and a user's guide is contained in the documentation.

Ongoing work concentrates on three topics.:

- A driver programme that is to provide guidance through the steps of an analysis and is to assist in the choice from the various options offered by the programmes in the package. It also needs to take care of the data transfer between these programmes and is to provide a framework for the inevitable design extensions by parameters that cannot be readily handled via MEDUSA. Finally it needs to provide for the two-stage procedure needed if Type A and B uncertainties are to be considered within the analysis.

- A Personal Computer version of the complete package together with practically relevant demonstration examples.

- A guide to a successful handling of the various aspects of expert judgement involved in steps 1 to 4 of the analysis.

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RADIATION PROTECTION PROGRAMME
Final Report

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Contract no.: BI6-F-295-UK

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Title of the research contract:

Investigation of the relationship between lung cancer and radon in houses.

List of projects:

1. Investigation of the relationship between lung cancer and radon in houses.

Title of the project no.. 1

Investigation of the relationship between lung cancer and radon in houses.

Head(s) of project:

M C O'Riordan

Scientific staff:

J C H Miles, B M R Green, P R Lomas, T Gooding

I. Objectives of the project:

To determine the magnitude of the risk associated with radon exposure in the home by carrying out a case-control study of lung cancer incidence in relation to past and present household radon concentrations in SW England.

II. Objectives for the reporting period:

To select appropriate community controls from the lists of Family Practitioner Committees. To continue interviewing cases and hospital controls and placing passive radon detectors in their present homes.

III. Progress achieved:

Study Centres

Interviewing of cases and hospital controls has continued in Truro and Plymouth, and started in Barnstaple, Torquay and Exeter in 1989. Thus all five study centres are now operational in SW England.

Cases and Hospital Controls

Some 480 patients with suspected lung cancer have been identified in this period (see Table 1). Thirty-nine patients were too ill to be approached for interview and, of the remainder, 25 refused to take part. One hundred and ninety eight patients had not lived in Devon and Cornwall for long enough to satisfy residence requirements, and 218 patients received a full interview. Review of hospital discharge diagnoses has been carried out for 250 of the 285 patients with suspected lung cancer who received a full interview so far in the study. For 176 (70%) the final diagnosis was lung cancer. The pathological review of microscopically confirmed cases (i.e. those with a positive histology or cytology) has begun, although these represent only about half of those with lung cancer as a final diagnosis.

A total of 381 potential hospital controls have been identified, 304 of them in this reporting period (see Table 1). Twelve patients were too ill to be interviewed, and a further 9 refused to take part in the study. Of the remainder, 136 did not meet the residence criteria, and 215 received full interviews. Review of the hospital discharge diagnoses has been carried out for 195 of the 215 hospital controls with full interview. For 5 patients the final diagnosis rendered them ineligible as controls, as they turned out to have diseases strongly related to smoking: cancer of pancreas (1); cancer of bladder (3); aneurysm of iliac artery (1).

Community Controls

Lists of the names, addresses and General Practitioners for patients born on days selected by the Imperial Cancer Research Fund have now been received from both Devon and Cornwall Family Practitioner Committees. Interviewers based in Devon have begun work on community controls: 4 people have received a full interview; 6 further potential controls did not satisfy the residence requirements; 2 people selected from the FPC lists had died. Interviewing of Cornish community controls will commence soon.

Measurements

Of the 504 cases, hospital controls and community controls who have received a full interview, the interviewers have reported that detectors have been successfully installed in the homes of 401. The distribution of radon concentrations found in results available so far is consistent with the distribution found in previous radon surveys. Measurements in previous addresses of subjects will begin in 1990.

It should be noted that a smaller proportion than expected of lung cancer cases is eligible for the study, and that it will therefore take longer than expected to recruit the required numbers to the study.

CASES OF SUSPECTED LUNG CANCER

Year - quarter		Full interview	Short residence	Patient refused	Patient too ill	Total
1988	2	5	21	0	4	30
	3	31	25	8	9	73
	4	31	26	5	3	65
1989	1	31	33	3	10	77
	2	68	47	11	10	136
	3	88	84	8	11	191
	4	31	34	3	8	76
TOTAL		285	270	38	55	648

HOSPITAL CONTROLS

Year - quarter		Full interview	Short residence	Patient refused	Patient too ill	Total
1988	2	1	0	0	0	2
	3	14	3	1	1	19
	4	36	16	3	0	56
1989	1	28	12	2	0	43
	2	37	29	0	1	67
	3	76	52	2	5	137
	4	27	24	1	5	57
TOTAL		215	136	9	12	381

Table 1: Numbers of patients with suspected lung cancer or with other diseases suitable for comparison purposes identified in Devon and Cornwall.

IV. Objectives for the next reporting period:

To continue interviewing cases and hospital controls and placing radon detectors in their present and previous homes. To commence interviewing community controls and to place detectors in their homes and in the previous residences of cases and hospital controls.

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

Imperial Cancer Research Fund
Cancer Epidemiology and Clinical Trials Unit
Oxford University
Gibson Building
The Radcliffe Infirmary
OXFORD OX2 6HE

(Emeritus Professor Sir Richard Doll, Dr S C Darby, Dr V Beral and Dr P B S Silcocks)

VI. Publications:

None.

RADIATION PROTECTION PROGRAMME

Progress Report

1989

Contractor:

Istituto Superiore di Sanità
Viale Regina Elena 299
I-00161 Roma

Contract no.: BI6-F-313-I

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

Dr. P. Comba
Lab. Igiene Ambienti Confinati
Istituto Superiore di Sanità
Viale Regina Elena 299
I-00161 Roma

Telephone number: (06) 4990

Title of the research contract:

Epidemiologic study on respiratory cancer among miners with low dose radiation exposures.

List of projects:

1. Epidemiologic study on respiratory cancer among miners with low dose radiation exposures.

Title of the project no..
Epidemiologic study on respiratory cancer among miners with low dose radiation exposures
n. B I 6 - F - 313 - I

Head(s) of project:
Dr. P. Comba
Lab. Igiene Ambientale
Istituto Superiore di Sanità
Viale Regina Elena 299 - 00161 Roma
Scientific staff:
S. Belli, D. Germani, M. Grignoli, S. Lagorio

I. Objectives of the project:

The present project is aimed at describing mortality from lung cancer among metal miners in Italy. A cohort study on pyrite miners in Tuscany showed an excess mortality from lung cancer among under ground workers (47 observed deaths versus 35.6 expected deaths); 13 extra cases per 10^6 person years and WLM were estimated (Battista et al., Scand. J. Work Env. Health 1988, 14:280-285). Other cohort studies are currently in progress in metal mines located in the Alps and in Sardinia. The purpose of the present project is the comparative analysis of these cohorts, with respect to radon daughters levels.

II. Objectives for the reporting period:

During the reporting period data collected in a zinc-leed-silver mine located in Gorno (Lombardy) were analyzed. This mine is located 10 Km away from the uraniferous ores of Novazza. The Etruscans exploited these ores in the IX - V century b.C., and the Romans continued the excavations. Mining in Gorno was discontinued in 1981. Exposure to radon daughters was estimated as ranging from 0.20 to 1.03 WL (ENEA) and from 0.10 to 0.90 WL (CEA). The mortality study was triggered by the observation of some cases of lung cancer among retired miners.

III. Progress achieved:

1. METHODOLOGY

The cohort includes all male subjects active in the Gorno Mine at 1950, and employed in the subsequent years. The source of data was represented by Company files. Information concerning name, data and place of birth, year of employment, number of years spent underground and year of dimission were collected for 1357 subjects. Vital Status at 1986 was ascertained for 95.6% of cohort members. The mortality experience of the cohort was contrasted to that of the Italian population, adjusting for age and calendar time. Cause-specific expected deaths and standardized mortality ratios were computed using the "Life Table Analysis Sistem" program, developed by NIOSH, installed on IBM 6660 computer.

2. RESULTS

Among underground workers over all mortality was significantly increased (348 observed deaths versus 217.11 expected); cancer mortality was significantly increased as well (95 observed versus 58.68 expected), mainly due to excessess of esophageal, gastric and lung cancer. There was a significant excess of respiratory tuberculosis (12 observed versus 3.71 expected) and of diseases of the respiratory system (68 versus 16.95); among these lasts 52 out of 68 were due to silicosis. Among surface workers non significant excess of mortality for all causes, all cancers, tubercolosis and respiratory diseases was observed.

Table 1 shows lung cancer mortality as a function of duration of employment and induction-latency time.

	Employment (Years)							
	Underground				Surface			
	20≤		>20		20≤		>20	
Latency (years)	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp
20≤	3	2.75	-	-	1	2.34	-	-
>20	12	5.64	18	8.22	2	3.56	7	4.99

As it can be seen, excess cases mainly occur after 20 years

of induction-latency time.

3. DISCUSSION

Among underground miners 16.4 extra cases of lung cancer were observed: given an average duration of underground work of 17.93 years, and a mediam value of exposure to radon daughters estimated as 0.60 WL (ENEA) and 0.36 WL (CEA), the extra cases were estimated as 9.78 - 16.31 per 10^6 person years and WLM. This figure is coherent with other estimates reported in the scientific literature.

IV. Objectives for the next reporting period:

During the next reporting period studies about mines located in other parts of Italy will be terminated. A comparison of data derived from these cohorts will be performed, with emphasis on risk estimates. The possible confounding role of silica exposure with respect to exposure to radon daughters will be discussed. The role of regional variation in mortality will be taken into account.

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

O. Axelson, Department of Industrial Medicine, Linköping, Sweden.
G. Battista, Institute of Occupational Medicine, Siena.
P.L. Cocco, Institute of Occupational Medicine, Cagliari.
M. Di Paola, ENEA, Rome
F. Gobbato & F. Larese, Institute of Occupational Medicine, Trieste.
R. Paganoni, Local Health Unit, Clusone
M. Ronchin, Local Health Unit, Albino

VI. Publications:

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Rijksuniversiteit Gent
Sint Pietersnieuwstraat 25
B-9000 Gent

Contract no.: BI6-F-112-B

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

Prof. Dr. A. Deruytter
Dosimetry Department
Laboratorium voor Kernfysica
Proeftuinstraat 86
B-9000 Gent

Telephone number: (091) 22.87.31

Title of the research contract:

Evaluation of the impact of the domestic environment on the population exposure to radon daughters.

List of projects:

1. Systematic analysis of the radon daughter equilibrium in houses.
2. Study of the behaviour and nature of radon daughter ions and clusters.
3. Investigation of the radon exhalation from building materials and soils.

Title of the project no.: 1

Systematic analysis of the radon daughter equilibrium in houses

Head(s) of project:

Dr H.Vanmarcke*

Scientific staff:

Dr.H.Vanmarcke*, Lic R.Van Dingenen, Dr A Poffijn, Ir.C Landsheere, Dr A.Janssens**

I. Objectives of the project:

Systematic study of the physico-chemical processes determining the fate of the radon decay products in the indoor environment Measurements will be performed in a representative number of dwellings Special attention will be paid to the influence of the ambient aerosol on the internal radon-radon daughter equilibrium In particular it will be investigated if the exposure of the public can be expressed in terms of radon concentrations rather than in terms of radon daughter concentrations.

II. Objectives for the reporting period:

* Now at SCK-CEN Mol, Belgium

** Now at CEC DGXI Luxemburg, Luxemburg

III. Progress achieved:

1. Optimization of the apparatus for measuring the decay products of radon (Vanmarcke, 1987, 1987a)

Our apparatus for measuring the low concentrations of radon decay products, typically found indoors, involves drawing air at a constant rate through a filter and counting the activity by means of alpha spectroscopy during the sampling, and during a decay time interval. The timing has been optimized by minimizing the mean value of the minimum measurable radon daughter concentrations (MMC)(Nazaroff, 1984), defined as the concentration at which the relative standard deviation in the measurement due to counting statistics is 20%, assuming the flow rate and the detection efficiency to be 28 l min^{-1} and 0.127 respectively. It was found that a long sampling time and a delay time

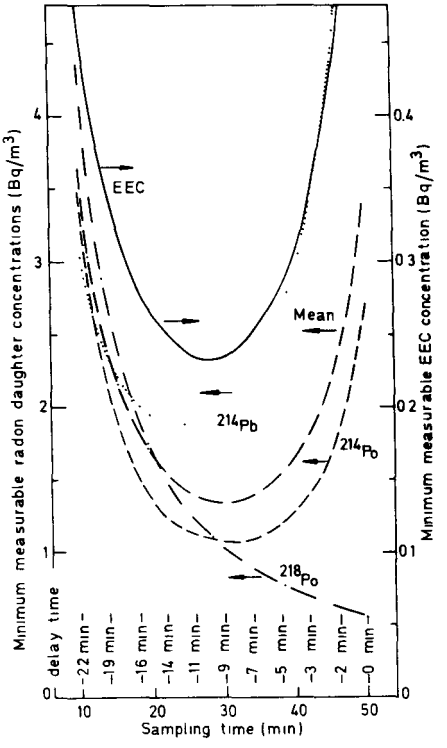

Fig.1. Optimized minimum measurable concentration of radon progeny as a function of sampling time. The mean of the MMC's is used as optimisation parameter and the total measurment time is fixed at 60 min.

longer than the minimum time needed to transfer the spectrum to a storage medium is favourable. As an example, fig.1 shows the optimized MMC's as a function of the sampling time. The total measurement time is fixed at 60 min.

The same kind of optimization has been performed for the thoron daughters In the calculations the sampling period was set at 30 min and the first decay time interval is started after the decay of the radon daughters (270 min). For a total measurement period of 16 hours, the optimized MMC of ^{212}Pb and ^{212}Bi are 0.02 Bq m^{-3} and 60 Bq m^{-3} (270-370 min, 540-960 min) respectively. Better results for ^{212}Bi are obtained with only one decay time interval and an estimation of the ratio ^{212}Pb to ^{212}Bi out of the removal processes (ventilation and deposition of the attached thoron daughters). The results of this analysis are plotted in fig.2 assuming the sum of the removal processes to equal 0.0 ± 0.1 h^{-1} and 1.0 ± 0.4 h^{-1}, respectively. It is seen that the influence of the removal rate is rather small. The steepness of the curves indicates that a longer counting time leads to a substantial improvement in measurement precision. For a total measurement period of 16 hours (counting interval . 270-960 min), the MMC of ^{212}Pb is about 0.014 Bq m^{-3}.

Measuring the ^{218}Po and ^{214}Po alpha decay during sampling improves significantly the precision, in particular for ^{218}Po and thus for the unattached fraction. However, at the same time part of the unattached activity is lost in the complex sampling-head geometry. This loss has been

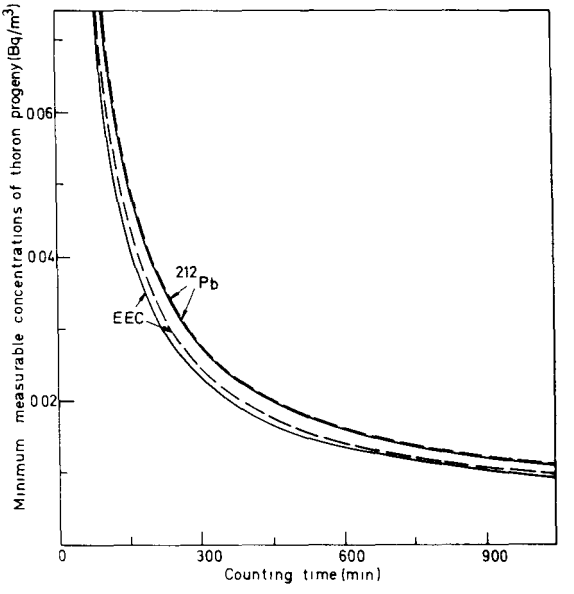

Fig.2. Minimum measurable concentration of thoron progeny versus counting time. The sum of ventialation and deposition of the attached daughters equals 0.0 ± 0.1 h^{-1} (full line) and 1.0 ± 0.4 h^{-1} (dashed line).

determined in the 1 m^3 box as a function of the flow rate. At 28 l min^{-1}, our standard flow rate in dwellings, the loss of the unattached daughters equals 0.22 ± 0.02.

2. The effect on the effective dose equivalent of the behaviour of the decay products of radon in the indoor environment (Vanmarcke, 1985,1987a,1989)

The radon daughter concentrations were periodically monitored at four different locations, with radon concentrations ranging from 7 to 111 Bq/m^3.
Together with radon daughter measurements, nearly continuous measurements of the ventilation rate were performed by means of the release of N_2O tracer gas and observation of its decay with an infrared spectrometer (Miran 101). Furthermore the aerosol concentration and size distribution were monitored every 20 to 30 min with an automated aerosol spectrometer.

Eighty-three radon daughter measurements were carried out on 18 different days. Eleven measurements were rejected from the optimization exercise because they were performed within 2 hours after the generation of a high aerosol concentration, so that no steady-state was reached.

As an example, fig.3 shows the data measured during one day in each of the four locations. The indicated ventilation rates are best fits to the N_2O decay curves and are considered as representative for the indicated time periods. There is some arbitrariness in the fitting leading to the discontinuities in the results. The accuracy is believed to be of the order of 20%. High aerosol concentrations with different size distributions were produced by burning a joss-stick, a bit of paper or by smoking or cooking. The attachment rate was calculated from the aerosol size distribution and has a systematic uncertainty of about 50%. The lower part of fig.3 shows the measured radon daughter concentrations.

The measured radon daughter concentrations were fitted by the room model to optimise the deposition rate of the unattached daughters. Then the fraction of unattached daughters (f_p), the equilibrium factor (F) and the activity median diameter (AMD), has been assessed in each measurement. These parameters are important in the dosimetric models. Finally, the effective dose equivalent is computed with the Jacobi-Eisfeld model (J-E) and with the James-Birchall model(J-B) (NEA experts' report, 1983).

The doses per unit radon concentration are plotted in fig.4 as a function of the equilibrium factor. The doses computed with the J-B model are up to a factor two larger than the ones computed with the J-E model, due to the higher dose conversion factor for the unattached fraction in the first model. The full lines are calculated for fixed parameters and changing attachment rates. As fixed parameters, the mean values of the measurements and two deposition rates of the unattached daughters are taken. The conversion factors of the NEA experts' report, (1983) and the ICRP (n 39, 1984) are proportional to the equilibrium factor and show a very different relationship compared to the J-E model and the J-B model. The main reason for this is that a low equilibrium factor is connected with a high unattached fraction. With the J-B model the dose per unit radon

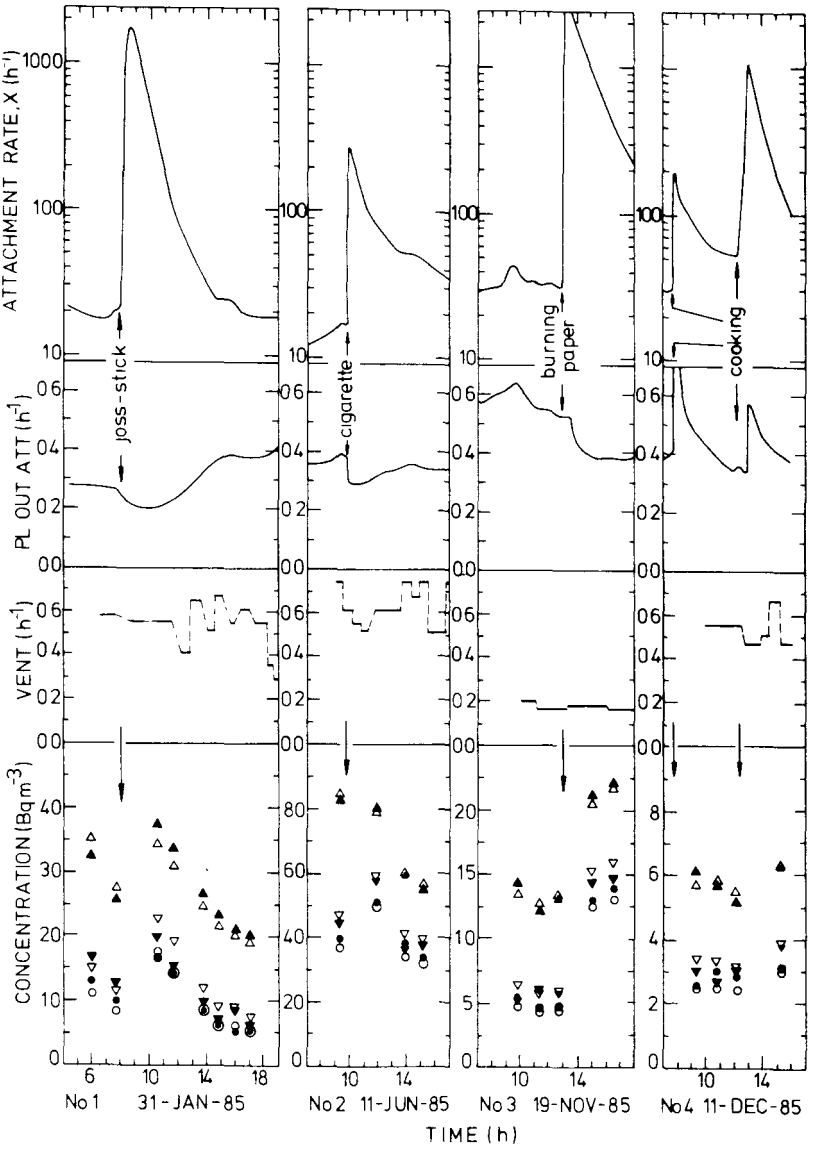

Fig.3. Evolution of the attachment rate, the deposition rate of the attached daughters, the ventilation rate and the radon daughter concentrations (▲ : ^{218}Po, ▼ : ^{214}Pb, ● ^{214}Bi measured, △ ▽ ○ fitted) during one day in each of the four locations.

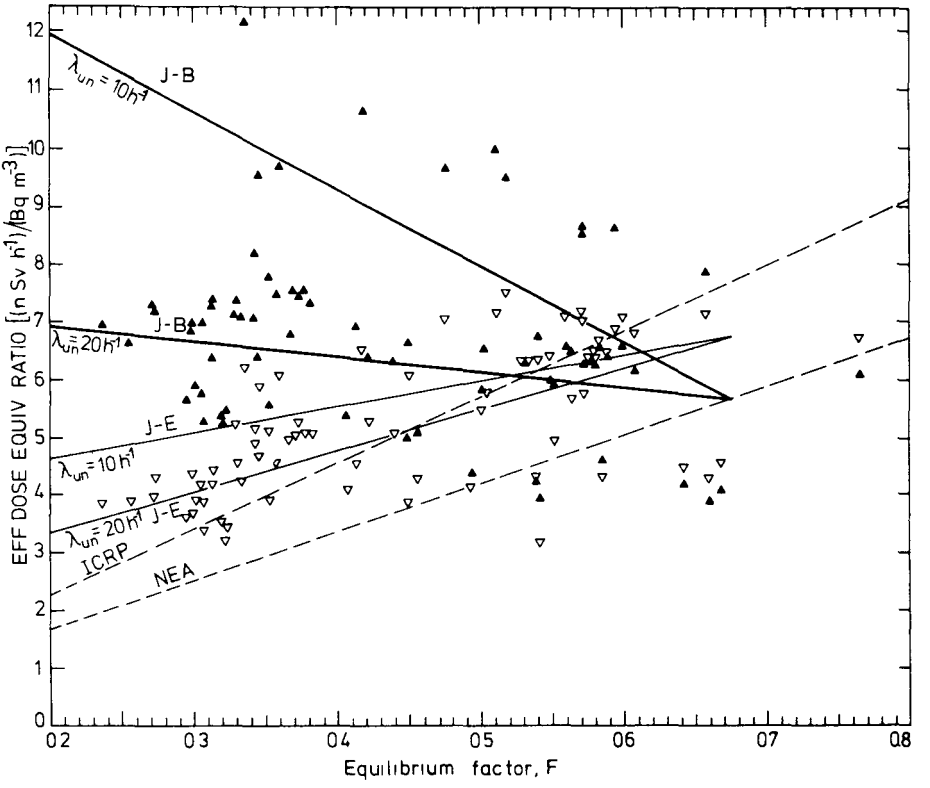

Fig.4. Effective dose equivalent per hour and per unit radon concentration (▲ J-B, ▽ J- E) as a function of the equilibrium factor. The full lines are calculated with the mean values of the 72 measurements.

concentration even decreases with an increasing equilibrium factor. Therefore it would be more adequate to introduce a constant conversion factor to the radon concentration.

Our analysis results in a conversion factor per unit radon concentration of 5.6 (nSv/h)/(Bq/m^3) or 50 (μSv/y)/(Bq/m^3). It is the mean value of the computed J-B and J-E doses (see Fig.4.). When we apply this conversion factor to the average radon concentration in Belgium (50 Bq/m^3) (Poffijn, 1985) and assume an occupancy factor of 80%, we obtain an average dose equivalent to the population of 2.0 mSv/y.

3. Comparison of methods for investigating indoor radon decay products (Vanmarcke, 1988)

An intercomparison with the university of Göttingen and the NRPB has been carried out to investigate the differences between our methodologies for determining the size distribution of the radon decay products. Twenty-one measurements were performed during three days in a house with radon concentrations ranging from 700 Bq/m^3 to 1200 Bq/m^3. The active and inactive size distributions were measured simultaneously with three screen diffusion batteries, with a Berner impactor and with a differential mobility particle sizer The aerosol concentrations were changed by burning a gas-stove, or by burning some candles, or by smouldering cigarettes.

Each participant performed radon and radon daughter measurements. Simultaneously, nearly continuous measurements of the ventilation rate were performed by means of the release of N_2O tracer gas and observation of its decay with an infrared spectrometer.

During the intercomparison, the ventilation rate in the room varied between 0.2 h^{-1} and 0 4 h^{-1}. The values noted during the night are at the high end, probably due to a greater difference between the outdoor and the indoor temperature

The unattached fraction was evaluated for each measurement. A value of about 10% was found without aerosol sources in the room . The unattached fraction decreased below 5% in the presence of aerosol sources. The different physical parameters of the radon decay products (attachment rate, deposition rate of the attached and unattached daughters, etc.) were also evaluated.

4. Uncertainties in decay product measurement arising from fluctuations in room parameters (Vanmarcke 1988a)

A model based on the Monte-Carlo technique 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 may be considered to consist of three parts.

First a realistic environment is simulated by varying the radon concentration and the parameters of the room model. Then the time evolution of the daughter concentrations is 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

In the last part of the model the deposition rate of the unattached daughters is fitted and compared to the input value

The fluctuations of the parameters of the room model increase the uncertainty on the measured daughter concentrations. In fig 5 all the parameters are varied simultaneously around their mean value. The standard deviation on the measured concentrations at zero variation (steady

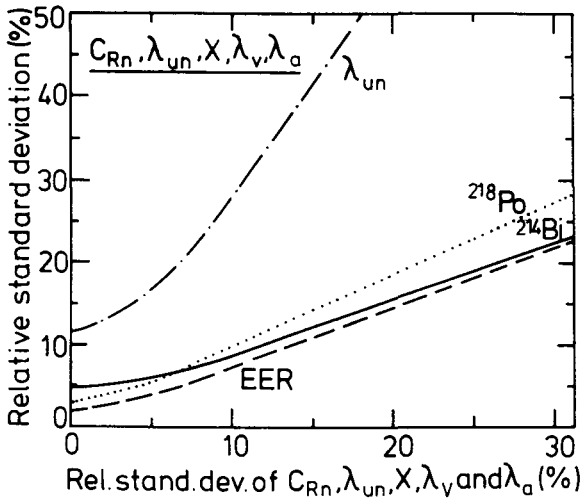

Fig.5. Relative standard deviation on the measured daughter concentrations and on the fitted deposition rate of the unattached daughters (λ_{un}) as a function of the random fluctuations on the radon concentrations ($<C_{Rn}> = 100 Bq/m^3$), the deposition rates of the attached ($<\lambda_a> = 0.2\ h^{-1}$) and unattached ($<\lambda_{un}> = 20\ h^{-1}$) daughters, the attachment rate ($<X> = 30\ h^{-1}$) and the ventilation rate ($<\lambda_V> = 0.4\ h^{-1}$).

state) is coming from counting statistics and from the variation on the flow rate (1%). It is seen that these errors are quickly dominated by errors induced by the random fluctuations of the parameters of the room model

The influence of a sudden change in the value of one or several parameters of the room model has also been investigated. Priority was given to realistic situations such as : smoking, cooking, opening or closing of a door or a window, etc.. It is shown that, without special precautions to keep a steady state situation in the room, the error easily exceeds 50%.

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Poffijn A., Marijns R., Vanmarcke H and Uyttenhove J (1985) "Results of a preliminary survey on radon in Belgium" The Science of the Tot Env 45 : 335-342

Vanmarcke H., Janssens A. and Raes F. (1985) "The equilibrium of attached and unattached radon daughters in the domestic environmet" The Science of the Tot Env. 45 : 251-260

Vanmarcke, H. (1987) "De bijdrage van het woonmilieu tot de blootstelling aan straling afkomstig van nucleiden uit de natuurlijke ^{238}U reeks" Doctoral Thesis, State University of Ghent

Vanmarke H., Janssens A., Raes F., Poffijn A., Berkvens P. and Van Dingenen R., (1987a) "The behaviour of radon daughters in the domestic environment Effect on the effective dose equivalent" ACS Symposium Series 331 : 301-323

Vanmarcke H., Reineking A., Postendörfer J. and Raes F., (1988) "Comparison of two methods for investigating indoor radon daughters" Rad Prot. Dos. 24 : 281-284

Vanmarcke H., Berkvens P., Poffijn A. and Raes F. (1988a) "Uncertainties in radon daughter measurement arising from fluctuations in room parameters" Rad Prot. Dos. 24 . 225-230

Vanmarcke H., Berkvens P. and Poffijn A (1989) "Radon versus radon daughters" Health Phys. 56 : 229-231

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

- Isotopenlabor der Georg-August-Universität, Göttingen
  (Dr.J.Postendörfer and Dr.A.Reineking, Burckhardtweg 2, Gottingen, F R G)
- National Radiological Protection Board
  (Dr.J.Miles and Dr.J Strong, Chilton, Didcot, Oxon OX11 ORQ, G B)
- Nuclear Research Centre SCK-CEN
  (Boeretang 200, 2400 Mol, Belgium)

V. Publications (1989) :

Vanmarcke H., Berkvens P. and Poffijn A. (1989) "Radon versus radon daughters" Health Phys. 56 229-231

Title of the project no. : 2

Study of the behaviour and nature of radon daughter ions and clusters

Head(s) of project:

Dr.H.Vanmarcke*

Scientific staff:

Dr.H.Vanmarcke*, Ir.C.Landsheere, Lic.R.Van Dingenen, Dr.F.Raes**, Lic.K.Van Laere

I. Objectives of the project:

Since the unattached radon daughters do not have a single and fixed size, the simple equilibrium model that uses a single deposition rate and attachment rate, may not be sufficient. It will be tried to work out a more detailed model based on the knowledge of radon daughter ions and clusters, gained from theory and laboratory experiments. In particular it will be investigated how charge and size of the radon daughters depend on the environmental conditions like humidity, tracer gases, aerosol loading and turbulence.

II. Objectives for the reporting period:

* Now at SCK-CEN Mol, Belgium

** Now at CCR Ispra, Italy

III. Progress achieved:

1 Numerical modelling of the size distribution of radon decay products in the indoor environment (Raes, 1985, 1987).

The numerical code AERO1A, discussed in the previous Annual Report has been extended to describe the size distributions of the three relevant short lived decay products of radon · ^{218}Po (RaA), ^{214}Pb (RaB), and ^{214}Po (RaC'). Most of the relevant processes were taken into account, i.e. the physical ones (ventilation, attachment, deposition of the attached and unattached fraction and recoil) as well as the physico-chemical ones (clustering and growth and neutralization of the free radioactive ions); patterns of air circulation and degrees of turbulence , which may also affect the deposition of airborne material, are not considered. Application of the model in some selected conditions showed that the active size distributions and the amount of airborne radioactivity is largely affected, not only by the aerosol loading of the atmosphere, but also by its chemical composition, as well as by the dielectric/conductive characteristics of the surfaces in the room. This is demonstrated in the figures 1 and 2, showing the size distributions of the non-active room aerosol and of the radon decay products. Two extreme conditions are considered . a steel room for laboratory conditions (fig.1) and a normal living room (fig.2), resulting in quite different size distributions. It was concluded that clustering and growth of the free radioactive ions are not too relevant in domestic environments

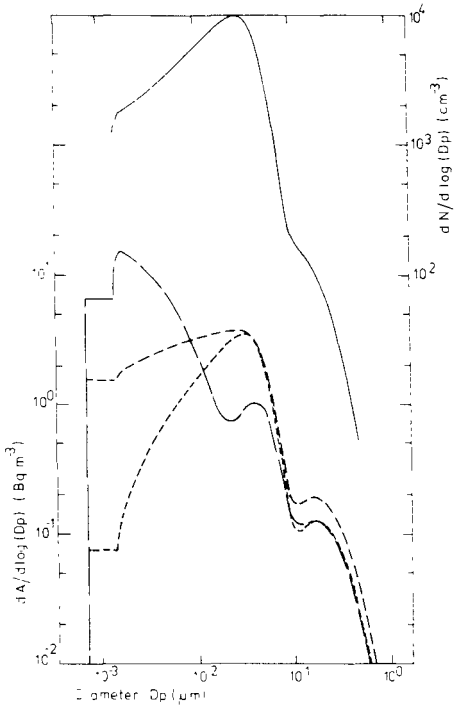

Fig.1. Size distributions in laboratory conditions (steel room). Aerosol size distribution (——) and activity size distributions for RaA (— —), RaB (— — — —) and RaC' (- - - - -) after 1 hour, starting with only radon (12 Bq m^{-3}) and an initial aerosol concentration of 100 cm^{-3}. The formation rate of H_2SO_4 = 4 10^5 molec $cm^{-3}s^{-1}$, the NO_2 concentration = 0 ; the room has conductive walls, a surface to volume ratio of 0.02 cm^{-1} and a ventilation rate of 3 10^{-4} s^{-1}.

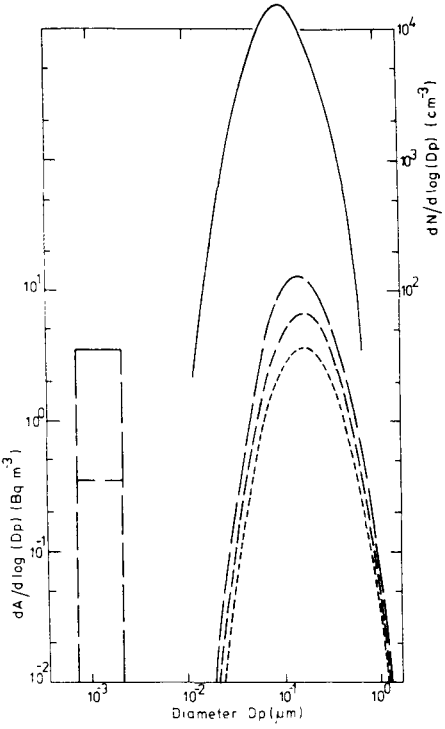

Fig.2. Size distributions in a domestic environment starting with only radon (12 Bq m^{-3}) and an initial aerosol concentration of 10^4 cm^{-3}. The H_2SO_4 formation rate is 10^5 molec cm^{-3} s^{-1}, the NO_2 concentration is 10^{11} cm^{-3} ; the room has dielectric walls, S/V = 0.02 cm^{-1} and a ventilation rate of 3 10^{-4} s^{-1}.

because of important competitive removal processes like neutralization by charge transfer and/or attachment and/or deposition by electrophoresis. On the other hand the charge properties of the radon decay products largely determine the amount of airborne activity in the domestic environment.

Our model calculations give a better understanding of the many factors that determine the behaviour of radon decay products, and they explain why such a large range of values is being found of diffusion coefficients of the unattached fractions, of equilibrium constants, plate out rates etc.

2. Deposition of gases and aerosols in different chambers (Van Dingenen, 1987, 1989 ; Holub, 1989)

Aerosol wall losses in a spherical batch reactor (2301) were investigated experimentally. The experiments were performed with monodisperse NaCl aerosol having a diameter between 0.02 and 0.2 μm.

Effects of irradiating the chamber and of initial particle charge distribution were investigated.

It was found that the input-charge distribution of the particles (neutral, singly charged or Fuchs-Boltzmann equilibrium) did not influence the deposition rate, which can only be explained by a small or zero electric field. In that case the time constant for establishing a charge equilibrium over the aerosol is small compared to the time constant for deposition of the charged particles

Time-dependent calculations showed that the observed deposition rates could not be explained by the presence of an electric field Therefore, the general deposition formula of Crump and Seinfeld (1981) was used Here, no electric field is taken into account and the eddy-diffusivity is defined by $D_e = k_e x^n$, where k_e is a measure for the turbulence in the reactor and x is the distance from the wall. For n, values between 2 and 3 are found in literature.

Fig 3 illustrates the error which may occur in the assessment of gas-deposition constants when the wrong value for n is adopted, based on aerosol experiments only. It highlights the necessity of supplementing the aerosol measurements with data for (100% sticking) gaseous

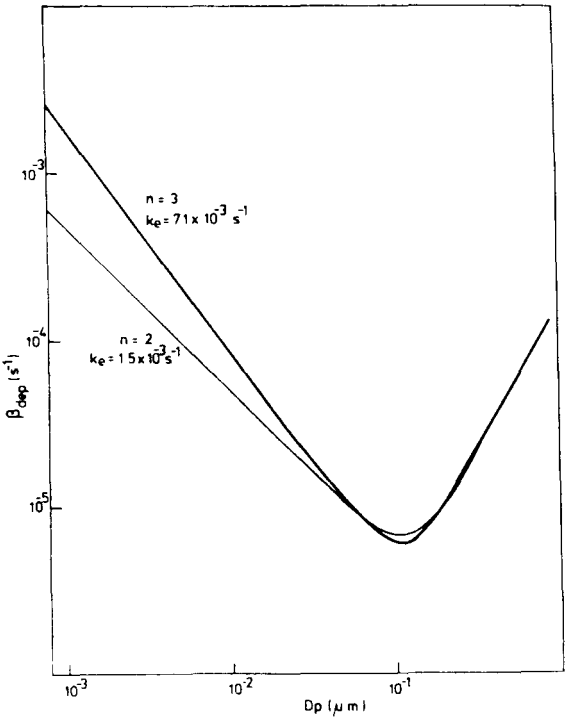

Fig.3. Theoretical deposition constant calculated with Crumps'theory for n = 2 and n = 3, k_e is taken in order to obtain a good agreement between the curves in the "aerosol region".

components which are also important for the modelling of aerosol formation in smog chambers With this purpose, a new set of experiments on gas deposition was performed. The deposition constant of gaseous components was determined by measuring the plate-out activity of ^{218}Po on a well defined glass surface in the same vessel.

Finally, the best fit to all measurements (gas + aerosol) was obtained by using Crump and Seinfeld's theory with k_e and n as adjustable parameters. We found $k_e = 3.46\ 10^{-2}$ and n = 2.6. This value for n is very close to the value of n = 2.7, found by Okuyama et al. (1985) in a stirred reactor. Afterwards,our result was confirmed by similar experiments performed by the Bureau of Mines, USA (Holub, 1988). Deposition of aerosol particles and unattached Rn daughters in a $4m^3$ cylindrical chamber, stirred with propellers, was perfectly explained by Crumps' theory with n = 2.6. It may be concluded that Crumps' theory with n = 2.6 is capable to explain particle deposition over almost three orders of magnitude with respect to size as well as to turbulence. Our final result, together with Holubs' is presented in fig 4.

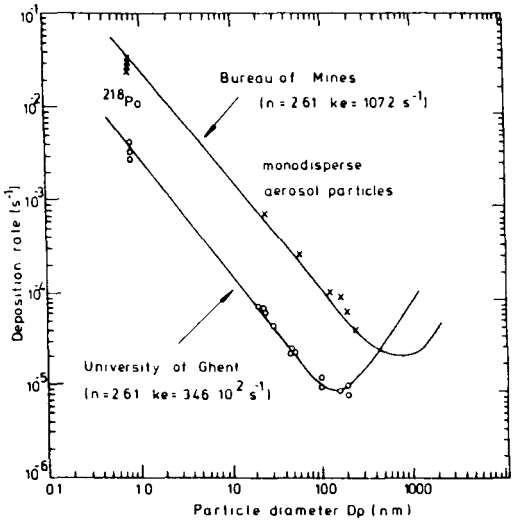

Fig.4. Gas and aerosol deposition constant, experimental results (0) and Crump & Seinfelds'theory fitted to the experimental values.

3. Influence of the degree of turbulence on the deposition rate constant of the unattached decay products of radon.

The values of the deposition rate constant of the unattached radon decay products reported in literature, vary over two orders of magnitude. In order to classify some of this variability we

investigated the influence of the degree of turbulence on the deposition rate constant of the unattached daughters The experiments were carried out in our 1 m^3 radon chamber. The turbulence in the chamber was induced by closed circuit ventilation and/or by generating heat The aerosol concentration was less than 10 particles/cm^3, so that the attached fraction could be neglected. The deposition rate constants were derived from the radon concentration and the three decay product concentrations. The values for ^{218}Po were found to be about three times the size of the values for ^{214}Pb, which means that the associated diameter of the ultrafine ^{214}Pb particle is about twice as large as the diameter of the ultrafine ^{218}Po particle.

We applied a modified form of the formula of Crump and Seinfeld (1981) to compute the corresponding diffusion coefficients. The results in the case of closed circuit ventilation are shown in fig.5 as a function of the lifetime of the ^{218}Po isotope and the ^{214}Pb isotope in the radon chamber. We expect the diffusion coefficient to decrease with increasing lifetime. However, we only observe a difference between the two nuclides. The horizontal lines in the figure represent the weighted mean values of the experimental points. The differences in diffusion coefficient could be due to the chemical properties of the two elements.

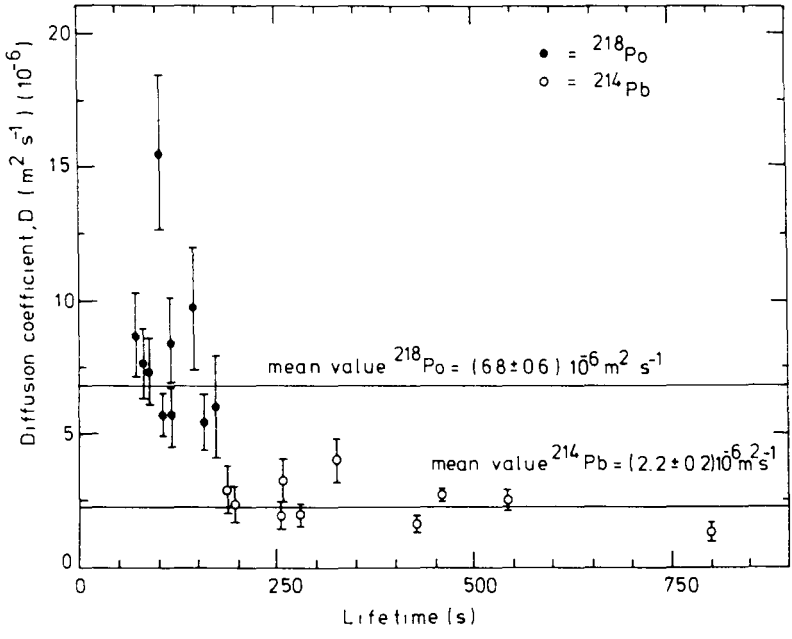

Fig.5. The diffusion coefficient of ^{218}Po and ^{214}Pb as a function of the lifetime of ^{218}Po and ^{214}Pb in the chamber. A diffusion coefficient for ^{218}Po of 7.10^{-6} m^2s^{-1} is assumed in the calculation of the k_e values with the theory of Crump and Seinfeld.

The models for predicting the concentrations of the decay products in the indoor environment assume the deposition rate constants of ^{218}Po and ^{214}Pb to be equal. However a significant lower deposition rate constant for ^{214}Pb would explain some of the difficulties we found in fitting the room model to the data we collected during our case studies in houses. Indeed, when we apply the deposition rate constant of the unattached ^{214}Pb to the unattached ^{218}Po we systematically underestimate the radon concentrations.

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Van Dingenen R., Raes F. and Vanmarcke H. (1989) "Molecule and aerosol particle wall losses in smog chambers made of glass" J. Aerosol Sci. 20, 113-122.

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- Lund University Hospital, Department of Radiation Physics
  (Dr.C.Samuelsson, S-22185, Lund, Sweden)
- Nuclear Research' Centre SCK-CEN
  (Boeretang 200, 2400 Mol, Belgium)

V. Publications (1989):

Van Dingenen R., Raes F. and Vanmarcke H. (1989) "Molecule and aerosol particle wall losses in smog chambers made of glass" J.Aerosol Sci. 20, 113-122.

Title of the project no. : 3

Investigation of the radon exhalation from building materials and soils

Head(s) of project :

Dr.J.Uyttenhove

Scientific staff :

Dr J.Uyttenhove, Dr.H Vanmarcke*, Dr A Poffijn, Dr.Ir P.Berkvens**, Lic K Van Laere

I. Objectives of the project :

The main objective is to improve our knowledge of the radon source term and exhalation in Belgian houses This is accomplished by a nation-wide survey, together with specific surveys to identify correlations with various parameters

The contribution of radon and thoron exhalation from building materials will be investigated A thorough theoretical treatment of the radon transport mechanism will accompany these investigations.

II. Objectives for the reporting period :

* Now at SCK-CEN Mol, Belgium

** Now at ESRF Grenoble, France

III. Progress achieved :

1. Radon exhalation from building materials (Berkvens, 1988 ; Poffijn, 1988).

The knowledge of radon exhalation rates for building materials is of fundamental importance in the assessment of possible radon daughters concentrations in dwellings. Measurements of exhalation rates are usually performed using small samples, often in closed can set-ups. Since these laboratory conditions are very different from the actual walls, we solved the mathematical problem of three-dimensional steady-state radon diffusion in rectangular samples, and established the relationship between exhalation from such samples and the one-dimensional exhalation from walls. This implies that one has to solve the well known differential equation, in three dimensions, with the appropriate boundary conditions. The analytical solution of the problem is found by means of a triple fourier series. Fig.1 gives a typical bound exhalation concentration profile thus obtained.

Of more importance however is the exhalation rate. The results obtained from an exhalation measurement on a sample can be interpreted directly in terms of radon exhalation from walls (the latter being a one-dimensional process) only if the calculated one-dimensional exhalation rate from the sample agrees with the three-dimensional experimental results. In fig.2 this ratio between one- and three-dimensional exhalation rates is shown, and the discrepancy with increasing thickness is obvious. The exact analytical three-dimensional solution is however very tedious, and therefore we introduced a modified one-dimensional formalism. It is evident that the difference between one- and three dimensional results with increasing sample-thickness is due to the fact that in the one-dimensional formalism all radon has to exhale through the $x=\pm l_x$ planes, whereas in reality for an increasing part of the sample the radon diffuses to another more adjacent plane. Therefore we defined an equivalent sample geometry with a reduced thickness to take this effect into account, and then applied a pure one-dimensional model to this reshaped sample. It is evident that this reduced thickness l'_x will be chosen as four times the mean value over the sample volume of the closest distance from a point in the sample to the surface.
The exhalation E1dim , which will occur when using the material in a wall of the same thickness as the measured sample is then given by.

$$\frac{E_{3dim}}{E_{1dim}} = \frac{l'_x}{l_x} \frac{\tanh(l_x/L)}{\tanh(l'_x/L)}$$

with E_{3dim} the measured exhalation from the sample, and L the diffusion length.
As can be seen from fig 2 this modified one-dimensionsal formalism gives very accurate results, and allows for the interpretation of exhalation measurements on samples with geometries that can no longer be considered as one-dimensional.

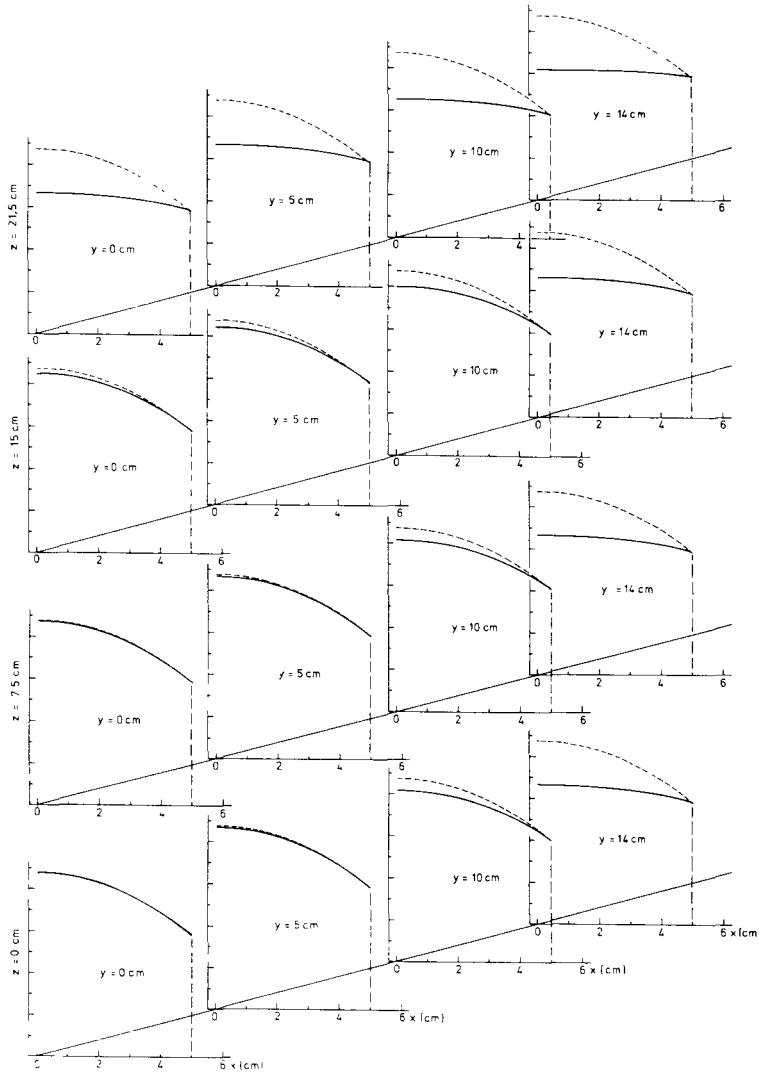

Fig. 1. Three dimensional radon concentration profile in rectangular sample.

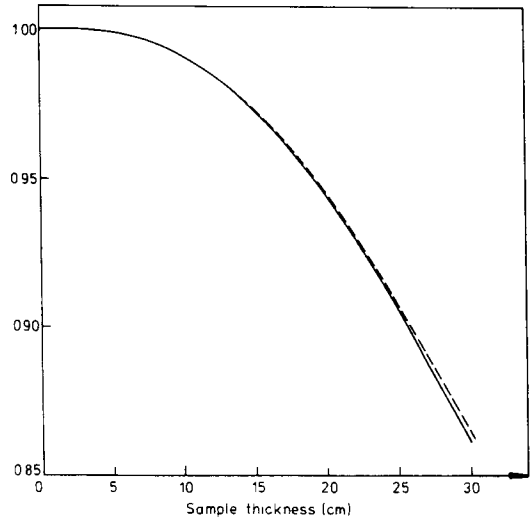

Fig.2. Ratio between one- and three-dimensional exhalation rates (full line) and ratio between one-dimensional and modified one-dimensional exhalation rates (dashed line).

2. National survey (Poffijn, 1985, 1989)

In collaboration with the Belgian Building Research Institute (BBRI), a national survey was organised in 290 dwellings. The dwellings are representative for the population distribution and for the type of building material. They were monitored during six-month periods. One detector was placed in the living room and in half of the houses a second detector was placed in a bedroom. The average radon concentration was found to be 48 Bq/m^3, which is not significantly different from the 53 Bq/m^3 found in our pilot study.

Our national survey indicated that the average radon concentration in dwellings in the geological Ardennes, including the Condroz and the Entre-Sambre-et-Meuse (zone II in fig.3) is much higher than in the rest of the country (zone I). Applying our dose conversion factor of "20 Bq/m^3 radon concentration = 1 mSv/y" and assuming an occupancy factor of 80%, results in an average dose equivalent to the population of 3.05 mSv/y (see fig.3) (Vanmarcke, 1989). The other sources of radiation, including corrected cosmic exposure and population averaged exposure of patients to medical radiation are estimated at 1.45 mSv/y. In the rest of the country the average exposure is estimated at 3 8 mSv/y.

Not shown in fig.3 is the range of doses within the population. The variation between extremes is for radon higher than for any other natural source of radiation. On the basis of our

national survey it is estimated that 5 to 15% of the dwellings in zone II exceeds 150 Bq/m^3 or 6.0 mSv/y, the lowest action level in the U S. Statistical considerations indicate that there are hundreds of houses where the average dose equivalent is higher than the occupational limit of 50 mSv/y (1250 Bq/m^3, 80% occupancy factor).

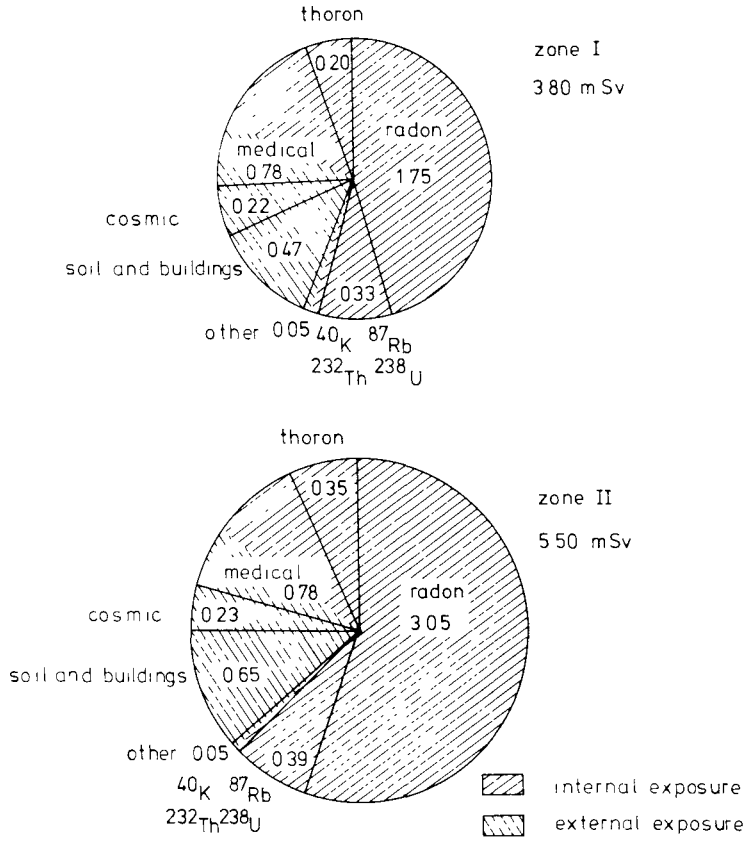

Fig.3. Average effective dose equivalent to the population in the two geological zone's of Belgium. Zone II : Ardennes, Condroz and Entre-Sambre-et-Meuse. Zone I : rest of the country.

3. Local surveys (IWONL, 1989)

Infiltration of radon from the ground is suspected to be an important source of radon in houses. Evidence about this was found by means of a case - control study. In 51 houses built on or close to an uranium anomaly a radon detector was placed in the living room and in about half of the houses a second detector was placed in the cellar. The houses were monitored for one year. Forty-nine similar houses in a neighbouring village were monitored in the same way. The seasonal averages are presented in fig.4. The radon concentration in the houses on or close to the anomaly

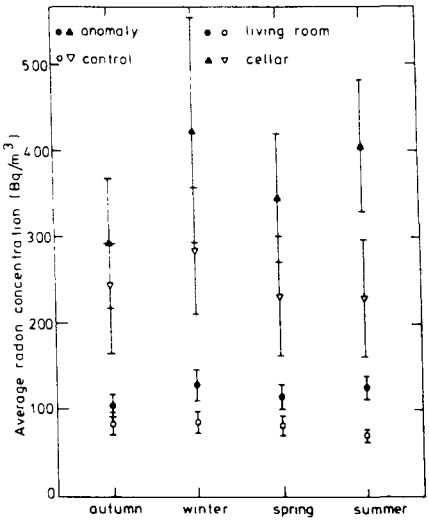

Fig.4. Seasonal averaged radon concentration in the living room and in the cellar of the houses build on or close to the uranium anomaly and in the control houses.

is significantly higher than in the control houses. In about 30% of these houses the radon concentration exceeds 150 Bq/m^3, so that the area around the uranium anomaly has to be classified as a high risk area. Houses built in this area should be designed "radon-safe".

A second survey has been carried out in a municipality where high radon concentrations are expected because of the geological properties of the earth. In 54 houses, selected ad random out of the telephone book, a radon detector was placed in the living room and if possible a second detector was placed in the cellar. The houses were monitored for one year.

The seasonal averaged radon concentrations in the living rooms vary between 30 and 4000 Bq/m^3 and between 35 and 14500 Bq/m^3 in the cellars. The radon concentration is in 10% of the living rooms in excess of 150 Bq/m^3.

We measured the radon concentrations in three houses close to the house with 4000 Bq/m^3 in the living room, which corresponds to an effective dose equivalent of 160 mSv/y (occupancy factor 80%) We found average concentrations of 3400 ± 200 Bq/m^3, 360 ± 20 Bq/m^3 and 165 ± 15 Bq/m^3 respectively, so that the highest value of our ad random study is not an isolated case.
Mitigation measures, which mainly consist of a sub-floor ventilation system, are installed in the houses with a high radon concentration. The installations are paid for by the municipality The first results show a marked decrease in radon concentration (less than 20% left).

4. ^{222}Rn and ^{220}Rn exhalation of phosphogypsum (IWONL, 1989)

New types of phosphogypsum are sold as building material in Belgium because industry has switched over from Maroccan phosphate ore to South African ore. The radioactive properties of two of these new types of phosphogypsum have been tested. From each type 30 plates of 0.295x0.210 m by 0.005 m and 30 plates of 0 295x0.210 m by 0 020 m were made. Three properties were assessed.

1 The specific ^{226}Ra and ^{232}Th activities were measured by means of high resolution gamma spectroscopy :

type1 = ^{226}Ra : 75 ± 5 Bqkg^{-1}; ^{232}Th : 230 ± 20 Bqkg^{-1}

type2 = ^{226}Ra : 155 ± 10 Bqkg^{-1}; ^{232}Th : 160 ± 15 Bqkg^{-1}

The thorium activity of the new types of phosphogypsum is much higher than the thorium activity of the phosphogypsum of Maroccan origin For the radium activity it is just the reverse.

2. The ^{222}Rn exhalation rate was evaluated by closing the plates in airtight barrels and measuring the equilibrium concentration by means of the Lucas method :

type1 : 12 Bq kg^{-1} s^{-1}

type2 47 Bq kg^{-1} s^{-1}

3. The ^{220}Rn exhalation rate of the plates was determined by means of the ^{220}Rn decay product concentration in an airtight 1 m^3 chamber The chamber was ventilated in a controlled way with outside air, in order to obtain sufficient aerosol particles inside the chamber Each sample was measured 3 to 4 times. The reproducibility of the equilibrium equivalent thoron concentration is about 25%, which is almost entirely due to fluctuations in the aerosol concentration in the chamber. The thoron exhalation is, as expected from the short half-live of ^{220}Rn, independent of the thickness of the samples.

The effect of covering the entire surface of the plates with two layers of a common Latex paint was also investigated. The thoron exhalation after painting was 10 to 20 times lower.

In order to evaluate the ^{220}Rn exhalation rates of the phosphogypsum plates, we converted the radon chamber results using the following "realistic" assumptions :

- room dimensions : 4x5x3 m (60 m^3),
- ventilation rate : 0.5 h^{-1},
- walls and ceiling covered with the new types of phosphogypsum (74 m^2),
- occupancy factor : 80%.

The effective dose equivalent received by an occupant due to the thoron exhalation of uncovered gypsum is 1.8 ± 0.4 mSv/y for type 1 and 0.9 ± 0.2 mSv/y for type 2. Such a high dose, originating from one building material, can not be brought into agreement with the ALARA principle. Painting the gypsum, however, lowers the ^{220}Rn dose by a factor of 10 to 20.

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Vanmarcke H., Berkvens P. and Poffijn A. (1989) "Radon versus radon daughters" Health Phys. 56 : 229-231.

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

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  (Ir P Wauters, Ir.G.Demets, Av. Pierre Holoffe 21, 1342 Limelette, Belgium)
- Faculté Polytechnique de Mons, Département de Mines
  (Prof.Charlet, Rue de Houdain 9, 7000 Mons, Belgium)

V. Publications (1989) :

Poffijn A. and Vanmarcke H. (1989) "Indoor radon in Belgium" Proc of the conf. on Present and Future of Indoor Air Quality, 14-16 februari, Brussels.

Poffijn A., Mak R., Vanhoorne M , Weynants P. and Prignot Y. (1989) "Radon and lung cancer : a case-control study in southern Belgium" to be published in the Proc. of the conf in Present and Future of Indoor Air Quality, 14-16 februari, Brussels

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Netherlands Institute for
Sea Research (NIOZ)
P.O. Box 59
NL-1790 AB Den Burg Texel

Contract no.: BI6-F-328-NL

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

Prof. E.K. Duursma
NIOZ
Netherlands Inst. for Sea Research
P.O. Box 59
NL-1790 AB Den Burg Texel

Telephone number: 02226-541

Title of the research contract:

Research on processes controlling the regional distribution of Po-210, Pb-210, Ra-226, Pa-231 and Thorium isotopes in estuaries.

List of projects:

1. Research on processes controlling the regional distribution of Po-210, Pb-210, Ra-226, Pa-231 and Thorium isotopes in estuaries.

Title of the project no.:
Research on processes controlling the regional distribution of ^{210}Po, ^{210}Pb, ^{226}Ra, ^{231}Pa and Thorium isotopes in estuaries.

Heads of the project:
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Dr. D. Eisma
Ing. G. W. Berger
Address: PO Box 59, 1790 AB Den Burg, Texel, The Netherlands

Scientific staff:
Dr. D. Eisma
Ing. G. W. Berger

Objectives of the project:

The aim of the project was to get insight into the radiological impact of the releases of non-nuclear industries with the emphasis on the behaviour of the radionuclides in the estuaries of the main European rivers. Special attention was given to the distribution and behaviour of the radionuclides related to the release and disposal of phosphogypsum. This project served as a pilot study in the Dutch New Waterway and the Rhine estuary in the framework of an European wide investigation program to be carried out from 1990 and on.

Objectives for the reporting period:

The objectives for the reporting period were
-to develop and carry out a sampling scheme in the New Waterway and its estuary. This should be done in such a way that the pathways, distribution and behaviour of the radionuclides of interest could be modelled.
-to gain insight into the radiological consequences on the food chain to man
-to aquire knowledge about the impact on the quality of bottom sediments in river, harbours, estuary and the marine environment.

III. Progress achieved:

Author: G. W. Berger, Neth. Inst. for Sea Res., Texel

1. Methodology:

General approach: The area of interest was seen as to a "box", and divided in small compartments to get insight in the sources, dynamics of distribution and budgets of the radionuclides involved.

Shipboard procedures, sampling: for the sampling of sediments a 30 kg Van Veen grab sampler was used. The sediment sample obtained this way was mixed and subsampled. For the sampling of water and suspended matter a 30 liter Niskin bottle was used. To obtain suspended matter the water was filtered (10 to 20 liter), using 145 mm diameter, .45 μm pore size filters. The filtered water was used to determine the concentrations of dissolved ^{226}Ra, $^{210}Po/^{210}Pb$ and Th isotopes.

Analytical procedures: The methods of analyses are based on the standard analytical technics used in geochemistry and oceanography and will be described briefly:
Sediment samples and filters containing the suspended material were dissolved in a mixture of concentrated acids in teflon bombs. The solution was evaporated. For ^{210}Po analyses, the residue was redissolved in 1 M HCl and the Po was plated on a silver disc and analyzed using alpha spectroscopy (Bacon et al.,1976, Berger et al., 1987).
A selection of the plating solutions was stored for ingrowth of a new generation ^{210}Po to estimate the amount of ^{210}Pb.
Dissolved ^{210}Po was analyzed using the Cobaltnitrate/APDC method as described by Bacon et al.(1976).
^{226}Ra in dissolved form was analyzed by draining the filtered water through a column filled with MnO_2 coated acrylic fibre and measuring the amount of ^{222}Rn, generated in a closed system via scintillation counting (Moore, 1976, Mathieu et al., 1980, Berger et al, 1988).
^{226}Ra in sediments or suspended material was analyzed by measuring the generated ^{222}Rn in a closed system after dissolution of the samples.

Two sampling cruises were carried out. During the first sampling cruise (February 1989) the aim was to take sediment samples in such a way that each compartment could be described regarding natural and increased concentrations of the radionuclides of interest.
Besides, during the first cruise water- and suspended material were sampled. Special attention was given to the location of the sources of discharges of radionuclides.
The second cruise (August 1989) was used to take water samples along the North Sea coast, to do some additional sediment sampling based on the results of the first cruise

and to study the behaviour and the dynamics of the distribution of the isotopes in the estuary: what is leaving the box and in which form.
During this cruise an anchoring station was occupied in the mouth of the estuary (km 1029, Hoek van Holland) over one tidal cycle. Vertical profiles of suspended matter concentrations, salinity, temperature, water current velocity and current direction were measured in combination with water- and suspended matter sampling.
More than 200 sediment samples and 125 water- and suspended matter samples were taken.
Fig. 1 shows a map of the New Waterway with the location of the phosphate processing plants and the anchoring station near Hoek van Holland.

2. Results

Sediments

^{210}Po-concentrations in sediments collected in the New Waterway, harbour basins and in the Rhine estuary range from 8 to more than 3000 Bq kg^{-1}. The highest concentrations are found in the immidiate vicinity (< 50 m) of the discharge pipes of the two phosphate processing plants: between 400 and 1000 Bq kg^{-1} on the North bank of the New Waterway and between 500 and 3400 Bq kg^{-1} on the South bank.
The harbour basins in the neighborhood of the plants show high concentrations of ^{210}Po: up to 1000 Bq kg^{-1} in the 1^{e} Petroleum harbour (< 1 km from the phosphate processing plants) and around 400 Bq kg^{-1} in the harbours at a distance of up to 6 km from the discharge pipes of the plants.

^{226}Ra in sediment samples are about 30 Bq kg^{-1} upstream, 2700 Bq kg-1 in front of the discharge pipes and around 200 Bq kg^{-1} in the harbour basins near the plants.

^{210}Po concentrations adsorbed on suspended material are in the range from 37 Bq kg^{-1} (twenty km upstream the phosphate plants) to 12000 Bq kg^{-1} (close to the discharge pipe of the phosphate plant on the North bank). ^{210}Po concentrations adsorbed on suspended material downstream of the plants in fresh surface water range from 400 to 2000 Bq kg^{-1}.
^{226}Ra concentrations adsorbed on suspended material are 97 (upstream) to 1800 Bq kg^{-1} close to the factories. Downstream of the plants ^{226}Ra concentrations of several hundreds of Bq kg^{-1} are common.

^{210}Po concentrations dissolved in water range from .4 Bq m^{-3} (upstream and in the North Sea) to 32 Bq m^{-3} close to the discharge pipes. In the river downstream of the phosphate plants up to the mouth of the estuary the concentration in the surface water is around 1.5 Bq m^{-3}.

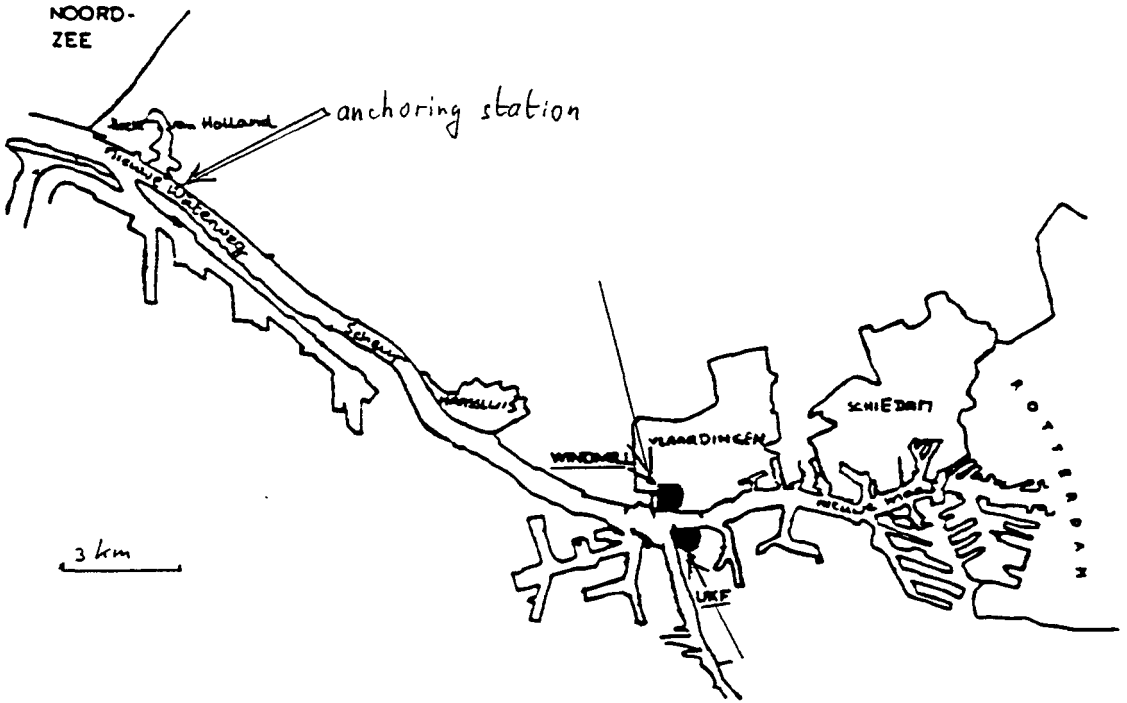

Fig. 1. The New Waterway. Indicated are the location of the anchoring station and the two phosphate processing plants.

^{226}Ra concentrations dissolved in water range from around 5 Bq m^{-3} (upstream and North Sea water) to more than 500 Bq m^{-3} in front of the discharge pipes. Values of around 20 Bq m^{-3} are common in the surface river water downstream of the phosphate plants.
Current measurements: Vertical profiles of water current velocity and direction were measured in combination with salinity over one tidal cycle. The location of this station was near Hoek van Holland, km 1029, in the mouth of the estuary. In combination with these measurements water- and suspended material samples were taken.
The hydrographic profiles at the anchoring station over one tidal cycle show the following:
The topmost few (3) meters of water have a salinity gradient from 4 to 18 promille, while the bottom water show salinities between 27 and 30 promille. The concentration of ^{210}Po adsorbed on suspended material in the surface layer is between 500 and 1700 Bq kg^{-1} (average value 1000 Bq kg^{-1}) while the concentration in the bottom layer is between 200 and 500 Bq kg^{-1} (average value 300 Bq kg^{-1}). There is a clear relationship between salinity and ^{210}Po adsorbed on suspended material.

Samples taken along the North Sea coast: ^{210}Po concentrations in suspended matter north of the New Waterway along the coast are between 290 and 170 Bq kg^{-1}. There is a clear relationship with salinity. This seems the result of simple mixing: the water in this area consists for approximately 10% of Rhine water in which the ^{210}Po concentrations are about an order of magnitude higher. South of the mouth of the New Waterway the concentration is around 70 Bq kg^{-1}, in the Central North Sea around 50 Bq kg^{-1}, in the Wadden Sea 140 Bq kg^{-1}, in the German Bight 150 Bq kg^{-1}.

The data obtained in this environmental study may form the basis for a study on the radiological impact on the food chain to man in the freshwater- as well as in the marine environment.

3. Discussion and conclusion

In the reporting period we studied the behaviour and dynamics of the distribution of radionuclides released by phosphate processing plants in the New Waterway.
Although not all of the samples taken in the reporting period have been analyzed or interpreted, some conclusions can be drawn.
The concentrations of ^{210}Po and ^{226}Ra in sediments, suspended matter and dissolved in water, as found in front of and in the immidiate vicinity of two phosphate processing plants situated between km 1012 and 1014 in the New Waterway

clearly indicate the source of the increased concentrations. The harbour basins in the vicinity of these plants acts as a sink for the isotopes released.
The lowest values of ^{210}Po and ^{226}Ra in sediments, adsorbed on suspended matter and dissolved in water, are found upstream of the phosphate processing plants. These values we assume to be the "natural background" to calculate the amount of excess ^{210}Po and ^{226}Ra. For ^{210}Po this is 40 Bq kg^{-1} in sediment, 50 Bq kg^{-1} in suspended matter and .5 Bq m^{-3} dissolved. For ^{226}Ra these values are 30 Bq kg^{-1}, 100 Bq kg^{-1} and 5 Bq m^{-3}, respectivily.
The phosphate processing plants in the New Waterway yearly discharge 1600 GBq ^{210}Po (Deeladvies comm. van de Gezondheidsraad, 1987) into the New Waterway. If we assume that 1.7×10^{9} kg a^{-1} suspended matter leaves the estuary per year (Eisma, 1981) and the average concentration of ^{210}Po excess is 500 Bq kg^{-1} (this study), 1000 GBq ^{210}Po per year is being transported to the North Sea adsorbed on suspended material.
The total amount of ^{210}Po excess, adsorbed on sediments in the area at a distance of less than 6 km from the phosphate processing plant is about 1000 GBq.
In the area with enlevelled ^{210}Po concentrations, dredging activities remove about 400.000 m^3 sediment per year (data provided by the Council of Rotterdam). If we assume a dry sediment weight of 400 kg per m^3 and an average concentration of 400 Bq kg^{-1}, this means that yearly 80 GBq ^{210}Po a^{-1} will be removed this way.
Dissolved in water 60 GBq ^{210}Po and 600 GBq ^{226}Ra is transported to the North Sea in dissolved form, with the assumptions that the anual discharge of the Rhine is $6*10^{10}$ m^3 (van Bennekom, 1975) with an average ^{210}Po excess dissolved in water is 1 Bq m^{-3} and 10 Bq m^{-3} ^{226}Ra (this study).

The amount of ^{210}Po adsorbed on suspended material is in general 50% higher than the ^{226}Ra concentration. In sediments, the ^{210}Po concentration is two times higher than the ^{226}Ra concentration. Dissolved in water, the Ra concentration is about ten times higher than the ^{210}Po concentration.

In the reported period most attention was paid to the analyses of ^{210}Po. The reason is that if one wants to study Po/Pb equilibria it is important that the time between sampling and analyses of ^{210}Po is as short as possible. Besides, the period required for ingrowth of a new generation of ^{210}Po is at least six months. Only part of the samples will be analysed for Th- and ^{231}Pa isotopes. The selection of these samples will be based on the ^{210}Po data.

The modelling of the data and the radiological impact study

will be performed in cooperation with the RIVM, Bilthoven, The Netherlands.

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IV. Other research groups collaborating activily on this project:

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3720 BA Bilthoven
The Netherlands

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Commissariat à l'Energie
Atomique, CEA
B.P. n° 6
F-92265 Fontenay-aux-Roses

Contract no.: BI6-F-332-F

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

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CEA-CEN de Fontenay-aux-Roses
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Telephone number. (1) 4654.75.41

Title of the research contract.

Mise au point d'un banc étalon de radon 222 et de ses descendants à vie courte dans l'air.

List of projects:

1. Mise au point d'un banc étalon de radon 222 et de ses descendants à vie courte dans l'air

Title of the project no.: 1

Mise au point d'un banc étalon de radon 222 et de ses descendants à vie courte dans l'air.

Head(s) of project:

J. CHARUAU

Scientific staff.

J. CHARUAU - M. AMMERICH - G. CHUITON - M. GUELIN

I. Objectives of the project:

L'instrumentation développée actuellement dans le monde destinée à la mesure du radon 222 et/ou de ses descendants à vie courte dans l'air, en particulier dans les habitations, est très diverse. Cependant, l'étalonnage de ces systèmes de mesure est un problème encore mal résolu, cela étant dû à l'absence de source étalon de radon 222 dans l'air. Ceci explique les nombreuses campagnes d'intercomparaison.

Le projet comporte deux aspects :

- qualification d'une source de radon 222 dans l'air susceptible d'être adoptée comme source étalon, et d'un nouveau dispositif qui réalise des mesures de référence de ce gaz,
- adaptation d'une installation de calibration en aérosols radioactifs "ICARE", existant au CEA-Saclay afin de pouvoir disposer d'activités volumiques bien étalonnées en radon 222 et ses descendants à vie courte dans le but de calibrer les instruments de mesure.

II. Objectives for the reporting period.

Durant l'année 1989, les objectifs du projet étaient de :

- concevoir l'adaptation d'un nouveau périphérique sur le banc d'essais "ICARE", permettant de calibrer en fonctionnement dynamique les instruments de mesure du radon dans une gamme d'activité volumique étendue et réaliste (de 4 à 4 000 Bq/m^3 environ),
- réaliser et qualifier des sources, considérées comme étalon, de radon 222 et des dispositifs de mesure de référence de ce gaz,
- réaliser un circuit aéraulique comportant ces nouvelles sources, raccordé sur l'installation ICARE.

L'équipement complémentaire du banc d'étalonnage permettant de produire et de mesurer les descendants du radon 222, ainsi que la chambre de test des instruments, font l'objet d'un contrat pour 1990.

III. Progress achieved:

Le banc d'étalonnage d'instruments de mesure du radon 222 et de ses produits de filiation à vie courte dans l'air doit être conçu de manière à s'adapter à un banc d'essais rendu récemment opérationnel dans un laboratoire de haute activité du centre CEA de Saclay /1/. Il s'agit d'une Installation de Calibration à l'aide d'Aérosols Radioactifs Etalons (ICARE). Elle fut conçue pour qualifier en situation réelle les performances des capteurs de la contamination atmosphérique par des aérosols radioactifs artificiels alpha ou bêta, et notamment des moniteurs d'alarme utilisés pour les laboratoires, usines, réacteurs nucléaires, environnement.

Dans ICARE les capteurs sont soumis à des aérosols de chlorure de césium marqués avec du plutonium 239 (alpha) ou du césium 137 (bêta). L'activité volumique peut varier de 0,08 à 12 Bq/m^3 pour le Pu-239 (1 à 150 LDCA*) et de 10^2 à 10^5 Bq/m^3 pour le Cs-137 (0,05 à 50 LDCA*). Le diamètre médian aérodynamique en activité peut être choisi égal à 0,4 µm ou à 4 µm, avec un écart-type géométrique de 1,4.

La limite de sensibilité de ces capteurs vis-à-vis des aérosols radioactifs artificiels dépend bien souvent de la radioactivité naturelle ; aussi sont-ils généralement conçus pour séparer et éliminer le "bruit de fond" dû aux descendants à vie courte du radon. Au début de la recherche, objet de ce rapport, le banc ICARE comprend une source de radon obtenue à partir d'un minerai d'uranium naturel et un système de vieillissement, ce qui permet d'injecter dans une veine gazeuse, en amont des générateurs de plutonium 239 et de césium 137, des aérosols radioactifs naturels ayant une activité volumique comprise entre 6 et 60 Bq/m^3. Dans cette veine gazeuse, dont le débit est de 60 Nm^3/h, diverses buses de prélèvement sont disposées en aval des systèmes de génération, leur diamètre étant adapté à une plage de débit des capteurs de contamination compris entre 6.10^{-2} et 60 Nm^3/h.

Ni la source de radon, ni le dispositif de mesure (chambre d'ionisation différentielle) équipant primitivement ICARE ne pouvant être considérés comme des moyens de référence, il est nécessaire de doter ce banc d'étalonnage de tels moyens, donc de concevoir et de réaliser de nouveaux systèmes de production et de mesure du radon 222.

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*LDCA : Limite Dérivée de Concentration Atmosphérique

III.1 Circuit aéraulique des sources de radon 222

III.1.1. Méthodologie

La figure 1 présente le nouveau circuit aéraulique des sources de radon 222 à raccorder au volume de vieillissement dont disposait primitivement ICARE. En aval de ce volume, sera installée une chambre de test des appareils de mesure du radon 222 et de ses descendants ; ces appareils pourront être testés en écoulement dynamique, et soumis à une activité volumique de radon 222 variable entre 4 et 4 000 Bq/m^3 ; le débit d'air traversant cette chambre sera voisin de 20 m^3/h.

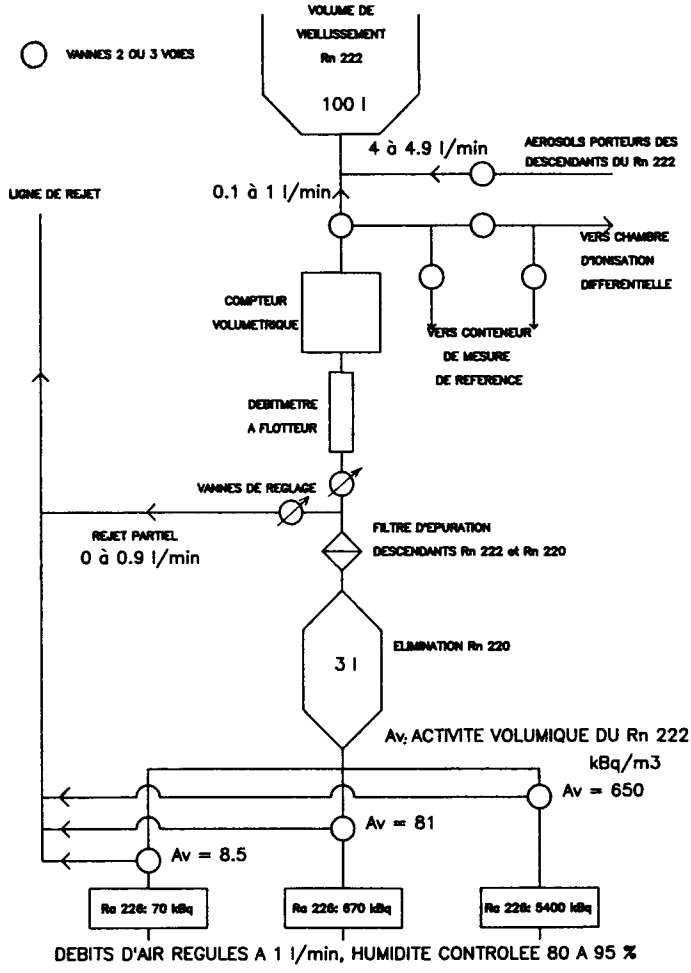


figure 1 - Circuit aéraulique des sources de radon 222

La méthode retenue pour atteindre cet objectif est de balayer trois sources solides constituées à partir de radium 226 ayant des activités voisines de 60, 600 et 6 000 kBq, avec trois débits d'air filtré et régulés à 1 l/min. De plus, un système de rejet partiel de 0 à 0,9 l/min de cet d'air permettrait d'injecter dans le volume de vieillissement 10 à 100 % de l'activité émanant des sources, donc de couvrir toute la gamme d'activité volumique désirée dans la chambre d'essais, avec une dynamique de 1 000.

III.1.2 Résultats

L'ensemble du circuit aéraulique des sources de radon 222 équipe maintenant le banc d'essais ICARE ; cependant, l'introduction des aérosols porteurs des descendants du radon n'est pas encore raccordée à un système de génération et de mesure de référence ; elle le sera comme prévu au cours de l'année 1990.

L'air de balayage des trois sources, alimenté par le circuit d'air comprimé du laboratoire, détendu, filtré est distribué sur trois voies avec un débit ajusté à 1 l/min sur chacune par trois régulateurs de débit massique. L'air est porté à une humidité relative comprise entre 80 et 95 % en traversant un barboteur installé sur chaque voie ; une sonde mesure en amont de chaque source radioactive le taux d'humidité relative et la température de l'air.

L'étude de conception et la réalisation des sources de radon 222 obtenues à partir de dépôts solides de radium 226 font l'objet du chapitre III.2.

Malgré la pureté du radium en isotope 226 assurée par le fabricant, un volume d'élimination (3 litres) du radon 220 émanant éventuellement de la source est disposé en série dans chaque circuit ; compte tenu de la période courte (56 s) ce volume élimine environ 90 % de l'activité de cet isotope. Un filtre de haute efficacité à fibres de verre (GELMAN A/E) situé en aval assure l'épuration des descendants formés.

Par un jeu de vannes et d'une ligne de rejet, il est possible :

- de sélectionner l'une des trois sources (faible - moyenne - forte activité)
- d'utiliser la totalité de l'activité du radon 222 (1 l/min) ou d'une fraction (0,1 à 1 l/min) ; un débitmètre à flotteur et un compteur volumétrique permettent d'ajuster et de mesurer le débit de l'écoulement d'air.

L'activité volumique peut être estimée à l'aide d'une chambre d'ionisation différentielle (2 x 10 litres). Une mesure précise exige son étalonnage à partir d'une mesure de référence. Un nouveau dispositif, décrit au paragraphe III.3, permet de le réaliser.

III.1.3 Discussion

Le circuit aéraulique est totalement réalisé selon le programme contractuel. Son fonctionnement ne pose pas de problème particulier. Seul, l'étalonnage de la chambre d'ionisation différentielle reste à faire.

III.2. Sources solides de radon 222

II.2.1 Méthodologie

Les sources solides de radon 222 ont fait l'objet d'une étude décrite en /2/ ; celle-ci a permis d'élaborer des procédures de fabrication et de contrôle de qualité. Nous décrirons succinctement les différentes phases de réalisation de ce type de source qui est constituée d'un dépôt solide homogène de radium 226 dans un disque de feutre acrylique imprégné d'oxydes de manganèse. Le dépôt est obtenu à partir d'une solution étalon de radium 226 dont les atomes sont retenus sur les oxydes de manganèse.

Préparation du revêtement d'oxydes de manganèse

Les feutres acryliques sont plongés dans une solution de permanganate de potassium à 50 g/dm^3 portée au préalable à 60°C. L'ensemble est maintenu pendant 24 heures à la température de 60°C dans une étuve. Les feutres sont ensuite lavés et séchés ; le lavage est important car il permet d'éliminer des particules d'oxydes de manganèse non fixées à l'intérieur et à la surface des feutres, ces particules pouvant nuire à la stabilité mécanique des sources. La masse d'oxydes de manganèse retenue est évaluée par pesée.

Une étude des mécanismes d'oxydo-réduction responsables de la formation de ces oxydes a permis de mettre en évidence la participation de la matière acrylique dans ces réactions.

Répartition du radium dans les feutres

Le radium 226 se trouvant primitivement en solution acide, milieu peu favorable à la stabilité des oxydes de manganèse, nous avons dû élaborer une technique permettant d'assurer la stabilité radiochimique des sources en radium 226. Elle consiste essentiellement à neutraliser

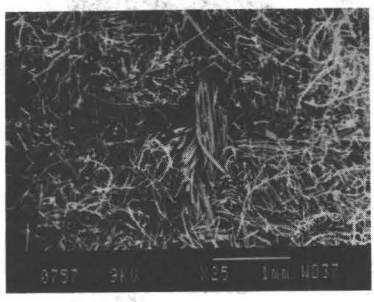
de façon partielle la solution initiale.

Pour une surface de feutre donnée, l'activité maximum du radium 226 qui peut y être déposée est limitée principalement par la quantité importante d'ions hydrogène présents dans les solutions d'origine.

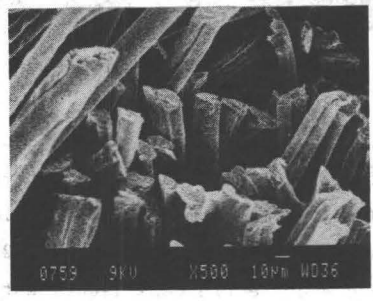
Après complète évaporation de la solution, nous vérifions la stabilité chimique et mécanique de cette source en procédant à des lavages successifs avec de l'eau bidistillée. On mesure dans ces eaux de rinçage l'activité totale de radium, qu'il soit sous la forme soluble (instabilité chimique) ou insoluble (instabilité mécanique). La stabilité est jugée acceptable lorsque l'eau de lavage contient moins de 1 % de l'activité déposée dans le feutre.

Les quatre clichés de la figure 2 présentent :

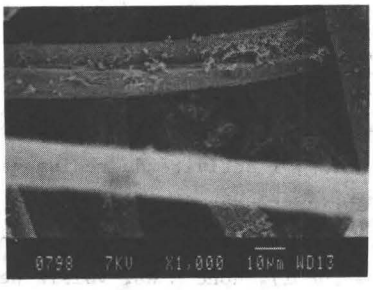
- en a et b : des fibres acryliques vierges (vues en coupe du feutre)
- en c et d : des fibres traitées, où l'on peut voir la répartition des oxydes de manganèse (parties claires des clichés) au contact des fibres.



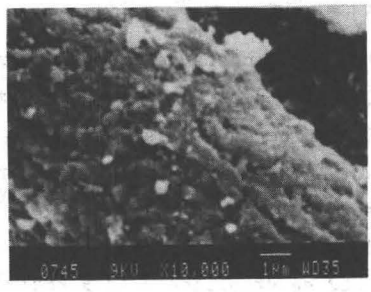
a - grossissement 25



b - grossissement 500



c - grossissement 1 000



d - grossissement 10 000

Figure 2 - Fibres acryliques vues au microscope

Emanation du radon 222

Chaque feutre contenant le dépôt solide de radium 226 est monté dans un dispositif porte-source, tel que montré sur la figure 3. Un débit régulé d'air filtré (1 l/min) le traverse en continu.

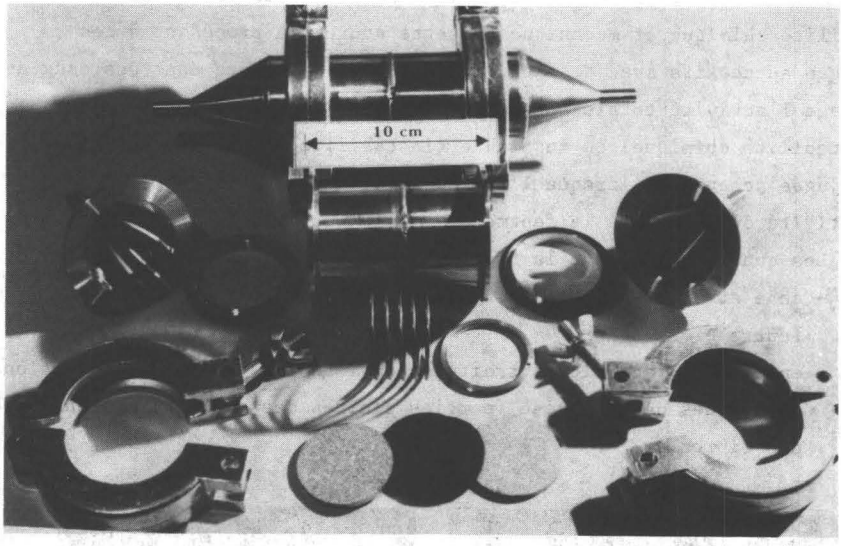


Figure 3 - Ensemble porte-source et feutre imprégné de Ra-226

III.2.2 Résultats

La figure 4 ci-après montre que si l'on fait croître le taux d'humidité de l'air (THR), le facteur d'émission (E) croît jusqu'à atteindre son maximum de façon stable à partir de THR = 50 %.

L'activité volumique du radon 222 (Av en Bq/m^3) dans le gaz de balayage d'un feutre dans lequel est déposée une activité A_{Ra} (en Bq) de radium 226, est donnée par la relation :

$$A_v = \frac{\lambda_{Rn222} \times A_{Ra}}{Q_v} \times E$$

avec λ_{Rn222} : constante de désintégration du Rn-222 = $2{,}1.10^{-6}$ s^{-1}

Q_v : débit volumique du gaz de balayage, m^3/s

La courbe de la figure 4 a été obtenue à partir de cette relation en mesurant A_v à l'aide de ballons scintillants ; l'activité du radium utilisé (240 kBq) est certifiée par le fabricant pour une incertitude relative de 3,7 %. Pour un débit de 1,7 l/min, l'activité volumique en radon 222 se stabilise à 17,9 kBq/m^3 (E = 100 %), donc E est voisin de 100 %, c'est-à-dire que tout le radon produit dans le feutre passe dans l'air de balayage.

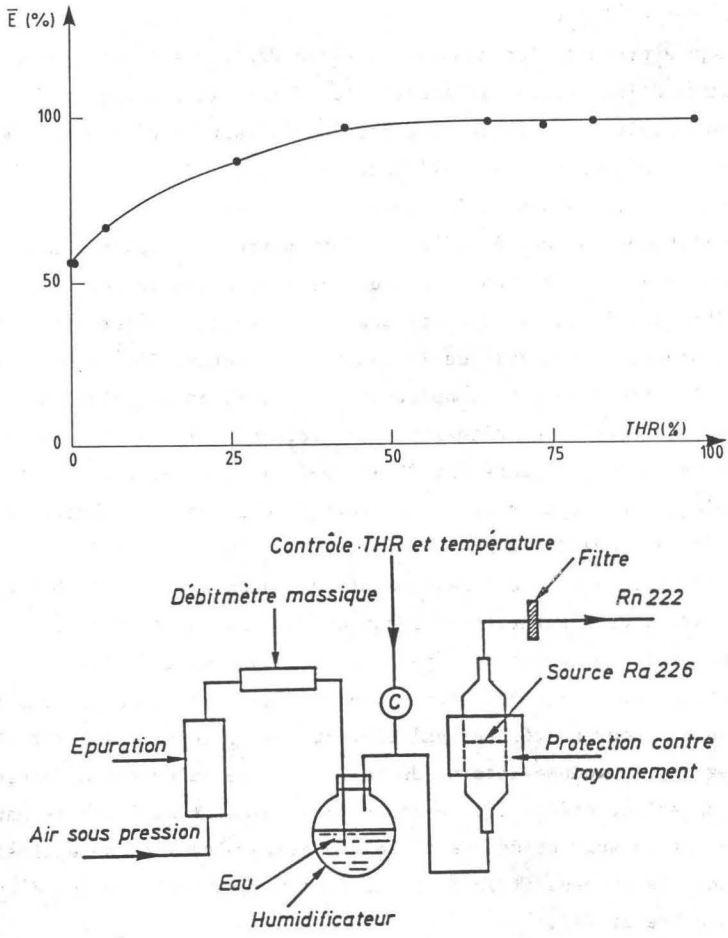


Figure 4 - Dispositif de balayage et facteur d'émission du Rn-222

III.2.3 Discussion

Trois sources solides de radon 222 ont été fabriquées et équipent maintenant le banc d'étalonnage. Les activités des dépôts de radium 226, calculées par la relation ci-dessus, sont égales à :

- source d'activité faible : 70 kBq
- source d'activité moyenne : 670 kBq
- source d'activité forte : 5 400 kBq

Les activités volumiques de radon 222 obtenues permettront d'atteindre l'objectif fixé : 4 à 4 000 Bq/m^3 dans la chambre de test des appareils de mesure radon et descendants du radon.

III.3 Dispositif de mesure de référence du radon 222

III.3.1 Méthodologie

Jusqu'à présent, les mesures de radon 222 ont été effectuées avec une chambre d'ionisation différentielle. Malgré la bonne reproductibilité des mesures faites par cette méthode, la réalisation d'un banc d'essai d'étalonnage nécessite une qualification à partir d'un dispositif de référence raccordé à des étalons.

La méthode consiste à prélever l'air porteur de radon 222 en aval d'une source dans un conteneur adéquat et d'attendre la montée à l'équilibre des descendants à vie courte (3 heures). L'activité volumique du radon prélevé est déduit de la mesure de l'activité du plomb 214 et du bismuth 214 effectuée par un spectromètre gamma, et du volume du conteneur. Ce système de détection est étalonné à l'aide de sources de gaz étalons émetteurs gamma (Kr-85 et Xe-127) fournies par le Laboratoire de Métrologie des Rayonnements Ionisants du CEA dans les conteneurs normalisés /3/ modifiés comme suit.

L'activité mesurée des descendants du radon n'est véritablement représentative de l'activité du radon qui est gazeux que si les atomes formés par désintégration se répartissent dans tout le volume du conteneur, et non uniquement sur les parois latérales. Cette condition indispensable pour se référer valablement aux étalons gazeux est obtenue en aménageant le volume interne de telle manière qu'un grand nombre d'alvéoles soient créées et que les atomes puissent se fixer de manière homogène sur la surface de ces alvéoles par le mécanisme de diffusion brownienne. Ce procédé et le dispositif de mesure font l'objet d'une demande de brevet /4/.

III.3.2 Résultats

La figure 5 montre la modification de structure interne d'un conteneur normalisé du type SG 500, de volume 500 cm^3 (capot retiré). Le gaz arrive au fond du conteneur par le tube d'injection visible sur la photo ; il circule à travers les performations des plaques superposées dont les plis sont disposés successivement à angle droit, et sort par l'orifice visible. Après une circulation du gaz porteur du radon à mesurer correspondant au minimum à 5 renouvellements du volume, l'entrée et la sortie sont isolées du circuit aéraulique.

Les plaques sont faites à partir d'aluminium déployé dont l'épaisseur et la dimension des perforations ont des dimensions voisines de 0,3 mm. La hauteur des plis est égale à 3 mm. Grâce à l'agencement de 18 plaques, dont 2 sont légèrement plus petites pour s'adapter à la courbure du fond du capot, plus d'un millier d'alvéoles sont ainsi pratiquées.

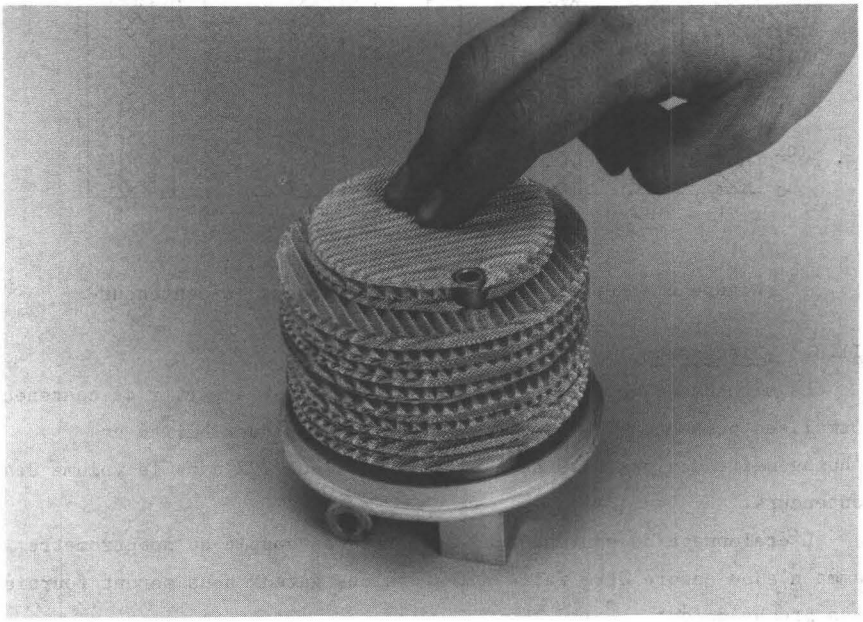


Figure 5 - Structure interne du conteneur radon

L'homogénéité de l'activité des descendants du radon dans le volume du conteneur a été qualifiée en interposant entre le fond du conteneur et la surface de détection du spectromètre un écran de plomb circulaire dont un secteur de 45° est évidé. La rotation de cet écran permet donc d'examiner la répartition de l'activité selon 8 secteurs (figure 6). Une légère hétérogénéité peut être notée dans le secteur 4 où se trouve le passage du tube d'injection.

La reproductibilité des mesures déjà faites sur des prélèvements de radon de la source de forte activité (activité volumique calculée : 5 400 kBq) est bonne, compte tenu de l'incertitude relative des comptages estimée à 2 %. Cette observation est confirmée par les mesures faites à l'aide de la chambre d'ionisation différentielle sur les trois sources de radon : variations inférieures à 5 %.

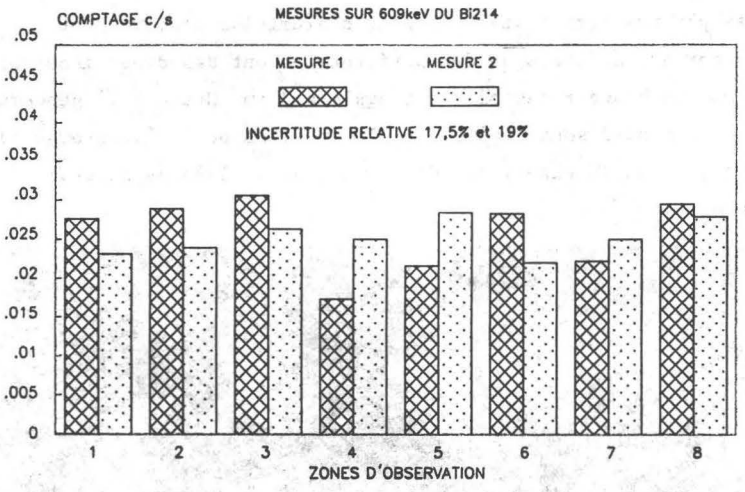


Figure 6 - Répartition de l'activité dans le conteneur

III.3.3 Discussion

La méthode de mesure et le dispositif réalisé à partir de conteneurs normalisés présentent de bonnes qualités de reproductibilité et d'homogénéité des produits de filiation du radon 222 dans le volume des conteneurs.

L'étalonnage du conteneur de mesure radon couplé au spectromètre gamma n'a pu encore être faite ; les étalons gazeux nous seront fournis très prochainement.

IV. Publications

/1/ M. AMMERICH
Réalisation d'une installation d'étalonnage de moniteurs de contamination atmosphérique à l'aide d'aérosols radioactifs calibrés (ICARE)
Rapport CEA-R-5484, juin 1989

/2/ G. CHUITON
Etude des caractéristiques physico-chimiques d'une source solide de radon 222.
Rapport interne CEA-IPSN-DPT-SPIN, décembre 1989 (rapport CEA à paraître)

/3/ Groupe de Travail de normalisation (GTN.5)
Normalisation de conteneurs gaz utilisés en spectrométrie gamma.
Rapport CEA-R-5384, 1986

/4/ J. CHARUAU, M. GUELIN, J. LE GAC
Procédé et dispositif de mesure de la concentration des différents isotopes du radon dans une atmosphère gazeuse.
Brevet EN8907109, 30 mai 1989

RADIATION PROTECTION PROGRAMME
Final Report

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. Galvào
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. 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.

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 Engenharia do Ambiente, Universidade Nova de Lisboa (DCEA/UNL). (1986/1987)

Scientific staff:

A. O. Bettencourt
L. Canelas (DCEA/UNL)(1986/87)
Nuno Nascimento
M. M. G. R. Teixeira
A. Brogueira
M. C. Faísca
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:

Continuation of the indoor radon survey and its enlargement to the whole granitic region.

Extension of the survey to other regions of the country.

Measurement of outdoor radon concentrations, in order to calculate its contribution to radon inhalation by the critical groups. Mathematical simulation of outdoor radon concentrations, and collection of meteorological data.

III. Progress achieved:

1. METHODOLOGY

For the determination of radon concentrations in Portuguese dwellings, Kodak LR-115 films were exposed for 1-3 months periods, collected, chemically etched with NaOH at constant temperature, and measured with a spark counter. The method sensivity is 1.4 tracks.cm^{-2}/kBq.m^{-3}.h and the detection limit is 6 Bq.m^{-3}.

For a secondary calibration, the detectors are irradiated by direct contact with thick standardised uranium sources.

The quality of the results was confirmed by the participation in two intercomparison exercises organized by CEC/NRPB (1987 and 1989). We have also participated in two bilateral intercomparison exercises with the IPSN/CEA: in the first one LR-115 films were exposed at the IPSN, in a rich radon atmosphere. In the second, several films were exposed, together and in identical conditions, in houses situated in the vicinity of the abandoned radium salts factory (Barracão). In both cases, half of the dosemeters were processed in each one of the laboratories.

A first selection of houses to be surveyed in the vicinity of the uranium tailings (at Urgeiriça) and of the old radium salts factory (at Barraçao), was made. Due to some high values found in dwellings from these two zones, this survey was pursued. The programme was also enlarged to some towns and villages from the granitic region of Beira Alta, and in 1989 to the whole granitic region.

Simultaneously some measurements started being performed in other regions from the country, in order to contribute to the European Atlas on natural radiation.

Questionaries 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 system, etc..

To avoid or minimize unwanted exposures, the films in transit or storage are kept in sealed lead plastic bags.

This programme was performed with the co-operation of the local high-schools. In a statistical base, (1 dosemeter for 2000 inhabitants) the dosemeters were delivered to the high-schools teachers, who proceeded to their distribution; the films were recollected 1-3 months later and sent to LNETI. Whenever possible, measurements were performed twice in each house, except in

what concerns the above mentioned high natural radioactivity regions, where they were repeated several times.

A study on the contribution of radon emanation from building materials, to the indoor concentrations, was also considered.

For this purpose, some of the main building materials, (sand, cement, brick and gypse) were crushed and placed into plastic boxes, up to 3 cm of the top. LR-115 films were fixed under the cover (3 cm far from the materials) and the boxes were closed and sealed. The same building materials were also analysed by Ge (Li) gamma spectrometry.

Several soil samples from the technologically enhanced radioactive zone and from uranium tailings were analysed by Ge (Li) gamma spectrometry, in order to characterize the source-terms.

Concerning the evaluation of outdoor radon concentrations, measurements in the vicinity of the uranium mining facilities were made, with the assistance of the KFK/FRG to study the horizontal and vertical dispersion of the radon emanated from the tailings.

KFK dosemeters were used for this purpose, and a grid of 100m x 100m within an area of 500m x 500m was established.

They were exposed for three months and processed by the Karlsruhe Nuclear Research Center. Gamma background measurements were also performed with a pressurized ionizing chamber, in each one of the grid points.

For the implementation of a model to simulate the radon dispersion, sampling of local meteorological data is a request.

After a careful evaluation of different available equipments for the measurements of meteorological parameters, an anemograph with specifications, in accordance with the foreseen needs of a simulation model, was selected and ordered.

Several algorithms for prediction of radon concentration were analysed. A final one was selected and implemented to predict concentrations at very short distance (below 100 meters) and run at LNETI computers.

2. RESULTS

Results on radon concentration corresponding to 52 counties (34 in the granitic region and 18 in the non granitic ones) and concerning 700 surveyed houses, have been obtained during the period of this project.

These results were grouped by district, and in Table I we present the corresponding geometric means, together with the range of observed values in the

houses of each district and the number of surveyed houses.

Table I: Indoor radon concentrations, by districts

		^{222}Rn ($Bq.m^{-3}$)	
		Region	
DISTRICT	nº of houses	Granitic	Non Granitic
Aveiro	17	--	52 (16-186)
Beja	3	--	24 (12-46)
Braga	35	126 (32-1574)	--
Castelo Branco	48	171 (35-1450)	--
Coimbra	13	--	81 (14-121)
Faro	13	--	9 (6-20)
Guarda(*)	179	176 (28-1344)	--
Lisboa	64	--	34 (22-76)
Portalegre	13	160 (47-498)	--
Porto	4	50 (28-74)	--
Setúbal	5	--	33 (19-52)
Viana do Castelo	21	126 (66-429)	--
Vila Real	29	101 (35-181)	--
Viseu(*)	226	161 (36-2682)	78 (50-180)

(*) Except the technologically enhanced radioactive zones

Besides these results, about 2000 dosemeters are already distributed or being processed.

The highest mean values correspond to houses nearby the old radium salts factory (907 $Bq.m^{-3}$, ranging from 107 to 2795 $Bq.m^{-3}$), and the uranium mining and milling facilities (755 $Bq.m^{-3}$, ranging from 363 to 2201 $Bq.m^{-3}$). Nevertheless, some individual values of the same order of magnitute were also observed in some other localities from the granitic region.

In the granitic region (with the exception of these high zones) a geometric mean of 108 $Bq.m^{-3}$ was found with values ranging from 50 to 176 $Bq.m^{-3}$. In the non granitic one the geometric mean of all the results is 41 $Bq.m^{-3}$ ranging from 9 to 81 $Bq.m^{-3}$; the lowest concentration concerns a region at the south of the country, with clayish and calcareous soils, and is close to the background values. The frequency distribution of the indoor radon concentrations, observed up to now, is presented in Figure 1. However, as this programme was started by the regions where higher concentrations were expected,

a certain deviation in this histogram might be observed, when more measurements become available.

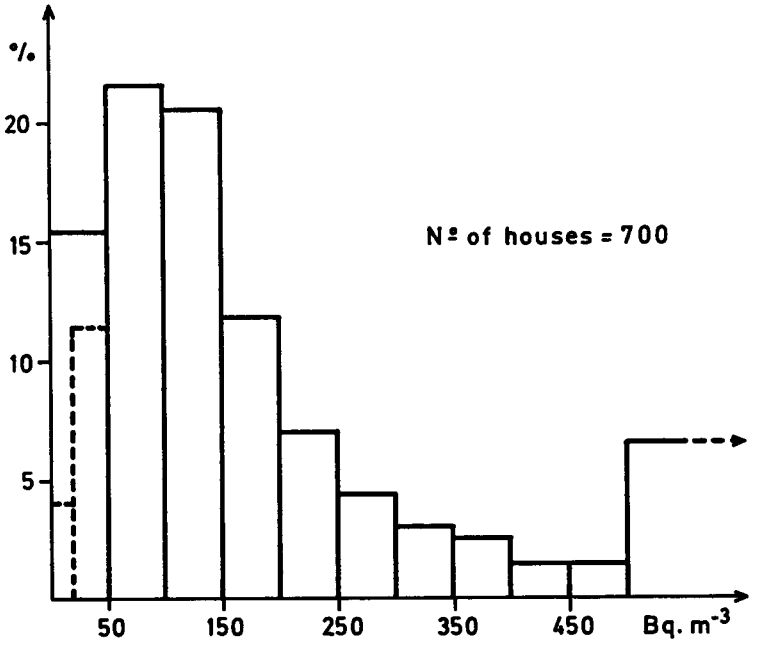


Figure 1: Frequency distribution of indoor radon concentration

Comparing individual values of rooms situated in the ground floor and in upper ones, it was observed that in ground floors the values are always higher. This may be illustrate by the following mean values:

	^{222}Rn ($Bq.m^{-3}$)		
	Guarda	Arcos de Valdevez	Nelas
Ground floor	507 (19)	243 (10)	408 (32)
Upper floor	177 (55)	92 (22)	109 (36)

( ) Number of surveyed houses

Concerning the radon emanation from different building materials, the values observed for some of the most significant ones were:

Sand - 2.0×10^{-5} Bq m^{-2} s^{-1}

Cement - 9.0×10^{-6} Bq m^{-2} s^{-1}

Gypse - 1.6×10^{-6} Bq m^{-2} s^{-1}

Brick - 1.2×10^{-5} Bq m^{-2} s^{-1}

The contents of ^{226}Ra, ^{232}Th and ^{40}K obtained from the same materials, analysed by gamma-spectrometry were:

Material	^{226}Ra ($Bq.kg^{-1}$)	^{232}Th ($Bq.kg^{-1}$)	^{40}K ($Bq.kg^{-1}$) ($x10^{-2}$)
Sand	25 ± 3	4 ± 1	5.5 ± 0.3
Cement	42 ± 5	11 ± 2	2.5 ± 0.4
Brick	58 ± 4	26 ± 2	12.0 ± 0.7
Gypse	65 ± 4	--	≤ 0.1

The results of ^{226}Ra concentration obtained from some soils samples collected at Barracão, range from 300 to 63000 $Bq.kg^{-1}$ dry soil.

The contents of ^{226}Ra from samples collected at the tailings of uranium range from 2500 to 20000 $Bq.kg^{-1}$.

The frequency of outdoor radon concentrations performed in the vicinity of the uranium mining facilities, along the horizontal grid, are shown in Figure 2.

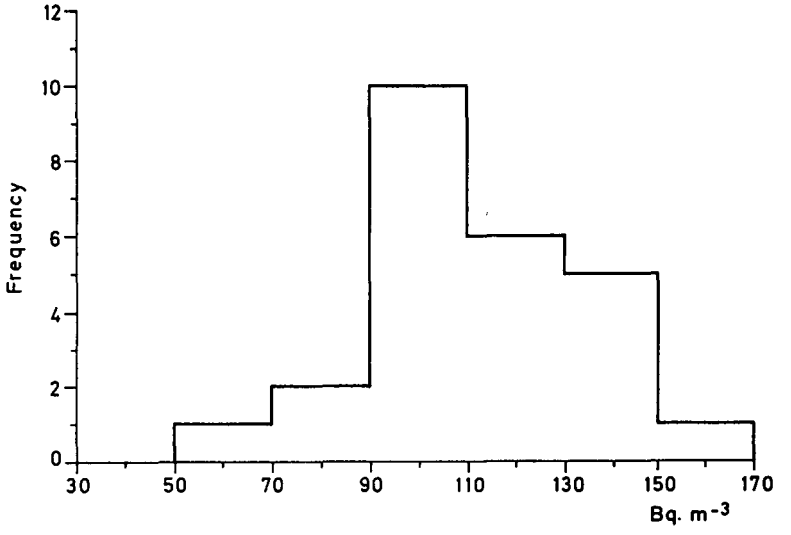


Figure 2: Radon distribution Urgeiriça
(Exposure 89/06/08 - 89/09/20)

The following results of intercomparison exercises with NRPB, expressed in $kBq.m^{-3}.h$, are presented:

1987		1989	
NRPB	LNETI	NRPB	LNETI
37 ± 4	25 ± 7		
208 ± 21	167 ± 39	233 ± 23	248 ± 15
2120 ± 211	1571 ± 261		

Concerning the intercomparison with I.P.S.N., a deviation < 10% was found. The acquisition of the anemograph was accomplished after the introduction of several important improvements by our request, in view of the parameters to be measured.

The gaussian mathematical model is now running and the scientific staff is familiar with it. Simulations of short term scenarios have been done using available regional meteorological informations.

3. DISCUSSION

In general, the objectives proposed for this project were attained.

Concerning indoor radon concentrations, the results clearly show three different levels of activity, accordingly to the studied regions:

- the highest one is due to the influence of wastes from the old radium salts factory (907 $Bq.m^{-3}$) and from the uranium tailings (755 $Bq.m^{-3}$)
- the granitic region (108 $Bq.m^{-3}$)
- the non granitic region (41 $Bq.m^{-3}$)

Comparing the geometric means of indoor radon concentration in the granitic and non granitic regions a ratio of about 3 can be observed.

The corresponding doses to the population due to radon inhalation would be on the order of 40, 5, and 2 mSv a^{-1}, for each of these regions, respectively.

In Portugal, the granitic regions are situated in a climatic zone colder than most of the non granitic ones. So, the high values found in those regions could be explained by the geological composition of the soils, probably enhanced by a poor ventilation of the dwellings.

Observing all the individual values, it was verified that 4% of dwellings have indoor radon concentration below 20 $Bq.m^{-3}$ and ≈10% present levels higher than 400 $Bq.m^{-3}$. The most frequent values are situated between 50-150 $Bq.m^{-3}$ (about 40%) (Figure 1).

The obtained results seem to confirm that the main cause for the elevated

indoor radon concentrations in houses would be the influx of radon through the soil beneath the houses.

Concerning the outdoor radon concentrations and the gamma dose measurements, there is not a clear agreement between both values. So, these measurements should be repeated, and the corresponding meteorological data should obtained in order to get a better understanding of the situation.

The main objective of implementing a mathematical model for predicting the atmospheric dispersion of radon has been accomplished. However, it was not yet possible to obtain site-specific meteorological data to run the model in realistic conditions, due to difficulties in installing the anemograph at Urgeiriça. Actually, it was not possible to install it in the zone of the old radium salts factory, as initially foreseen, due to the removal of the contaminated sands from the site, which occurred about one year after the starting of this project. On the other hand, there were also some difficulties concerning the zone around the uranium tailings, but it is expected to proceed soon with its installation there, in order to obtain more accurate site-specific meteorological data for running the mathematical model.

As soon as results become available, they will be published with reference to this project.

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

In 1986/87:
Departamento de Ciências e Engenharia do Ambiente

Universidade Nova de Lisboa

Quinta da Torre

P-2825-Monte da Caparica

V. Publications:

M. C. Faísca and A. O. Bettencourt

Preliminary Survey of Indoor Radon Concentrations in Portuguese Houses from High Natural Radioactivity Regions

Radiation Protection Dosimetry, Vol. 24, Nº 1/4, pp. 353-355 (1988)

M. Conceição Faísca, M. Manuela Ribau Teixeira

Influência dos Materiais de Construção nas Concentrações de Radão Medidas no Interior de Habitações. (Poster)

Conferência Nacional de Física, Aveiro, September 1988

M. Manuela Ribau Teixeira, M. Conceição Faísca

Radão no Interior de Habitações (Poster)

Conferência Nacional de Física, Aveiro, September 1988

Comparação de níveis de radão no interior de habitações de regiões graníticas e não graníticas

M. Manuela G. R. Teixeira

M. C. Faísca

LNETI/DPSR-B-Nº5 (III Série)

Canelas, L. (1987)

Avaliação da Exposição a Radão de Populações nas proximidades de Instalações de Tratamento de Urânio - Contributos para a Modelação Matemática

Publicações DCEA, Quinta da Torre, 2825 Monte da Caparica

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Risø National Laboratory
DK-4000 Roskilde

Contract no.: BI6-F-175-DK

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

Dr. H.L. Gjørup
Health Physics Dept.
Risø National Laboratory
DK-4000 Roskilde

Telephone number: (2) 37.12.12

Title of the research contract:

Shielding factor calculation for plume radiation

List of projects:

1. Shielding factor calculation for plume radiation.

Title of the project no.:

SHIELDING FACTOR CALCULATION FOR PLUME RADIATION

Head(s) of project:

Per Hedemann Jensen

Scientific staff:

Søren Thykier-Nielsen

I. Objectives of the project:

Development of a computer model for calculating indoor and outdoor protection afforded by buildings against gamma radiation from a passing radioactive plume. Identification of important parameters such as building structure dimensions, window apertures, plume/building geometry and photon energy by means of parametric studies. Calculations of shielding factors for typical European houses and recommendations of representative shielding factors for European housing conditions.

II. Objectives for the reporting period:

Shielding factors have been calculated for indoor residences in selected European single-family and multistory houses and outdoor residences in built-up urban areas. Parametric variations have been performed to identify the most important factors responsible for affording protection indoors against plume gamma radiation. The parameters varied were the thickness of outer and inner walls, floors and roofs. Recommendations have been made for representative shielding factors for indoor residences in a wide range of European building types as well as for outdoor residences in urban and suburban areas.

III. Progress achieved:

1. METHODOLOGY

If radioactive material is released from a nuclear facility into the atmosphere the surrounding population will be exposed to radiation emitted from the radioactive plume (cloudshine) as well as to radiation emitted from radionuclides deposited on building and ground surfaces (groundshine).

The indoor gamma doses from both exposure pathways will be less than the outdoor doses due to the shielding effect of the buildings. Building shielding factors for gamma radiation from deposited activity have been studied in great detail, both theoretically and experimentally. Due to a greater complexity of the source-detector geometry in the calculation of shielding factors for a radioactive plume, extensive work has not previously been performed.

Shielding factors for cloudshine have been based on simplified calculations, mainly for American housing conditions. More realistic shielding factors were therefore needed, also because the cloudshine exposure pathway might be relatively more important than previously assumed.

The fundamental calculation method used in this study is based on the exponential point attenuation kernel that links the radiation flux density at a given detector position to a point source strength. Attenuation resulting from geometrical spreading with increasing distance from the source as well as exponential attenuation and scattering of the photons are taken into consideration.

The point kernel method presumes that both the source and shielding medium are isotropic, i.e. that the source radiates uniformly in all directions, and that the medium attenuates equally in all directions. As far as photon scattering is concerned, it is assumed that both source and detector are located within an infinite homogeneous medium. The scatter contribution is calculated by the use of a dose build-up factor which depends on both the photon energy and atomic number of the absorbing medium.

Building types are described as a composite of cubic boxes. The thickness of outer walls, inner walls, partition walls and floors/ceilings can be varied independently of each other. Attenuation and scattering in the different walls and floors having projected thicknesses along the direction from each plume subvolume to the detector point are accounted for in the numerical integration over the relevant part of the plume volume. The attenuation of photons passing through window apertures is neglected.

The shielding factor S for plume radiation is defined as the ratio of the dose rate (or kerma rate) at a given indoor (or outdoor) location to an outdoor reference dose rate. The reference dose rate is defined here as the dose rate 1 meter above ground level without buildings present and with the same horizontal position to the plume as that of the location considered.

Building data

Shielding factors have been calculated for European single-family houses and multistory buildings. The dimensions and building data for the single-family houses are shown in Table 1 and Figure 1.

TABLE 1. Structure dimensions in kg per square meter for selected European single-family houses

	Outer walls	Inner walls	Roof/ceiling
Great Britain	340	238	119
France			
-before 1920	748	595	408
-prefabricated	357	289	323
Fed. Rep. Germany			
-ground floor	564	34	493
-first floor	85	34	68
Greece	187	153	255
Denmark	400	220	200
Sweden	155	51	100
Norway	34	20	82
Finland	51	43	100

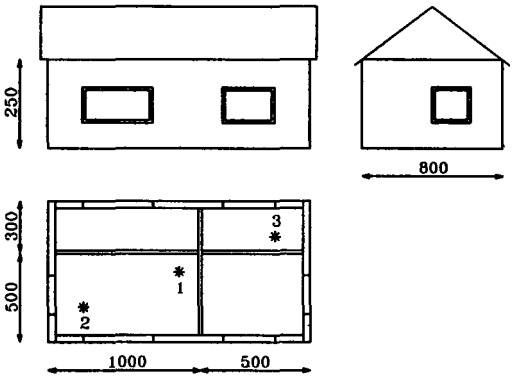


FIGURE 1. Dimensions of a "standard" European single-family house in cm

The dimensions and building data for the multistory buildings are shown in Table 2 and Figure 2.

TABLE 2. Structure dimensions in kg per square meter for selected European five-story buildings

	Outer walls	Inner walls	Partition walls	Floors	Roof/ ceiling
Great Britain	439	186	254	342	180
France	418	272	374	418	418
Fed. Rep. Germany	425	210	374	425	470
Greece	300	147	147	200	255
Denmark	595	220	255	170	255
Sweden	305	100	325	425	255
Norway	390	30	360	440	80
Finland	360	255	440	440	100

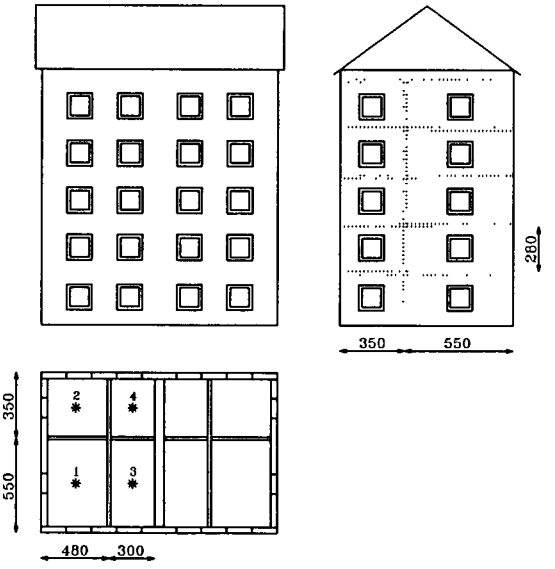


FIGURE 2. Dimensions of a "standard" European five-story building in cm

In the calculations of shielding factors in this study it is assumed that the window fraction, i.e. the fraction of the total outer wall area that is covered by windows, is 20-25%. The dimensions and placement of the windows can be selected freely, exemplified in Figures 1 and 2.

2. RESULTS

2.1. Shielding factors for European houses

For the single-family houses shielding factors were calculated for a photon energy of 0.68 MeV and for the three different locations shown in Figure 1. The results are shown

in Table 3. The variation in the shielding factors expresses the dependence of the position in the house.

TABLE 3. Shielding factors for European single-family houses for a photon energy of 0.68 MeV and a semi-infinite cloud

Great Britain	0.08-0.18
France	
-before 1920	0.01-0.02
-prefabricated	0.02-0.06
Fed. Rep. Germany	
-ground floor	0.09-0.27
-first floor	0.28-0.46
Greece	0.07-0.16
Denmark	0.05-0.09
Norway	0.56-0.66
Sweden	0.17-0.30
Finland	0.27-0.52

For the multistory buildings shielding factors were also calculated for a photon energy of 0.68 MeV and four different locations at each story as shown in Figure 2. The results are shown in Table 4. The variation expresses the dependence of the location at the given story. It appears from the table that the story level position is an important parameter in so far as the protection drops at higher stories.

TABLE 4. Shielding factors for European five-story houses for a photon energy of 0.68 MeV and a semi-infinite cloud

	bottom story	3rd story	5th story
Great Britain	0.020-0.056	0.060-0.10	0.11-0.21
France	0.015-0.068	0.028-0.092	0.045-0.13
Fed. Rep. Germ.	0.015-0.047	0.038-0.088	0.090-0.11
Greece	0.019-0.10	0.086-0.13	0.17-0.26
Denmark	0.013-0.056	0.032-0.097	0.081-0.13
Norway	0.031-0.069	0.065-0.11	0.27-0.36
Sweden	0.026-0.083	0.050-0.12	0.15-0.29
Finland	0.016-0.054	0.024-0.12	0.17-0.33

2.2. Parameter variations

Some of the parameters were varied in the calculations to identify their influence on the shielding factors. For a simple blockhouse without windows and submerged in a semi-infinite cloud, both the wall/roof thickness and photon energy were varied within relevant ranges. The blockhouse geometry is shown in Figure 3. In the calculations the density of the walls is 1.7 kg/l.

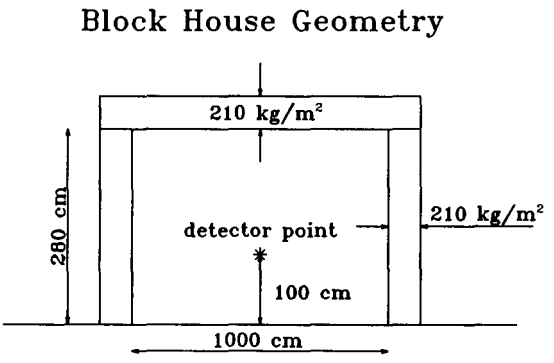


FIGURE 3. Geometry for a simple blockhouse

Figure 4 shows the calculated shielding factors for the blockhouse for different wall/roof thicknesses and photon energies. Both parameters have a significant influence on the calculated shielding factors.

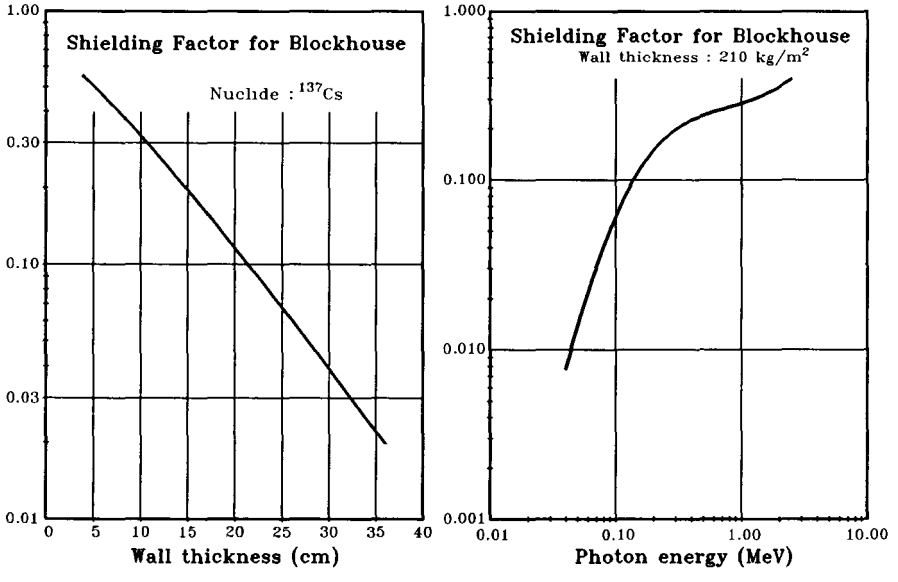


FIGURE 4. Shielding factors for a simple blockhouse for different wall/roof thicknesses and photon energies

For the Danish single-family house the plume/building geometry was varied. Two different atmospheric stability categories (Pasquill D and F), two different heights (0 m and 100 m), four different downwind distances, and two different crosswind distances were used. The photon energy was 0.68 MeV. The results are shown in Table 5 for detector location no. 1 (see Fig. 1).

TABLE 5. Shielding factors for a Danish traditional single-family house for different plume/building geometries

Downwind distance (meter)	Pasquill D	Pasquill F
(crosswind distance=0 m)	(plume height=0 m)	
200	0.04	-
500	-	0.03
1000	0.06	0.06
10000	0.09	0.05
(crosswind distance=0 m)	(plume height=100 m)	
200	0.22	-
500	-	0.20
1000	0.15	0.19
10000	0.09	0.11
(crosswind distance=500 m)	(plume height=0 m)	
10000	0.09	0.04

For elevated plumes at short downwind distances, the shielding factor is increased compared to the semi-infinite plume; this is due to the less effective shielding of the roof, which constitutes the main part of the shielding in these situations. For ground level plumes at short distances an opposite effect is observed. The reasons are that (1) the dimension of the plume is still small relative to the semi-infinite plume and (2) the fraction of the total radiation penetrating the roof therefore is proportionally smaller. A similar effect can be observed for narrow ground level plumes (Pasquill F) with the house positioned at some crosswind distance from the plume. At large downwind distances the results become close to those for the semi-infinite cloud.

For the Danish single-family house submerged in a semi-infinite cloud, variations of the structure dimensions have been made. The thicknesses of the outer walls, inner walls, and roof/ceiling were varied in a range from 5 to 30 cm (85 kg per square meter to 510 kg per square meter). The results are shown in Figure 5.

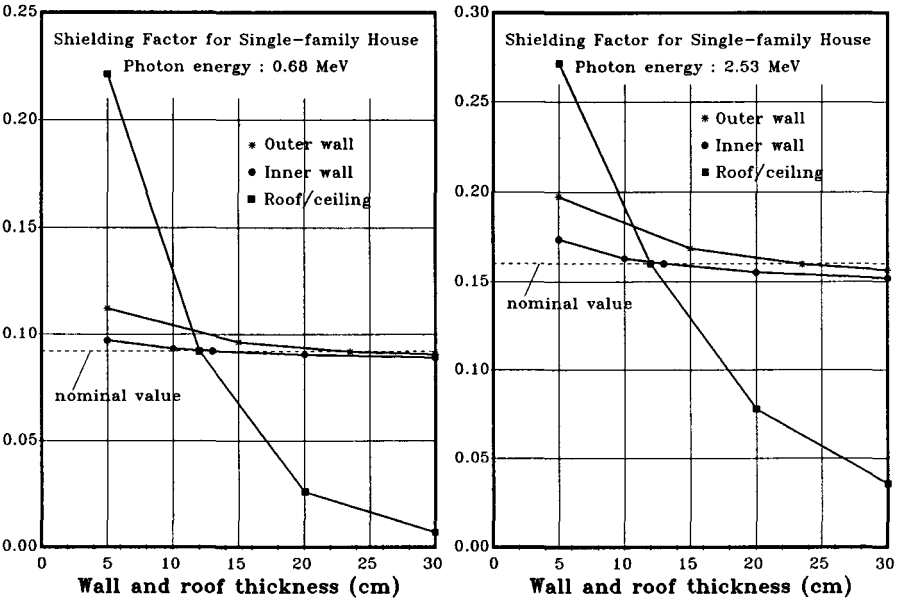


FIGURE 5. Shielding factors for a Danish single-family house for different wall and roof thicknesses and photon energies of 0.68 and 2.53 MeV

The main contribution to the indoor dose rate from a semi-infinite cloud originates from that part of the cloud lying above the roof plane; there is only a minor contribution from the part of the cloud below the roof plane. In a single-family house of standard dimensions, up to 90% of the indoor dose rate originates from the cloud above the roof plane.

This situation is reflected in Figure 5. It appears that the shielding factor is rather insensitive to variations in outer wall thickness, typically less than 30% for the given range. For the roof/ceiling, on the other hand, there is a strong dependence of the thickness, up to a factor of 10 for the given range.

2.3. Outdoor shielding factors for urban areas

A person staying out-of-doors in an urban area will be exposed to radiation only from a part of the plume; this is caused by the shielding effect of the surrounding buildings. In the calculations the detector point is placed one meter above the ground and in the middle of the street. The geometry is shown in Figure 6.

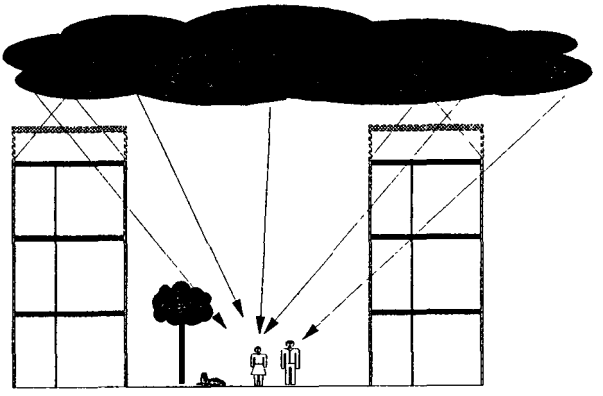


FIGURE 6. Geometry for outdoor exposure from a plume in an urban area with multistory buildings at the sides of the street

The shielding factor will decrease for increasing building height and decreasing street width. Calculations have been made for building heights as well as street widths of 10 m and 20 m. The results are shown in Table 6.

TABLE 6. Outdoor shielding factors for urban areas and a photon energy of 0.68 MeV

	Street width 10 m		Street width 20 m	
	Height 10 m	Height 20 m	Height 10 m	Height 20 m
	Buildings on both sides of the street			
Shielding factor	0.33	0.13	0.48	0.24
	Buildings on one side of the street			
Shielding factor	0.58	0.48	0.72	0.57

2.4. Monte Carlo calculations

The assumptions needed so the point kernel method may be used are not always fullfilled, so that in certain situations where the main part of the indoor dose rate originates from scattered photons, the point kernel results can be misleading. Therefore, some calculations have been made with the Monte Carlo code MCNP (Monte Carlo Neutron Photon) supplied by Oak Ridge National Laboratory. The purpose of these calculations was to investigate the influence of window and door apertures.

Shielding factors have been calculated for different photon energies and three different window fractions, 0%, 15%, and 50%. The plume was assumed to be of semi-infinite size. The MCNP results are shown in Figure 5 together with the corresponding point kernel results.

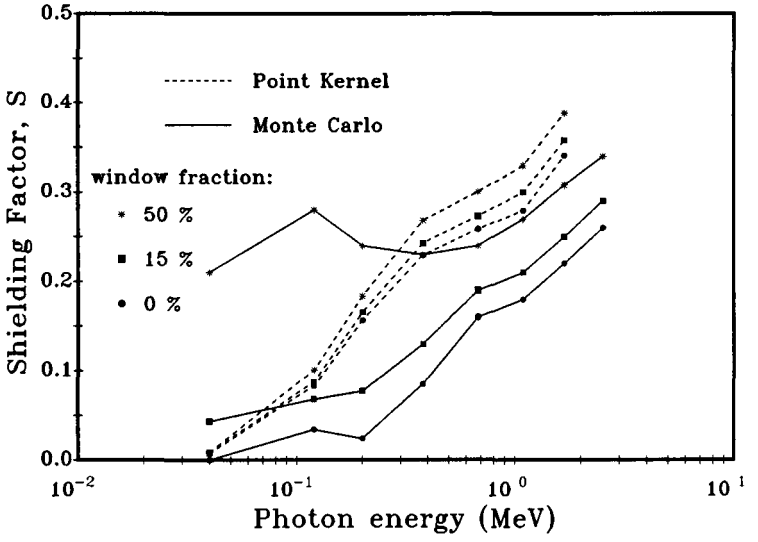


FIGURE 7. Shielding factors for simple blockhouse with dimensions as shown in Figure 3

The results indicate that window apertures could be more important than found by the point kernel method, especially at lower photon energies. On the other hand, the MCNP values tend to be lower than the corresponding point kernel values at higher photon energies.

2.5. Comparison with other works

Several authors have made both simplified and more detailed calculations of shielding factors for semi-infinite clouds containing gamma-emitting nuclides.

Kocher has made calculations for "igloo"-shaped buildings without windows. Shielding factors on the order of 0.7-0.02 were calculated for an outer wall thickness ranging from 5-20 centimeter concrete (116-470 kg per square meter). The calculations were performed by the point kernel method.

Burson and Profio have used the same method to calculate representative shielding factors of 0.6 for masonry houses, 0.4 for basements in masonry houses, and 0.2 or less for large office buildings.

Peter Jacob et. al. have made Monte Carlo calculations for a semi-detached house. Shielding factors were calculated in the range 0.1-0.3 in the ground floor, 0.2-0.5 in the first floor, and 0.001-0.02 in the basement for photon energies between 0.2 and 3.0 MeV. The outer wall was 23 centimeter thick and of brick (390 kg per square meter).

Le Grand et. al. have calculated shielding factors for dwellings in the range 0.3-0.4 for 0.5 MeV photons using Monte Carlo technique. The indoor doses were calculated to a cylindrical phantom. The outer walls and roof were of concrete, of thickness 10 and 3 centimeters, or 235 and 70 kg per square meter, respectively.

3. CONCLUSION

Based on calculations of shielding factors for different building types as well as parameter variations, the following shielding factors for plume radiation are recommended as conservative values covering a wide range of European building types.

	Single-family	Multistory
Great Britain	0.20	0.07
France	0.05	0.05
Fed. Rep. Germany	0.30	0.07
Greece	0.20	0.12
Denmark	0.10	0.07
Norway	0.60	0.15
Sweden	0.30	0.12
Finland	0.50	0.12

The dominating parameter for the shielding factor for single-family houses and upper stories in multistory buildings is the thickness of the roof because the main part of a large cloud lies above the roof plane. The best protection in multistory buildings is achieved in the lower stories in contradistinction from the best protection from radiation from deposited activity, which is achieved in the middle stories. The shielding factor dependence on photon energy is moderate. For standard buildings, the shielding factor will increase by a factor of 2-3 when the energy is increased from 0.2 to 2 MeV. A better protection is achieved in multistory houses compared to single-family houses, typically in the order of a factor of 2-4. Outdoor residences in urban areas with multistory buildings will give smaller cloudshine doses than in open areas. Outdoor shielding factors for urban areas in the range 0.2-0.7 are realistic, depending on how densely built-up are the areas. A value of 0.4 can be recommended as reasonably conservative for urban areas and 0.7 for suburban areas.

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

V. Publications:

Hedemann Jensen, P.; Thykier-Nielsen, S., Shielding Factors for Selected European Houses for Gamma Radiation from a Radioactive Plume. Risø-M-2849 (1990) (in preparation).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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and Technology
Exhibition Road
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Contract no.. BI6-F-108-UK
BI6-F-228-UK

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Title of the research contract:

Pathways and systems pertaining to the urban environment.

List of projects:

1. Pathways and systems pertaining to the urban environment.

Title of the project no.: BI6-F-108-UK (plus project number BI6-F-228-UK)

PATHWAYS AND SYSTEMS PERTAINING TO THE URBAN ENVIRONMENT
(AND SUPPLEMENTARY CONTRACT BI6-F-228-UK)

Head(s) of project: Professor A.J.H. Goddard, Dr. H.M. ApSimon,
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Scientific staff:

Dr. R.J. Cannell, Dr. K.L. Simms, Ms. J. MacCurtain

I. Objectives of the project:

The project has focussed upon aspects which may lead to enhanced exposure in the urban environment, and has had two aspects. The first aspect has been to obtain better data on the behaviour of particulate activity within dwellings and to evaluate exposure due to deposited activity indoors. The inadequacy of existing data led to the development of new experimental techniques under supplementary contract BI6-F-228-UK 'Experimental studies on aerosol transport processes in dwellings using inactive tracer techniques. The second aspect involved modelling contamination following a major nuclear accident; this contamination may be concentrated in areas where wet deposition in precipitation occurs. The objectives were to consider how the inhomogeneous nature of the wet deposition patterns could be assessed, and the implications for probabilistic risk assessment (PRA) studies of nuclear accidents.

II. Objectives for the reporting period:

Part A. A major part of the European population lives in urban areas and, in turn spend the majority of time indoors. The protection of people inside dwellings against the impact of a major nuclear accident must therefore have high priority. This protection must include an understanding of the shielding afforded by buildings against direct radiation, reduction of air exposure indoors from filtration process by the building fabric, reduction of the air exposure due to deposition on indoor surfaces and the possible enhancement of direct radiation from these deposits, together with subsidiary processes such as resuspension and mechanical transport indoors of material deposited outdoors. In circumstances where there has been historical outdoor deposits, the last two processes must ultimately dominate the long term exposure of those indoors.

The project began with an assessment of existing data for the behaviour of particulate activity, in aerosol form, indoors relative to corresponding concentration outdoors. It was established that there was essentially no data of scientific quality upon which models for dose assessment could be constructed. Under these circumstances, two routes were followed. The first consisted of developing experimental facilities in order to obtain individual data items under controlled conditions. The second was to carry out full scale experiments in dwellings, and for this purpose contacts and collaboration were established with the Danish Riso National Laboratory; this led to extremely fruitful experimental collaboration which has been continued to the present time. Imperial College work was conducted, over the period of contract, by three research students, Dr. Cannell, Dr. Simms and Ms. MacCurtain and therefore had valuable research training content - which is one of the strengths of university participation.

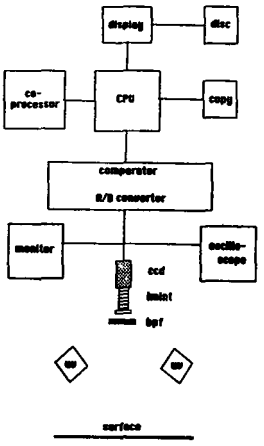
The project has been aided by a collaboration in the development of aerosol technology with Imperial College's Reactor Centre. There, the range of experimental work has been in progress, centred around the MAFF wind tunnel, to characterise, label and disperse monodisperse aerosols as part of the study of their behaviour in the natural environment. The method, developed at the Reactor Centre for labelling monodisperse silica particles has been adapted for use in studying the indoor environment.

Modelling work by other contractors, to the Radiation Protection Programme, had shown that, within the range of uncertainties of data, doses to the occupants of dwelling due to radioactive aerosols deposited indoors could give comparable direct exposure to that from outdoor deposits, when due account is taken of the shielding effects of the building structure. Thus, attention must clearly be given to indoor deposits. This deposition process indoors is also of significance in its role in reducing air exposure due to infiltrated aerosols. Deposition on indoor surfaces therefore became the main item of technical interest. This involved decisions on the type of tracer and on the best means of sampling its surface deposition.

Aerosol Labelling and the Scanning System

While stable tracer techniques normally offer the best combination of sensitivity and convenience, the difficulties of sampling from a surface led to development of a non invasive surface scanning technique.

Fluorescence under ultra violet illumination was the selected scanning technique; a stilbene compound which was readily adsorbed from solution onto silica particles was employed; this exhibited strong dry state fluoresence. Silica particles were available within the size range 1-20 microns and a particular size showed satisfactory mono dispersicity. A prototype surface scanning system was built comprising a CCTV camera feeding an analogue to digital converter interfaced to a microprocessor for image processing. This served to show the promise of the technique and under the supplementary contract a development system was built and tested. In order to increase sensitivity while discriminating in favour of the fluoresced irradiation, a micro channel plate image intensifier was preceded by a narrow band pass interference filter. The output from the intensifier passed to a charged coupled device camera with a wide angle lens, giving a field of view of about 45^{o}. The camera output again passed to an a/d converter and a micro computer with storage and hard copy facilities. This system was shown to have acceptable linearity and sensitivity (of the order 10 $\mu g\ m^{-2}$ of deposited particles).



KEY: CCD charge-coupled device camera
uv ultra-violet lamp
disc mass storage
CPU data acquisition processor
copy graphics printer
imint micro-channel plate image intensifier
bpf narrow bandpass interference filter

DIADEM development system for non-invasive scanning of aerosol deposition on surfaces

Early Applications

The scanning system was applied in a range of mechanical transport, decontamination and resuspension studies. To investigate mechanical transport, square tracks of various flooring materials were designed so that the scanning system could establish the particle loading on each element making the track. Flooring surfaces investigated were carpet tiles, vinyl floor tiles, concrete and wooden surfaces, thereby encompassing the most common types of surface to be found in dwelling or like commercial premises. The majority of measurements were conducted with 3 μm particles although measurements were made with 0.1 and 1.0 μm particles and with poly disperse tracer. Various shoe types were also investigated. The results may be used to infer transfer rates from outdoors to indoors and within the dwelling; for example with standard polyamide carpet tiles of an area 0.16 m^2, a fractional mass transfer rate of about 0.4% per step was indicated. This scanning technique may also be used to study the effectiveness of surface decontamination.

Normal vacuum cleaning served to remove approximately 37% of material from carpet tiles which had been trodden on and 61% of tracer from those which had not. Even an intensive cleaning period only removed approximately 50% of particulate material from tiles where the particulate had been applied mechanically. It thus seems unlikely that more than 50% of any material transported into a dwelling and incorporated into carpeted surface could be removed by conventional means. In collaborative research with AERE Harwell, the scanning technique was used to investigate resuspension from road surfaces caused by vehicular traffice. Patches of fluorescent labelled mono disperse aerosol were deposited in the middle of a road surface at night so that the scanning system did not suffer interference. The density of deposition was established by scanning and a car was driven repeatedly at a standard speed over the patch with, at intervals, the remaining deposit established by scanning. The results clearly established the dependence of resuspension of vehicular traffic of dry deposits, upon particle size and time of exposure.

Aerosol Test Chamber

In order to seek better understanding of the detailed processes involved in deposition of particulate material indoors, an aerosol test chamber has been built. This is an aluminium room in the form of a cube of size 2 m and is provided with two doors, in the top and the side for man access; these doors also house silica windows to allow uv illumination on an opposite surface and to allow viewing by the scanning system of that surface. Artificial surfaces may be mounted in the field of view as required. An external circuit connected to the chamber allows circulation of air at a chosen air exchange rate and also allows the introduction into the ducting of fluorescent labelled aerosol with the aid of a brush type powder dispersion generator. Gross particle loadings at two points within the chamber are also measured as a function of time with optical particle counters. The experimental method involves measuring the time integrated air concentration of aerosol during the injection of aerosol and the subsequent time die away; this, in comparison with the mass deposited on the surface viewed, gives the appropriate deposition velocity. Measurements using representative indoor surfaces mounted on a side wall gave results of the expected order. Modifications to the chamber are currently in hand to equalise uv illumination on both floor and side walls so that accurate cross comparison tests may be made; computerised logging of optical particle counter readings is also being incorporated.

Collaboration with Riso National Laboratory

During 1986 an opportunity for collaboration with the RISO National Laboratory, Denmark became available. The research involved the use of a staff house adjoining the Riso site for the purpose of measuring particle deposition velocities under realistic conditions. Under conditions of constant air exchange and infiltration, and for a non decaying contaminant, the relative indoor outdoor concentrations of an aerosol bearing the contaminant, under steady conditions, is influenced both by the effective deposition velocity indoors and by the building filtration factor. To determine these factors it is necessary to separate them experimentally. In these collaborative experiments the building structure filtration factor was removed by ducting external air directly into the building. The effective indoor deposition velocity may then be determined from the ratio of indoor and outdoor concentrations, the air exchange rate and building volume and internal surface area. The house used was of typical Danish design: a single storey double brick cavity construction with double glazing, a good standard of

insulation and draft proofing. Two experiments were conducted; in the first the house had all the normal fixtures and fittings but was left completely unfurnished, while in the second experiment tests were carried out on the same house in order to determine the effect if any that furnishings would have on the overall deposition velocity. Sulphur hexafluoride sampling was used in prior experiments to determine the air exchange rate. During the experiment proper, air filter sampling both in the incoming ducted air and at points within the house activity of cosmogenic Be-7 associated with aerosol also Cs-137 and yielded Cs-134 also associated with aerosol material. Other workers have indicated that these activities are likely to be associated with aerosols of aerodynamic diameter of about 0.5 μm. Selected deposition velocities inferred from the data, in units of 10^{-4} ms^{-1} were: Be-7 unfurnished 0.33 + 0.8, furnished 0.10 + 0.06; Cs-134 furnished 1.03 + 0.26, unfurnished 0.76 + 0.19. Air exchange rate in both experiments was close to 0.7 h^{-1}.

Simulation Model

A computer model, DHOMO, has been written and embodies all the main transfer processes within a simple representation of a dwelling. Air exchange rate, building structural shielding, a structural filtration factor and indoor deposition velocity are all shown to be important parameters in sensitivity tests. In a representative situation of accidental release to the atmosphere, mechanical transport and resuspension only become significant contributors to indoor exposure over a long time (beyond about 1year). This is analogous to long term contamination from operational releases; in such a situation mechanical transport have been shown to contribute to indoor exposure. The simulation model is currently being rewritten for a modern microcomputer.

Continuation of the Research

In future research, close collaboration will be maintained between the RISO National Laboratory and Imperial College. During the last year, at Imperial College, new, sensitive, methods for labelling monodispersed aerosols with stable tracers have been developed, in the context of resuspension from natural surfaces. These techniques will be used in full scale experiments in dwellings on indoor deposition rates. This will remedy the lack of precise knowledge of aerosol size in the previous experiments and make possible experiments with a range of individual monodisperse particle sizes. At the same time work with the aerosol test chamber will continue in order to understand the important of flow conditions and indoor surface types on deposition velocities. In particular, research will employ both circulation through an external circuit, as has already been described, and time decay using fans mounted within the test chamber. The latter technique should permit a direct comparison between deposition velocity inferred from time decay parameters and that inferred by scanning of wall deposits resulting from the complete test.

Part B. Contamination following a major nuclear accident is concentrated in areas where wet deposition in precipitation occurs. The objectives were to consider how the inhomogeneous nature of the wet deposition patterns would be assessed, and the implications for probalistic risk analysis (PRA) studies of nuclear accidents.

Progress achieved

The Chernobyl accident has emphasised the potential importance of precipitation scavenging for the environmental consequences of a radioactive release.

A complicating aspect has been the extremely patchy nature of deposition with very localised areas of high contamination; it was this problem which has been the subject of this investigation.

The work began with a critical look at the traditional methods of assessing wet deposition patterns in PRA studies, using simple washout models combined with Gaussian plume simulations (ApSimon et al 1986). It was pointed out that these methods were equivalent to the material encountering fixed bands of rain at various distances downwind corresponding to periods of rain; and that this produced artificially high peaks at the leading edge of the rain bands where removal began. Treatments involving radionuclides drawn into moving cloud systems, whose dimensions might or might not spread right across the plume, would give quite different results. The deficiencies of the washout model, and its very simplistic representation of the complex dynamical, chemical and physical processes involved were also considered; including the vacuum cleaner effect of convective clouds drawing sucking large volumes of air in and depositing material in a concentrated patch beneath the core.

Weather radar database

To investigate the patchy nature it was felt necessary to use more realistic data on the spatial and temporal nature of precipitation. The introduction of a weather radar network in the United Kingdom provided the opportunity to assemble a substantial database of precipitation intensity with a fine resolution in space and time; this contained data from the new radar unit at Chenies to the west of London for the whole of 1985. The radar beam scans round at 4 elevations every 5 minutes, and from the reflected signal precipitation intensities are deduced with a spatial grid resolution of 5 hm out to a maximum of 200 km from the radar; thus giving coverage from the Chenies radar over a circular area covering southern England from the east coast across to the Bristol Channel on the west, on which 3 nuclear power stations are sited, and including the major populated areas of London.

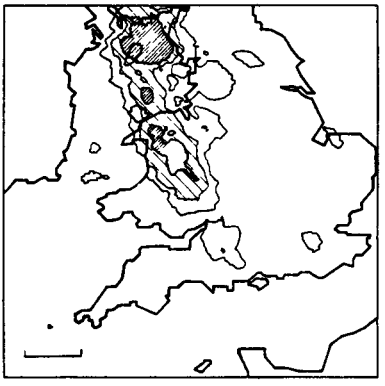
The RAINPATCH model

To use this data in risk analysis calculations a special computer model, RAINPATCH, was developed. This simulates the material released as a sequence of puffs, each following an independent trajectory and encountering precipitation accordingly. Each puff is differentially scavenged according to a simple washout prescription; simultaneously diffusion and turbulent mixing expands the puffs and redistributes the remaining material to some extent. Numerous tests were conducted to ascertain how many points are needed within each puff, and the number of precipitation classes required to represent different precipitation intensities with sufficient accuracy. To complement the weather radar data, windfield information was also accumulated for the same year 1985 over the same area, with assistance from the French and UK meteorological services. The resulting databases, when stored in processed and reduced form, still take up a formidable overall storage capacity equivalent to
20 x 2400 foot magnetic tapes.

Evaluation against the Chernobyl situation

Even with accurate meteorological data, there are still questions of the validity of such a model in view of the complexity of precipitation systems. It was therefore very

interesting to test how well the RAINPATCH model performed in the real situation of Chernobyl. For this the UK Meteorological Office made available data from their entire weather radar network at the time, giving good coverage over the whole of England and Wales. Despite uncertainties in the air concentrations within the cloud, the RAINPATCH model clearly picked out the most affected areas, showing high deposition over N.Wales and the Sellafield region, plus the Irish Sea where elevated levels were subsequently observed by MAFF in marine life. Detailed comparison with analyses based on raingauge data reveal some limitations, partly due to a few missing frames of weather radar data in transmission from the radar network, but also in some mountainous regions due to ground clutter interference with the radar beam.



Wet deposition from the RAINPATCH model contour levels over $\geq$ 500 Bq m^{-2} (close-hatched), 1000 Bq m^{-2} (hatched) and 100 Bq m^{-2} (from Ref.1)

Measured Cs-137 on grass in Bq m^{-2} produced by the Institute for Terrestial Ecology

However the value of weather radar data in emergency situations was clearly demonstrated, and will be incorporated in the new emergency capabilities under development in a collaborative project at the UK Meteorological Office. It was also significant that the estimated patterns of deposition are extremely sensitive to the timing of the passage of contaminated air, with implications for air monitoring requirements in an accident situation. It was also pointed out that monitoring devices are insufficient for this purpose. The results of this comparison have been reported in ApSimon, Collier and Simms, 1988 and ApSimon, Wilson and Simms 1989.

Applications of the RAINPATCH model in a PRA context

The RAINPATCH model has also been applied to a sequence of hypothetical releases over the period of the database collected from the Chenies radar (i.e.1985). To compare the effect of using full observed precipitation fields, a similar set of runs was also undertaken assuming that rainfall changed everywhere synchronously with that observed at the source. This is the normal data availabel in PRA calculations. The first set of runs used spatially varying winds, whereas the second used pseudo trajectories based on winds at the source. However since both treated the release in puff form there is only partial comparison with the Gaussian plume techniques commonly used in PRA (see discussion in ApSimon and Simms 1986, and ApSimon 1986).

A location on the Severn Estuary was adopted for the hypothetical releases, initiated at 29 hourly intervals through the year. This choice of source location was more subject to precipitation events associated with south-westerly flows, and there is higher land to the north west over the Welsh mountains giving orographic enhancement.

Maps of annual deposition were produced to see whether the use of spatially and temporally varying rainfall at different points within the moving puff (points mode) as opposed to source rainfall over the whole puff varying only in time (source mode) alters the geographical distribution of wet deposition systematically. It was expected for example that there might be significant enhancement over areas of higher land in Wales. However situations leading to transport in that direction accompanied by rainfall were infrequent and trajectories tended to bend round the mountains. The contours within 30 hm of the source appeared fairly similar for the points model and the source model. Further away the rather irregular contours tend to be dominated by individual events in spite of the large numbers of releases considered. The source mode tends to show areas at higher concentrations further away from the source than the points model. This is thought to be due to the assumption that in the source mode the whole puff is subject to heavy rainfall when this is observed at the source, whereas in the points mode heavy rain will affect only a portion of a puff. However the total population weighted collective dose, averaged over the 300 releases postulated, turned out to be higher for the points mode than for the source mode. This is because the source mode yields lower levels over the populated areas in the London region, possibly because the source lies within a local rain shadow of high land to the west and the source mode underpredicts precipitation over the London region.

Comparing the proportion of releases which lead to more than 10% of the Cs-137 being deposited in the map area (within about 200 km from the source), 84 out of 300 releases exceed this deposition within the map area in the source mode, and 79 out of 300 in the points mode. This is not a vast difference. Both modes showed the same seasonal trend, although this is affected by higher than average rainfall in June, and a drier than average October. It is difficult to deduce such trends from a single year sample.

Comparison of maximum levels of deposition in various distance bands for source and points modes showed no marked difference within 200 km, although there is a lightly steeper reduction with distance for the points mode. Note however that this is averaged over the widths of the puffs within the distance bands, and hot-spots may be higher in the points mode.

Thus although there are dramatic differences between individual releases in both points and source approximations there appear to be compensating factors so that statistically both show similar characteristics in the context of PRA analysis out to 200 to 300 km from the source. (Note however that plume models could give different results.) The most important factor is the correlation between rainfall and wind direction.

Continuation of the work

Although these results encourage confidence in present PRA analysis, at least as far as the limitations of using source rainfall data are concerned, other aspects may be important. Thus work is continuing on the development and use of detailed storm models to investigate the concentration mechanisms within precipitation systems due to the

dynamical and physical processes. This will indicate the differences of the simple washout model in relation to the vacuum cleaner effect of convective updraughts and the production of hot-spots. This continuation has been partially funded by the NII and by the NERC in the UK.

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

Riso National Laboratory, Denmark (J. Roed)
Environmental Sciences Division. AERE Harwell (K. Nicholson)

V. Publications:

Cannell, R.J. and Roed, J. Relationship between Indoor and Outdoor Aerosol Concentration following the Chernobyl Accident. Radiation Protection Dosimetry 21 (1987) 1/3 pp. 107-110.

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-F-106-B

Studiecentrum voor Kernenergie
SCK/CEN
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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

Optimization 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 exercises. 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:

- Optimisation of the user-system interaction
- Optimisation of the reporting module
- Documentation of the system by a descriptive report and a users'manual.

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).

2. RESULTS

2.1. Introduction

Following the general evolution in the world of nuclear safety analysis and emergency preparedness at nuclear sites the SCK/CEN has developed, as a result of several programmes coordinated by the European Communities, its own real-time emergency assessment software packages for use during accidental situations. Until 1984, those packages, known as CAERS-Computer Aided Emergency Response System , mainly performed atmospheric dispersion calculations.
During the 1985-1989 research campaign, the system has been optimized and extended to define realistic source terms to be included in dose assessments covering all exposure pathways of importance for decision making during the early emergency response phase. This main package is known as CAERSDOS.

2.2. CAERS

Basically the procedure CAERS, including an on-line version and an off-line version, computes using a bi-gaussian dispersion model, half hour mean concentration values and deposition fluxes for a defined receptor configuration. The on-line dispersion parameters are based on the Bultynck-Malet or SCK/CEN turbulence typing scheme and dispersion parameters (ref. 1). A meteorological mast provides all the necessary parameters to run the model. These parameters are stored in a monthly cycling data base, which permits the use of any half hour of the last 31 days. The off-line version allows other combinations of turbulence typing schemes and dispersion parameter sets (e.g. Pasquill, Vogt, Smith-Hosker, ...).
The windvector puff trajectory model determines the centre of most of individually released puffs using one minute values for the windspeed and direction stored in the meteorological data base. Graphical output on a map of the surroundings helps the measuring teams to decide where to measure.
The tri-gaussian puff dispersion model allows a variable source term combined with a variable meteorological input. A choice can be made between instantaneous and time averaged concentration calculations. The off-line version CAERSOFF requires the definition of the run-time options by the user as opposed to the on-line version CAERSON where all settings are done automatically.

For example in the on-line version the user just has to choose the installation, whose characteristics relevant to dispersion calculations are stored in a data base, for which he wants calculations to be done, together with its release conditions. CAERSON then automatically retrieves the installation parameters and the meteorological situation of the last half hour to make calculations in a square grid of 10 km side which is chosen in function of the wind direction. Graphical output in the form of isopleths is generated.
During the run of CAERSOFF the user has to choose amongst e.g.

* reflections on inversion layer
* plume rise
* turbulence typing scheme
* dispersion parameter set
* deposition parameter
* meteorological definition
* receptor set
* ...

2.3. CAERSDOS

CAERSDOS can be considered as the natural extension of CAERS towards a dose assessment model for use during accidental situations, as well as a tool to provide emergency scenarios for training purposes. The backbone of CAERS has been retained. The general structure is represented in fig. 1.

2.3.1. Exposure pathways

A study of the detailed requirements of the system lead to the selection of radionuclides and exposure pathways to consider, based on a review of existing safety studies. The radionuclides are classified according their importance for early effects, late effects committed during the passage of the cloud, those due to external irradiation by deposited materials and those due to consumption of contaminated food. The criterion of 1 % of the total dose due to a specific pathway, lead to the selection of 37 radionuclides to cover the four kinds of exposure. The list is extended on a qualitative base to include 44 radionuclides divided over seven physico-chemical groups as shown on table 1.

The selected exposure pathways are in order of importance : irradiation by deposited material, inhalation and external irradiation by the cloud. A subroutine, dealing with the dynamic behaviour of I-131 in the grass-milk pathway can be linked to the system. A more extensive and systematic handling of foodchain contamination will be included in the near future.

2.3.2. Source term simulator

A relative simple semi-analytical model has been developed to provide the core inventory for a large PWR-reactor in function of power level, burn-up and cooling-time. This model was checked against a large number of runs by the ORIGEN-code, varying the above mentioned input parameters. The semi-analytical model was completed with a numerical interpolation to match the ORIGEN-code data. This simulator is developed

to define source terms for the 44 isotopes for up to 21 time intervals of 1 half hour. The user has to define the release fraction of the inventory in function of time for each physico-chemical group of radionuclides.

2.3.3. Dose calculation module

For each half hour for which a source term has been provided, dose calculations are performed. The user can either introduce self-defined meteorological situations or rely on the meteorological data base. In the near future a small data base with 20 meteorological situations each consisting of 21 consecutive half hour meteorological data, covering a broad range of typical conditions recorded in Mol will be introduced in the Meteorological Data Simulator to provide realistic dispersion conditions to the trainees.
The doses are assessed for the following exposure pathways : cloud-shine (effective and skin), inhalation (effective, lungs, thyroid and red bone marrow), external exposure by deposits (effective and skin for integration intervals of 1 hour, 1 day, 1 year and 30 years).
The cloud-shine dose is performed using the numerically determined ratios of finite to semi-infinite cloud shine calculated by Gamertsfelder (ref. 2).
The user has to provide the fractions of elementary, organic and particulate iodine-131 to determine the effective deposition fluxes. The ingestion dose calculations, projected to be included very soon, will provide for the receptor with the greatest deposition the maximal concentration in milk, meat, green vegetables, grain and roots for the isotopes I-131, Cs-137, Sr-90 and Ru-106.

2.3.4. Reporting module

In the same way as for CAERSON the output is provided for a square grid with side of 10 km. The results are provided as well numerically as graphically in the form of isopleths for each considered exposure pathway.

3. DISCUSSION

The system, developed under this project, meets the initial objectives. However a detailed documentation and programme description is still lacking. During the prolongation of the contract, an extended foodchain module will be added. The opportunity of the addition of a decision logic with respect to countermeasures will be investigated.

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	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	KR 85M	...	...	...	...	...	...
2	KR 85	...	...	...	...	...	...
3	...	...	RB 86	...	...	...	...
4	KR 87	...	...	...	...	...	...
5	KR 88	...	...	...	...	...	...
6	...	...	...	...	SR 89	...	...
7	...	...	...	...	SR 90	...	...
8	...	...	...	...	...	...	Y 90
9	...	...	...	...	SR 91	...	...
10	...	...	...	...	...	...	Y 91
11	...	...	...	...	...	...	NB 95
12	...	...	...	...	...	...	ZR 95
13	...	...	...	...	...	...	ZR 97
14	...	...	...	...	...	MO 99	...
15	...	...	...	...	...	TC 99M	...
16	...	...	...	...	...	RU103	...
17	...	...	...	...	...	RH105	...
18	...	...	...	...	...	RU105	...
19	...	...	...	...	...	RU106	...
20	...	...	...	SB127	...	...	...
21	...	...	...	TE127M	...	...	...
22	...	...	...	TE127	...	...	...
23	...	...	...	SB129	...	...	...
24	...	...	...	TE129M	...	...	...
25	...	...	...	TE129	...	...	...
26	...	I 131	...	...	...	...	...
27	...	...	...	TE131M	...	...	...
28	...	...	...	TE132	...	...	...
29	...	I 132	...	...	...	...	...
30	...	I 133	...	...	...	...	...
31	XE133	...	...	...	...	...	...
32	...	I 134	...	...	...	...	...
33	...	...	CS134	...	...	...	...
34	...	I 135	...	...	...	...	...
35	XE135	...	...	...	...	...	...
36	...	...	CS136	...	...	...	...
37	...	...	CS137	...	...	...	...
38	...	...	...	...	BA140	...	...
39	...	...	...	...	...	...	LA140
40	...	...	...	...	...	...	CE141
41	...	...	...	...	...	...	CE143
42	...	...	...	...	...	...	PR143
43	...	...	...	...	...	...	CE144
44	...	...	...	...	...	...	ND147

Group 1 : noble gases
Group 2 : halogens
Group 3 : alkali metals
Group 4 : tellurium group
Group 5 : alkaline earths
Group 6 : transition metals
Group 7 : lanthanides and actinides

Table 1 : list of 44 isotopes included in 7 groups

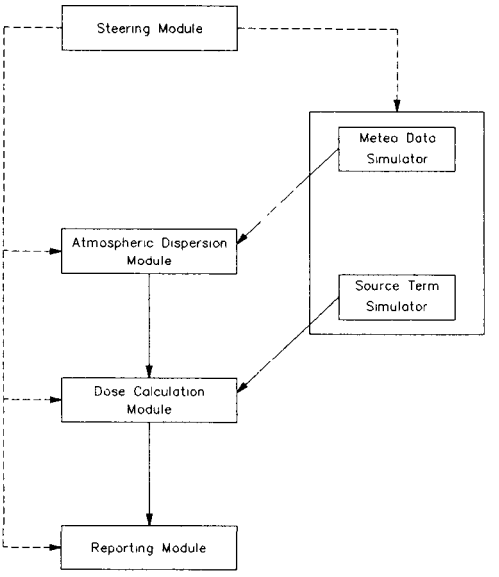

Fig 1 General structure of CAERSDOS

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

V. Publications:

P. GOVAERTS
Input Data to Real-time Computing of Consequences of Radioactive Releases to Atmosphere. Presented at the CEC-Workshop on Real-time Computing of the Environmental Consequences of an Accidental Release to Atmosphere from a Nuclear Installation, Luxembourg, 17-20 September 1985.

P. GOVAERTS, J. KRETZSCHMAR
Computer Aided Emergency Response System of the SCK/CEN Mol. XXIVth Symposium of the International Professional Association for Environmental Affairs, Luxembourg, May 1987.

Title of the project no.: 2

Feasibility study of feedback of survey results.

Head(s) of project:

P. Govaerts

Scientific staff:

A. Sohier

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:

- Documentation of feasibility tests
- Definition of generic probability functions to be assigned to environmental monitoring data
- Introduction of those distributions in the numerical feedback approach
- Discussion of problems related with use of on-line monitors

III. Progress achieved:

1. METHODOLOGY

- Simulation of environmental survey results to test parameter optimisation techniques by artificial data and data from tracer experiments.
- Development of an optimisation technique for simple applications.
- Demonstration of this technique.

2. RESULTS

2.1. Introduction

Following an accident at a nuclear facility an emergency staff will meet to ensure an adequate information flow to decision makers. Required countermeasures to the population will be based on the degree of contamination in the surroundings. Initially, only computer simulations based on real-time plant status and meteorological information will be available. An uncertain knowledge of model parameters will possibly lead to erroneous and cost-ineffective decisions.
At the same time mobile sampling teams will be guided in the most affected areas. Only a few reliable samplings will be performed per hour. Model based extrapolations to other places will still be necessary.
This study examines the possibility of feed-back of early environmental survey data to the model to increase the confidence on its results. Some aspects of importance concerning the sampling strategy will be pointed out. In any case the interaction measurements-computations will have to result in an accurate diagnosis of the particular situation at the time of the accident and not to make nice predictions which will be true only in a statistical sense.

2.2. Optimisation techniques

Virtually all environmental survey data available up to now consist of concentration data gathered during tracer experiments. Therefore our attention has mainly been focussed towards the interaction of air concentration measurements on predictions.
In simple terrain the main sources of uncertainty are :

- source term
- effective height
- wind direction
- dispersion parameters
- wind speed

The first optimisation scheme (fig. 1) is based on the comparison, using a subjective acceptance criterion, of measured and calculated data. The technique was decribed in the progress report of 1987. The main drawback of this procedure is the possible generation of solutions which fit well mathematically but without relations with the physical reality. Therefore a second optimisation scheme (fig. 2) was considered and which is based on a regression of measured data, assuming the validity of a general dispersion model. As will be shown in the tracer experiment examples, the best way to obtain a realistic set of model paramaters is to sample on a few arcs centered around the ground level projection of the plume axis and located at both sides of the effective height dependent location of the ground level concentration (glc) maximum.

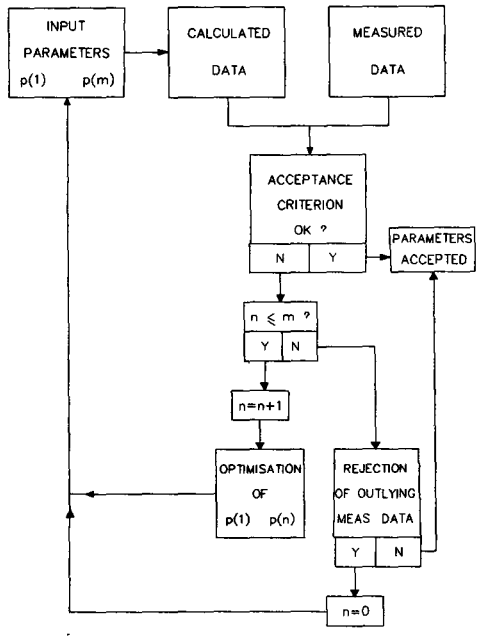

Fig. 1 General scheme of the optimisation system (first approach)

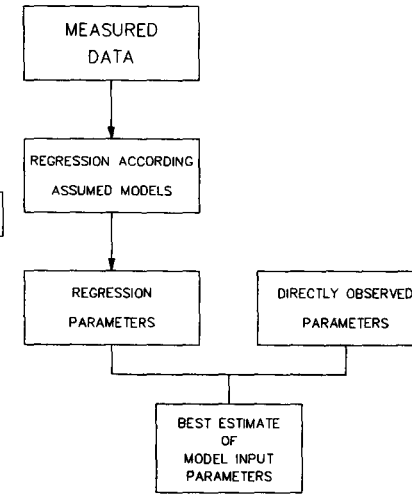

Fig. 2 General scheme of the optimisation system (second approach)

2.3. Relations between measurements and calculations of concentrations

2.3.1. Simulation of measurements with an exact bi-gaussian model

The insight of the processes of importance in a feed-back procedure can be increased in a first stage on a simplified subset of the physical reality. In a later stage the stringent conditions can be relaxated to face more realistic situations. Following assumptions are made in the first stage :

- one pollutant of which the effective height is reached instantaneously
- the measurements consist of exactly 1 half hour average glc
- the concentration distribution follows perfectly a bi-gaussian law with reflection at ground level
- the dispersion parameters are those of the SCK/CEN (ref. 1).

The N "measurements" will be found according the following expression

$$\hat{\chi}_i(x_i, y_i, o) = \frac{\hat{Q}}{2\pi \hat{u} \cdot \hat{ab}\, x_i^{0.796} \cdot x_i^{0.711}} \cdot \exp\left(-0.5\left(\frac{y_i}{\hat{a} \cdot x_i^{0.796}}\right)^2\right) \cdot \exp\left(-0.5\left(\frac{\hat{H}}{\hat{b} \cdot x_i^{0.711}}\right)^2\right)$$

in a coordinate system where the wind direction φ follows the X-axis. A cap above a parameter indicates a "true measured" value. In reality the 2 most unknown parameters will be the source term Q and the effective height H. The meteorological parameters (wind direction $\hat{\varphi}$, wind speed $\hat{u}$ and the horizontal and vertical dispersion coefficient ($\hat{a}$ and $\hat{b}$) will probably be known within a smaller uncertainty range. The optimization is done by varying the model parameters, according some rules, in the P dimensional space of P parameters $\{p_j, j=1...P\}$. The purpose is to find the parameter set $\{\hat{p}_j\}$ which minimalizes a norm (§ 2.4) which relates the difference between computations and measurements. Even in this ideal situation unicity is not guaranteed and depends on the sampling choice.

a) Independently of the sampling scheme, following relations will always hold

$$Q = \frac{\hat{Q}}{\hat{u}} u$$

However u is known within a smaller uncertainty range than Q.

b) In case of N samples on 1 arc transversal to the plume a dependent relation between H and b exists for which the relative chi square norm (§ 2.4) is equal to zero. Fig. 3 shows a family of such curves for several distances from the arc to the origin.

c) In case of N samples lying on the ground level projection of the plume axis one curve in the 3 dimensional space {a,b,H} reduces the relative chi-square norm to zero :

$$b(H) = \hat{b}/\hat{H}.H \; ; \; a(H) = \hat{a}.\hat{H}.1/H$$

d) For N random samples distributed as well horizontally as transversally a unique solution will emerge, except for the Q-u dependency.

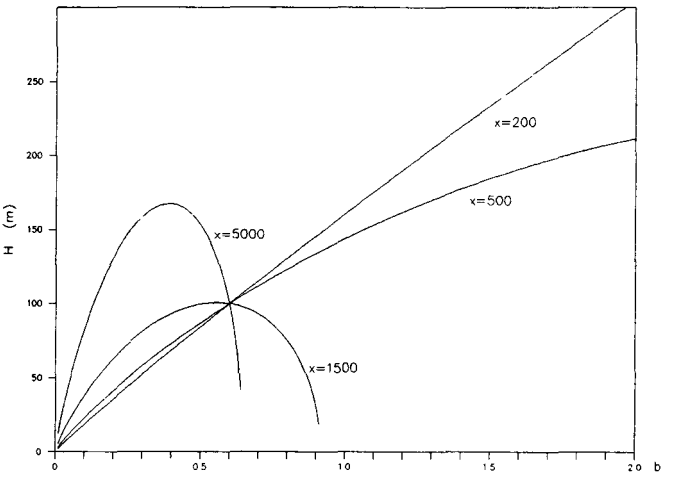

Fig 3 Variation of parameters (b,H) around $(\hat{b},\hat{H})=(0.6,100)$ for receptors on rings at several distances from origin, giving $\chi^2_{red} = 0$

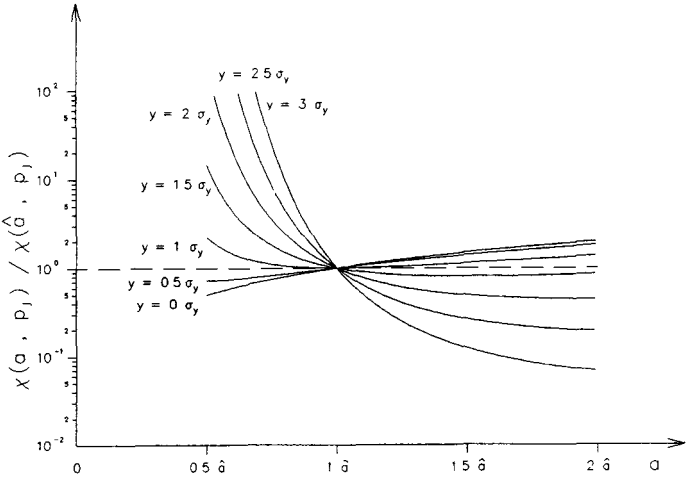

Fig 4 Sensitivity of simulated concentration on variation of a, for 7 different lateral distances from the receptor to the plume-axis

2.3.2.Simulation of realistic measurements

The removal of the restriction of the exact bi-gaussian distribution for the measurements leads, for a chosen norm, to a more complex parameter space with several local minima, none of them equal to zero. The selection of subdomain of the parameter space will partly necessarily rely on expert judgement of the physical process and partly on a judicious removal of out-lying data (§ 2.4, § 2.5).

In relation to this the sensitivity of the gaussian plume model for small variations of the parameters are very important in the optimization process. Fig. 4 shows e.g. the dependence of the concentration on the variation of a, for a receptor lying at several lateral distances from the centre of the plume.

2.4. The choice of a comparison criterion

The basic idea behind any regression scheme is the minimization of the difference between measured and the corresponding computed value of the same physical quantity on the base of the numerical value of some criterion. The adequacy of a particular criterion must be demonstrated. Three simple statistical measures or norms without arbitrary weights and with straightforward physical interpretation have been selected to establish conditions under which environmental data can be regressed under real time conditions.

2.4.1. Chi square, relative chi square and log norm

Given N physical distinct points for which measured as well as model based computed values of the same physical quantity (e.g. concentrations) are available, it is possible to relate the distance between the computations C_i and the measurements M_i ($i = 1...N$) using a norm :

* chi square : $$\chi^2 = \frac{1}{N} \sum_{i=1}^{N} (M_i - C_i)^2$$

The goodness of chi square grows point by point in favour of high concentration. Chi square is biased towards high concentration value optimization. This can clearly be seen in the tracer experiment simulations : although the maximum measured concentration is well matched, most of the time it fails to find the observed profiles. For this reason it is suggested not to use this norm in future feed-back schemes.

* relative chi square : $$\chi^2_{rel} = \frac{1}{N} \sum_{i=1}^{N} \left(\frac{M_i - C_i}{M_i + C_i} \right)^2$$

Identical relative differences are weighted identically, independently of the magnitude of the concentration. Each point for which data are available will be equally important in the optimization process. On the condition that the observations behave like the model, a good optimization can be expected. Practically, as suggested in the tracer experiment analysis, a cut-off of low concentrations (§ 5) has to be made.

* log norm : $$L = \frac{1}{N} \sum_{i=1}^{N} \left| \log (C_i / M_i) \right|$$

Again identical relative differences are weighted identically independently of the concentration magnitude. However, as opposed to relative chi square, there is no upper bound of the influence of the ratio of measured to computed concentrations on this norm. The optimization result is even more sensitive to the presence of outlyers (i.e. points which do not behave according the assumed model). The application of a cut-off remains a necessity.

Figures 5 and 6 show the effect of the contamination of a small fraction (f_2) of receptors with ratio C/M = a_2 on the 2 norms, in function of a_2 for several ratios C/M = a_1 for the remaining fraction (1 - f_2) of receptors. The presence of a small fraction (f_2 = 0.10 to 0.30) of outlyers has a very important influence on the norm. The optimization mainly results in a meaningless choice of parameters which reduce a_2 at the expense of a_1. This is even more true for the log norm.

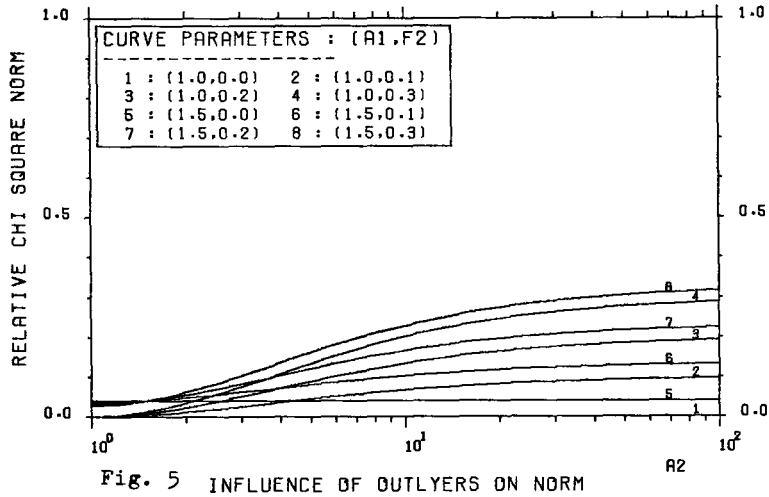

Fig. 5 INFLUENCE OF OUTLYERS ON NORM

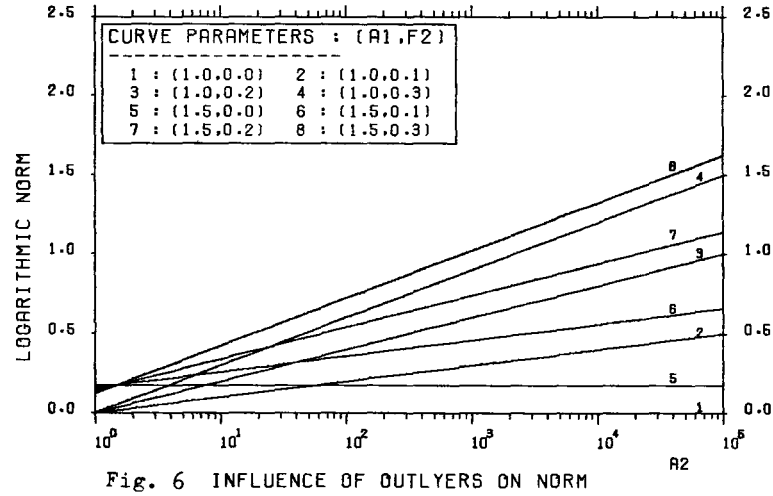

Fig. 6 INFLUENCE OF OUTLYERS ON NORM

2.5. Tracer experiments

The principles of feed-back have been applied to a variety of controlled tracer experiments involving 1 hour mean air concentration. Because of the nature of a tracer experiment Q and H were supposed to be well known. The dispersion coefficients a and b and the wind direction were authorized to vary. The exercise for several experiments under different conditions (ref. 2, 3, 4) reveals :

* The choice of input parameters only based on meteorological data can lead to wrong values.
* Feed-back feasability increases together with measurement angular resolutions.
* Optimization on different rings for the same experiment can lead to a different set of parameters (e.g. φ). An uncertainty factor must be included.
* Due to a lack of vertical profiles, the acquisition of σ_z values remains delicate.
* The possibility of physical meaningfull feed-back depends heavily on the validity of the assumed regression model. In most gaussian situations this requires a cut -off of low value tailing data which do not behave gaussianly. The cut-off occurs at a distance between 2 to 3 σ_y-values from the plume center (i.e. 10 % to 1 % of the maximum).
* In some (non gaussian) situations feed-back simply does not work.

As an example fig. 7 shows the feed-back for a Mol tracer experiment. All the measurements were considered and the 3 rings were considered as well separately as together. Fig. 8 shows the same process with the exclusion of tailing data. Fig. 9 shows the simulation with a parameter choice only based on meteorological data.

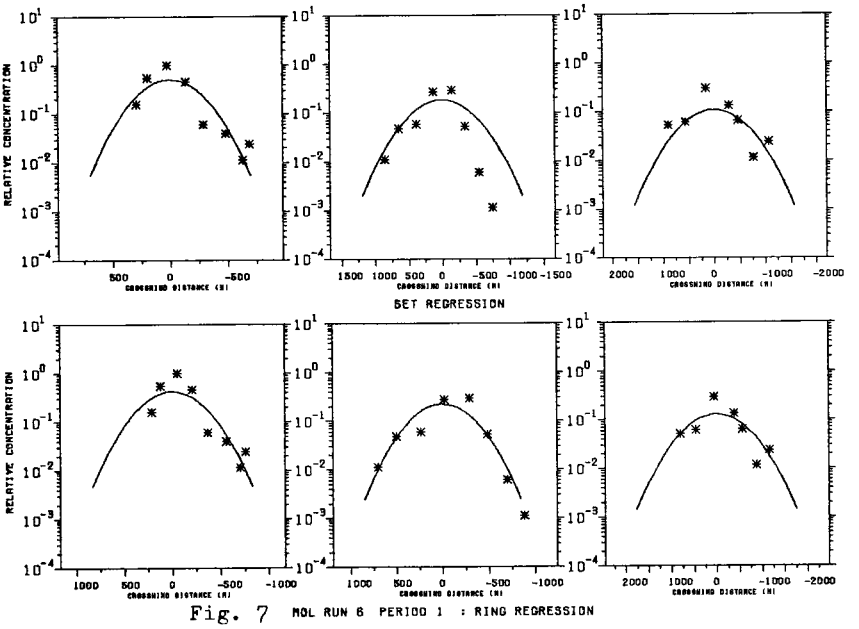

Fig. 7 MOL RUN 6 PERIOD 1 : RING REGRESSION

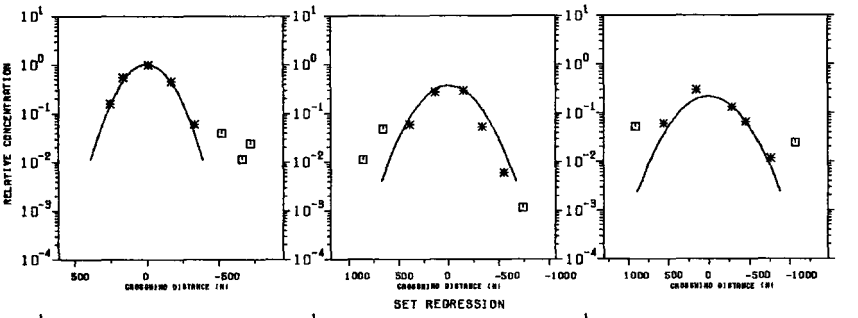

Fig. 8 MOL RUN 6 PERIOD 1 : RING REGRESSION

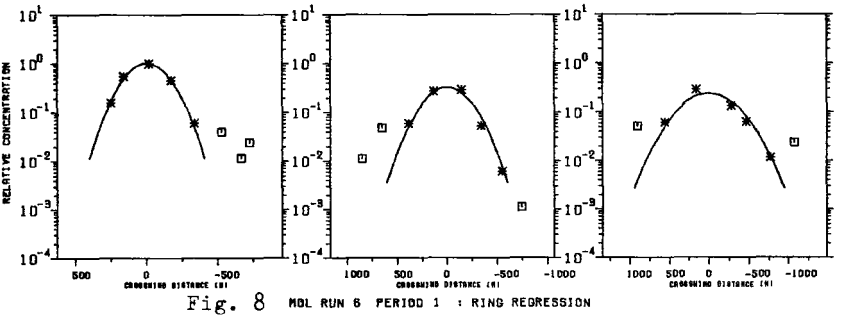

Fig. 9

3. DISCUSSION

Feed-back of environmental survey data can be applied in most non complex situations considering an adapted sampling scheme. An independant variation of model parameters using the relative chi square norm can fulfill the basic requirements. To reduce computer time a simple analytical (e.g. gaussian like) model is to be preferred. The sampling must be performed on several rings at several distances from the source. The samples must be within 2 to 3 σ_y-values from the ground level plume axis projection, rejecting a non gaussian background. A real time graphical output on video display can be very valuable. An inherent uncertainty factor, especially for the wind direction, has always to be considered. The effective plume height and the source term will have to be found from monitoring data and from the spatial variations of the glc. Still further investigations have to be performed in topics including :

* smaller sampling/averaging times, the effect of intermittency
* relation between concentrations and radiation measurements (2-dimensional and 3-dimensional integration)
* deposition for a complex source-term

Finally, it is believed that as long as an accidental situation remains simple enough feed-back will be a very valuable tool, although without being a universal panacea to resolve all unknowns in the early investigation phase.

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4. Agterberg R., Nieuwstadt F.T.M., Dispersion experiments with sulphur hexafluoride from the 215 m high meteorological mast of Cabauw in the Netherlands. Royal Netherlands Meteorological Institute, De Bilt, Van Duuren H., Hasselton A.J., Krijt G.D., Kema Laboratories, Arnhem.

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

V. Publications

P. GOVAERTS, A. SOHIER
Feedback of environmental survey data for the optimisation of the input parameters of assessment models during an emergency. Proceedings of the 17th International Technical Meeting of NATO-CCMS, Cambridge, September 1988.

P. GOVAERTS, A. SOHIER
Feedback of environmental survey data for the optimisation of the input parameters of assessment models during an emergency. OECD/CEC Workshop on Consequence Assessment Rome (Italy), 1988.

P. GOVAERTS, G. FIEUW, B. VANDERBORGHT
L'optimisation du contrôle de l'environnement en cas de rejets accidentels dans l'atmosphère.in "Emergency planning and preparedness for nuclear facilities", IAEA-SM-289O/45, 1986, p. 323-334.

P. GOVAERTS, A. SOHIER
Feasibility study on the optimisation of the input parameters of assessment models during an emergency by feedback of environmental survey data.
2nd International Workshop on real-time computing of the environmental consequences of an accidental release to atmosphere from a nuclear installation, Luxembourg, 16-19.3.89.

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
T. Zeevaert

I. Objectives of the project:

The model developed by the first project will be used to generate a set of scenarios for the purpose of emergency response training. The scenarios will be selected according the multiple objectives an emergency exercice can have. The scenarios are represented by the schedule of information flows between the different functions of an emergency response team, and will discuss the decision making process.

II. Objectives for the reporting period:

- Further development of a computer assisted system for scenario selection based on the perceived needs for training.
- Development of a standard format for the representation of the scenarios.

III. Progress achieved:

1. METHODOLOGY

In order to obtain a set of emergency scenarios, which can be selected in a logical way for the training of specific tasks of emergency response, following tasks can be distinguished :
1. Collection of a 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.

During this period, an example of scenario selection has been worked out based on the analysis of the emergency response organisation and the global set of potential objectives for an exercise.

2. RESULTS

2.1. PROCEDURE

The definition of an emergency response scenario requires :
- an analysis of the emergency preparedness organisation
- the description of the nature and severity of potential releases
- an analysis of the emergency decision logics

A more detailed analysis will list the functions to be tested and the corresponding elements of the scenario that have to be defined. This process will be performed in three steps :
1. Definition of a coarse release scenario
2. Definition of a detailed release scenario
3. Check whether the detailed scenario meets the exercise objectives
4. Detailed description of the time dependent input of information by the scenario to the different units of the emergency organisation

2.2. EMERGENCY PREPAREDNESS ORGANIZATION

The basic structure of the organization for which scenarios will be prepared is shown in Fig. 1. Two coordination units will be subject of testing ; the in-plant coordination unit (1), for which the nuclear facility operator is responsible ; and the off-site coordination unit (2), for which off-site authorities are responsible. The scenario has to provide the input information that is necessary for the unit to perform the function (1) to be tested. These functions can be the transformation of information or the execution of an action. This structure is used here only for the sake of showing the approach.

2.3. FUNCTIONS TO BE TESTED

The functions that are to be performed by the coordination units considered, may be classified into the following categories :

1. Communications

Communications between the coordination units
Communications between unit 1 and in-plant
Communications with off-site emergency centers and authorities
Communications with environmental survey teams

2. Activation of Emergency Organization (notification)

Alerting relevant staff of the operating organization and public authorities.

3. Gathering of information

Acquisition and analysis of data

4. Evaluation

Identification of the nature and importance of the accident.
Assessment of the situation : current status and potential development of the emergency
Determination and prediction of the quantities and rates of release and of the resultant radioactive contamination
Assessment of the projected exposure to personnel and to persons off-site

5. Implementation of actions (countermeasures)

5.1. Corrective/mitigatory actions

e.g. to prevent or reduce uncontrolled releases, fire control, ...

5.2. Protective actions

for plant personnel and off-site public

Early phase : sheltering, respiratory protection, administration of KI tablets, evacuation, anticipated harvesting, removal of animals from pasture, ...
Intermediate phase : sheltering, evacuation, relocation, control of access, control of foodstuffs and water, use of stored animal feed, ...
Recovery phase : decontamination, fixation of contamination, introduction of barriers to radionuclide transfer, controlled re-entry of the population, ...

5.3. Aid to affected persons

Removal of injured persons
Decontamination of exposed persons, first aid, medical treatment, transportation to medical services or hospitals, ...

5.4. Radiological survey in the environment

Direct radiation measurements
Air concentrations
Deposition on surfaces
Contamination of vegetation
Contamination of milk (e.g. foodstuffs)
Concentrations in surface waters (drinking water sources)
etc...

5.5. Information of the public, of authorities, of neighbour countries, of international organisations, ...

2.4. SCENARIO-ELEMENTS

The following elements defining the scenario, have to be assessed in accordance with the requirements for the testing of the functions concerned.

1. The type of the nuclear facility :
   standard : PWR
   variable : power, operation time (determining, together with the cooling time, the core inventory)

2. The type of incident :
   cooling time : determines the amounts and types of radionuclides available for release (core inventory)
   magnitude of release in function of time (expressed as percentages of the core inventory), energy content of the release, ...

3. The meteorological situation in function of time :
   parameters : atmospheric stability, wind velocity, wind direction, dry weather or rain

4. Demographic characteristics :
   geographical distribution of the population : density, places of concentrations (hamlets, towns, recreation sites ...)

5. Results of the radiological survey in function of time at specific reference points in the environment

2.5. DETERMINATION OF THE SCENARIO

The requirements for the functions concerned to be generated may be more or less stringent for the selection of the accident scenario.
Some functions (e.g. communications) do not require a specific scenario in order to be generated. Other functions require only a threshold on released quantities or on individual doses to be exceeded or a specific type of incident to have occured. Still other functions require a more detailed scenario e.g. individual doses situated between upper and lower bounds at certain places and with a certain distribution in time. Hereafter the scheme is explained, we followed to determine the elements of the scenario leading to the generation of a function of the last category, namely the implementation of protective measures for the public in the early phase of an accident. The ultimate criterion for the

decision on those measures is the individual dose. The decision-making can be schematised through a so-called decision tree. Decision trees or protective measures for the public (early phase) have been developed for two cases (the release has not yet started or is already ended), based on the reference levels of individual dose indicated in ref. 1.
Three basic principles are applied :

- for a certain countermeasure to be imposed, a specific reference level (threshold) of individual dose has to be exceeded ;
- with less drastic countermeasures the individual dose remains higher than this refererence level ;
- the imposition of the countermeasure has to bring about a sufficiently high benefit (= decrease of individual dose).

In view of a decision logic tree, the elements of the scenario can be determined in accordance with the countermeasure intended. As will be observed, a certain degree of freedom remains, depending on the action envisaged. It is possible to make use of this when several functions have to be tested simulateneously. The methodology foresees a first approach with a limited number of standard release scenarios and meteorological conditions. In this way the order of magnitude and the kind of meteorological sequence needed to reach the objectives of the exercise can be defined. In a second phase the elements of the scenario are to be refined.

- Type of nuclear facility and type of incidence :

These elements have to be chosen so that the required magnitude and time evolution of the release are generated.
A source term simulator has been developed, which is capable of calculating the core inventory of a standard PWR as a function of the power of the reactor, its operation time and the cooling time. In order to determine an order of severity of the releases, 4 typical classes of releases (Table 1) covering a broad range of accidents, were considered.

- Meteorological parameters in function of time :

The meteorological situation has to answer to the requirements of individual doses (through inhalation and external radiation), starting from the class of accident (= magnitude of release) assumed. Would it be impossible to yield the required dose values, another class of accident has to be chosen.
The meteorological situation may be selected from a meteo data bank consisting of 20 typical assemblies of 20 consecutive half hourly averages of the meteorological parameters observed at the meteo tower of the SCK/CEN.
The meteorological situation selected can be introduced into a computer aided emergency response system (CAERS-DOS) in order to convert the releases into dose values which may be graphically displayed on a monitor.
When applying the decision tree for the case concerned on these values, it becomes possible to delimit the zones where typical countermeasures for the public should be implemented. A graphical representation for two classes of accidents and two meteo situations is given in Fig. 2.

- Demographic characteristics

These characteristics may be adapted, according to the extent or the scale on which the action envisaged has to be carried out. If it is not possible to fullfil the requirements, another class of accident or another meteo situation has to be chosen.

- Results of radiological survey in function of time

The protection actions for the public to be decided on may require results of measurements in the environment (as a verification of the predictions). These results are to be in accordance with the releases and meteo situation assumed, taking into account a certain range of natural variability. Outliers, representing measurement or sampling errors may also be considered. The time delays for these results to become available are to be assessed on a realistic basis. A small basic-model was developed to determine the availability of monitoring data in function of time using realistic times for unit operations. A generic result for several preplanned monitoring circuits is shown in Fig. 3.

3. DISCUSSION

A generic methodology to define emergency response scenarios has been developed. This method should be implemented in an organised way on a P.C. and illustrated by a set of examples. This work is foreseen during the prolongation of the project during the 1990-1991 period.

References

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   Radiological Protection Criteria for Controlling Doses to the Public in the Event of Accidental Releases of Radioactive Material.
   Luxembourg, July 1982 (V15290/82)

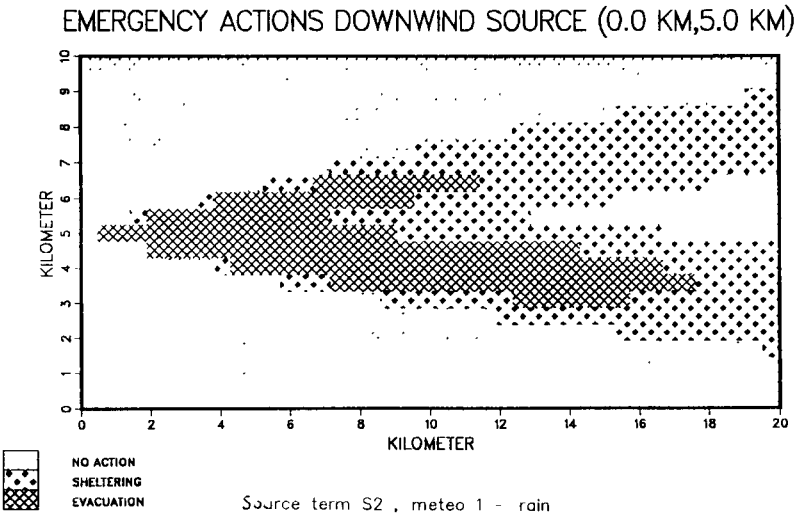
2. J. DUCO, J. BRISBOIS and D. QUEMIART
   French Safety Rationale for PWRs.
   Proceedings of OECD Specialist Meeting on filtered containment venting systems (CSNI Report 148), pp. 1-20, Paris, 17 and 18 May 1988.

Table 1

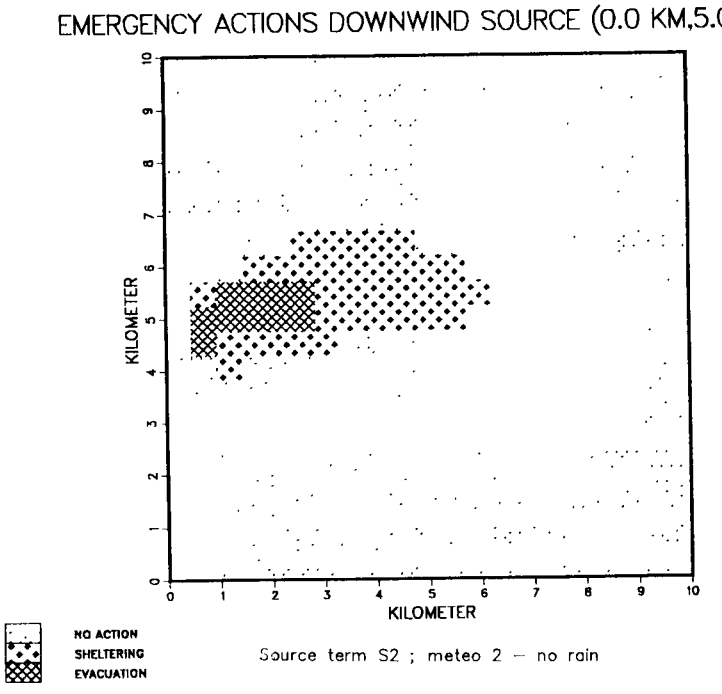
Typical classes of releases (expressed in % of the core inventory)

Class	Cooling time	Group I Noble gas	Group II Iodia		Group III Cs	Group IV Te	Group V Sr,Ba	Group VI Ru	Group VII La-act.
			Inorg.	Organs					
S_1 (1)	1 h	80	60	0.7	40	8	5	2	0.3
S_2 (1)	10 h	75	3	0.5	5	5	0.5	0.5	0.1
S_3 (1)	20 h	75	0.3	0.5	0.4	0.4	0.4	0.03	0.005
H (2)	1 h	3.5	4.0 E-5	2.0 E-5	0	0	0	0	0

(1) adopted from Ref. 2
(2) adopted from Ref. 3

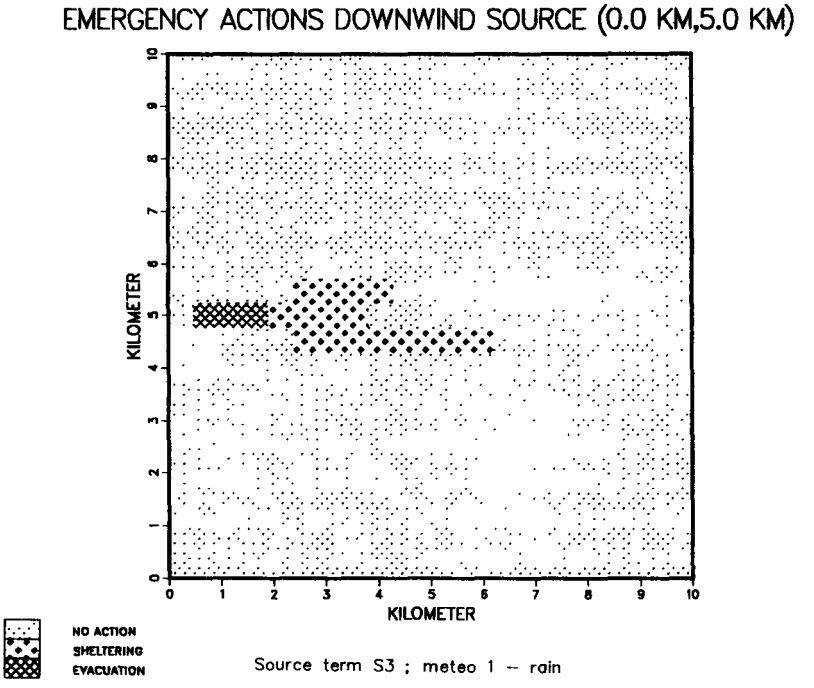


Source term S2 , meteo 1 - rain

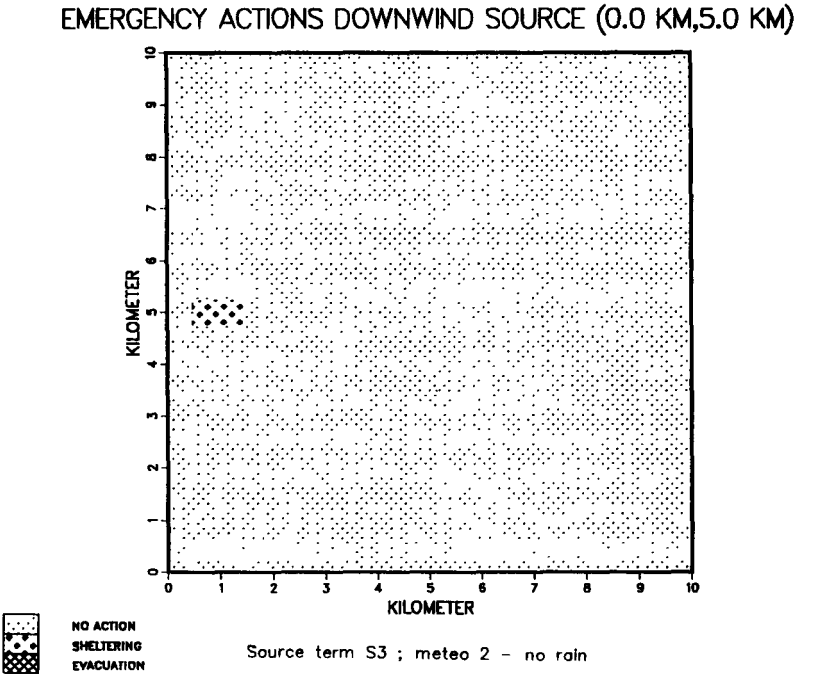


Source term S2 ; meteo 2 – no rain

fig. 2a



Source term S3 ; meteo 1 – rain



Source term S3 ; meteo 2 – no rain

fig. 2b

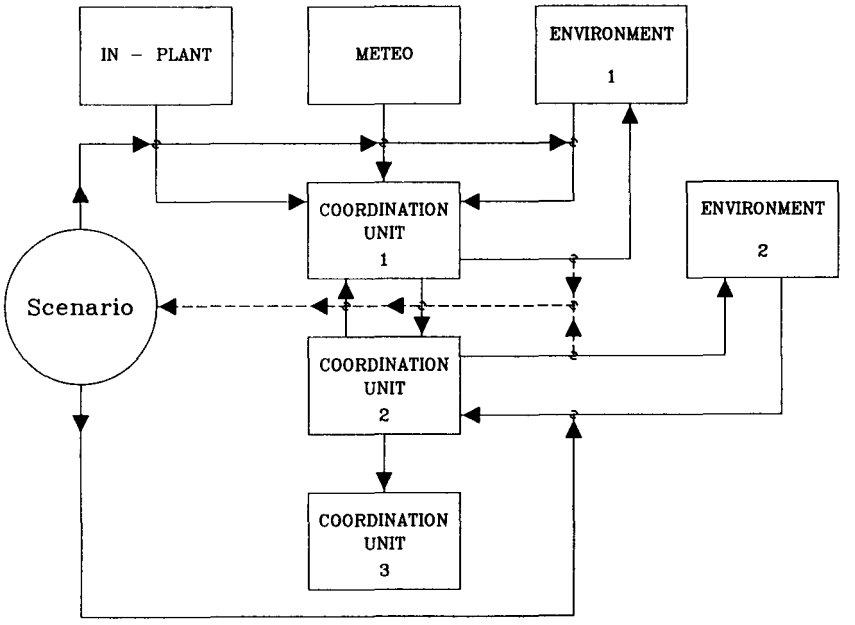


Fig. 1 : Basic structure for emergency response exercise scenarios.

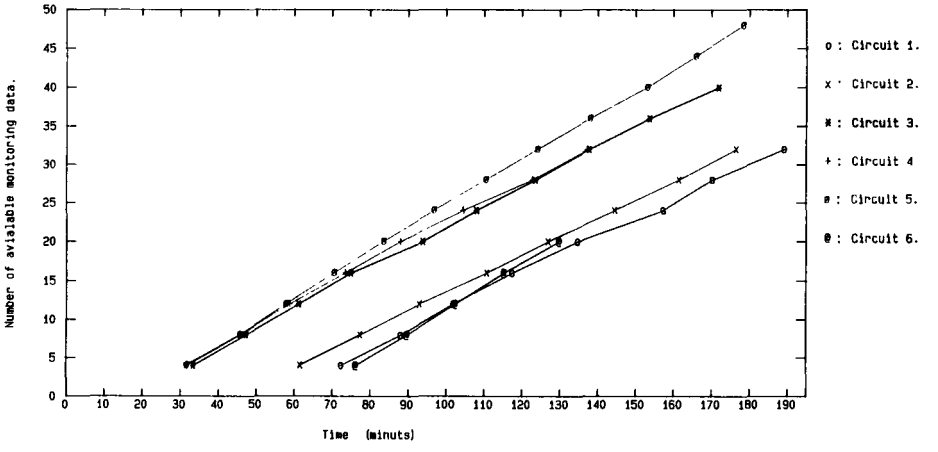


Fig 3. Typical simulation of the availability of monitoring data in function of time for several cicuits.

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

V. Publications:

P. GOVAERTS
Blootstellingswegen van de bevolking in geval van een ernstige radioaktieve lozing in de atmosfeer.
Annalen van de Belgische Vereniging voor Stralingsbescherming, Vol. 13, n° 3, pp. 205-222 (1987).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

United Kingdom Atomic Energy
Authority, UKAEA
11 Charles II Street
GB- London SW1 4QP

Contract no.. B16-F-131-UK

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

Dr. M.R. Hayns
Safety and Reliability Directorate
Wigshaw lane, Culcheth
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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:

B Y Underwood

Scientific staff:

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 assessment of the range of applicability of the simple models and enabling the formulation of appropriate modifications where necessary.

II. Objectives for the reporting period:

As above

III. Progress achieved:

Methodology

The work was subdivided into a number of tasks and the approach adopted for each task is detailed separately below:

(a) Dry deposition to vegetated surfaces

The principal aim here was to elucidate the dependencies of dry deposition velocity on the physico-chemical form of the pollutant, on the meteorological conditions (windspeed and stability) and on detailed characteristics of the surface cover.

Although a number of semi-empirical correlations exist in the literature, a sounder basis for specifying these dependencies is to develop a mathematical model describing how momentum and pollutant material are extracted from the airflow by the elements making up a stand of vegetation. Such a model was developed within this contract, building on earlier work in the literature. A feature of the model is the coupling together of the momentum equation - which yields the windspeed profile in the canopy - and the contaminant equation (the coupling arising because deposition at individual elements depends generally on the speed of the wind approaching an element).

Turbulent diffusion within the canopy is represented via a 'mixing-length' model which leads to second-order differential equations to describe the profiles of windspeed and pollutant concentration within the canopy. These were solved numerically. A number of analytical solutions to the equations, applicable after additional simplifying approximations, were also investigated.

As a check on performance, the model was used to simulate a set of high-quality wind-tunnel experiments on deposition of particles of various sizes.

(b) Dry deposition to the urban complex

The fundamental assumptions of vegetative-canopy modelling were examined to ascertain whether or not this approach had much to offer in the estimation of dry deposition to the urban 'canopy', ie to the array of buildings etc within a city.

This investigation then widened out into a critical review of experimental and theoretical approaches of potential use in calculating the deposition to arrays of bluff elements, including a discussion of the applicability of the concept of deposition velocity in the urban situation. This part of the work benefited from contact with Jørn Roed of Risø, who has made careful measurements of deposition to urban surfaces partly under the aegis of the Radiation Protection Programme.

(c) Interpretation of puff-trajectory modelling

Although the main thrust of the work in the contract as a whole has been concerned with deposition velocity and the coupling of deposition processes to dispersion modelling, task (c) focussed on an aspect of dispersion modelling per se. The origin of the work lay in the growing use of puff-trajectory modelling in probabilistic consequence assessment (PCA) codes as a way of circumventing the limitations of the straight-line Gaussian model, particularly for long-duration releases and when the pollutant has to be tracked over long distances.

A feature of puff-trajectory modelling is the use of a wind field (available at known spatial and temporal resolution) together with ensemble-average σ values (dispersion parameters). The nature of the concentration estimates thus obtained requires careful interpretation, and the main goal of the work was to develop such an interpretation from first principles. In the course of this development, it became possible to evaluate a variety of approaches and techniques that have been adopted in the past to implement puff-trajectory modelling.

(d) Deposition in foggy conditions

Foggy conditions have not been explicitly dealt with in probabilistic consequence assessment to date, and the aim of this task was to examine the potential impact of this omission.

The investigation was undertaken with reference to three scenarios, representing key foggy situations of interest: (i) release of radioactive pollutant into coastal advection fog, (ii) release into widespread radiation fog and (iii) release in clear conditions but with the pollutant being carried by the wind to regions of elevated terrain shrouded in ground-contacting cloud. The frequency of occurrence of these condi-

tions is an important parameter in the PCA context, and approximate estimates were made for typical sites in the UK, based on climatological data.

The impact of foggy conditions derives from part of the released contaminant becoming incorporated into fog droplets, thereby experiencing an enhanced deposition velocity compared to material not so incorporated. Thus the key quantities of interest are the fraction of material that is taken into droplets and the magnitude of the enhancement in deposition velocity. The latter was estimated for the three scenarios using both experimental data and theoretical considerations. Incorporation of particulate matter was discussed in terms both of attachment to existing droplets and of nucleation of 'new' droplets by the released particulate.

Using hypothetical source terms, a number of illustrative calculations were performed to indicate the potential impact of foggy conditions on the conventional end-points of PCA.

(e) Deposition of large particles

The objective of this task was to assess the adequacy of the simple prescriptions currently used in the PCA context for handling the dispersion-deposition of 'large' particles, ie particles with appreciable gravitational settling velocity.

This work built on the results of an earlier project undertaken within the MARIA I programme in which the predictions of eddy-diffusivity theory were used as the reference against which simple extensions to the Gaussian plume model were evaluated. The limitations of eddy-diffusivity theory for this purpose were recognised at the time, and the current work takes up the suggestion made there of using a Lagrangian-particle model as the reference, since the latter is better able to represent up-to-date understanding of turbulent dispersion in the atmospheric boundary layer. The solution to the model is obtained by Monte-Carlo simulation.

The version of Lagrangian-particle model utilised was an alternative to the more widely used Langevin-equation approach, on the grounds that the former enabled deposition to be investigated in a more intuitively understandable manner. The credentials of the reference model were first examined by detailed comparison of its predictions against available, albeit not plentiful, experimental data. The various simple models were then evaluated against the reference model over a

range of particle size. A technique was worked out for accounting in the reference model for the inability of large particles to follow completely the eddy motions of the air, and the impact of this was assessed.

In the course of this work, it became clear that there was no rigorously defined method for including the surface deposition of small particles and gases in Lagrangian-particle models. The formalism for this was worked out for the model used in the present work, paving the way for a calculation of deposition velocities within the Lagrangian-particle framework.

The investigation of models for 'heavy' particles described above was carried out for the case of the neutral surface layer of the atmosphere; the extension to layers with other turbulence characteristics requires a specification of how to handle a non-uniform turbulent-velocity scale, traditionally a difficult question for Lagrangian-particle models. The final task undertaken was to derive the correct method of including such a non-uniformity in the model employed.

Results

(a) Dry deposition to vegetated surfaces

The canopy model developed here performed well in detailed comparisons with wind-tunnel measurements of deposition velocity to an idealised vegetative canopy. The comparisons included data for particles in the size range 0.1-30 μm and for a contaminant gas. This work was published in a journal article /1/. The predictions for the partitioning of the deposit between canopy elements and the substrate were less successful, indicating the need for a better representation of turbulence in the lower reaches of the canopy.

The insights provided by this modelling activity were then used to deduce the broad aspects of the expected dependence of deposition velocity to vegetative canopies (within a specified range of types) on physico-chemical form of pollutant, on meteorological factors (windspeed and stability) and on canopy properties. In particular, this exercise shed light on the applicability and limitations of various semi-empirical correlations available in the literature and on the somewhat confusing statements that have been made in the past concerning dependence on stability. This work was published as an SRD report /2/.

(b) Dry deposition to the urban complex

Regarding the potential of vegetative-canopy modelling to yield an approach for estimating the overall deposition velocities to an urban complex, it was found that many of the key assumptions and simplifications operative in the vegetation case were no longer tenable in the urban case and that the models, therefore, had little to offer in quantifying the relationship between bulk urban deposition velocities and the properties of individual elements within the 'canopy'.

The concept of an overall urban deposition velocity itself was questioned and found to require careful handling in view of inhomogeneities and edge effects.

Wind-tunnel modelling of the deposition to arrays of bluff elements was considered as a means of providing correlations for use in assessment work, but it was found that important questions concerning similarity remain unanswered. It was also concluded that mathematical modelling of this situation has not developed sufficiently to provide a predictive tool nor a means of generalising from measurements made of particular contaminants depositing on particular urban surfaces. The 'naive' estimate of urban deposition velocity, which equates it to the area-weighted aggregate of the deposition velocities for individual surface types in the city, is not a rigorously defensible approach; it may turn out to be adequate for assessment purposes, but there is insufficient information to form a judgement.

The review of urban deposition was first published as an SRD report /3/ and subsequently in a revised form as a journal article /4/.

(c) Interpretation of puff-trajectory modelling

The work here developed an interpretation of puff-trajectory modelling which focuses on the relationship between lack of information on wind-field fluctuations of period less that a given value, T, and the spatial resolution of the estimates of dosage from a particular release. Quantitatively, the smoothing length of the dosage field obtained is closely related to the width of the ensemble of potential deviations of serially released particles from their trajectory positions arising from wind fluctuations with timescale less than T. These widths are the 'puff' σ values that should be applied.

Thinking through this interpretation enabled a critical assessment to be made of aspects of the constructions used in the past to implement puff-trajectory modelling, such as puff merging and splitting, and to suggest an alternative construction that takes seriously from the outset the question of the spatial resolution of the estimates obtained. This work was published as an SRD report /5/.

(d) Deposition in foggy conditions

This work concluded that the enhancement of deposition during foggy conditions could have a significant impact on a number of the conventional end-points of consequence assessment for some source terms and site characteristics. The sample calculations illustrated how increased deposition close to the release point could impact on consequences involving a threshold, whilst at the same time attenuating the amount of material remaining airborne for transport to centres of population. Taking into account both frequency of occurrence and potential impact, it appears that foggy conditions require explicit recognition and handling in probabilistic consequence assessment.

A major uncertainty was identified, however, in quantifying the impact of foggy conditions: it concerns the route by which released particulate becomes incorporated into droplets. Scoping calculations indicated that attachment to existing fog droplets is an inefficient process; the mechanism with the potential to create a major effect is the formation of fog droplets on the pollutant particles themselves. This process, however, is sensitively dependent on the physico-chemical characteristics of the released particles and on microphysical properties of the fog. For the former, this would require a greater level of detail in source-term characterisation than conventionally used in consequence assessment; for the latter, meteorological parameters would be required that are not routinely recorded. Further work on these points is necessary.

The work has been published as an SRD report /6/.

(e) Deposition of large particles

The 'reference' model developed in this work, a version of the Lagangian-particle model, was found to give results in good agreement with experimental data both for non-depositing

pollutant and for particles with appreciable settling velocity, giving confidence in its ability to shed light on the performance of simple extensions to the Gaussian model. In comparisons with the latter, it was found that the Source Depletion Approximation (SDA) - the extension widely used for small particles and gases - works well even for particles with appreciable settling velocity, up to the point where the latter reaches about one half the turbulent velocity scale in the atmosphere, whereas for larger particles the tilted-plume approximation (TPA) works better.

However, even the TPA becomes inaccurate as the ratio of settling velocity to turbulent velocity increases much beyond unity, leading to significant underprediction in the rate at which ground-level concentration falls off with distance beyond the point of maximum concentration (and the latter concentration is also underestimated, but not by a large factor). The discrepancy is aggravated by the failure of larger particles to faithfully follow the eddy motions of the air. A report of this work has been submitted to the CEC in a preliminary version /7/ and is about to be published as an SRD report.

The search for a formalism to include surface deposition of small particles and gases in the Lagrangian-particle model reached a successful conclusion, and the technique for calculating deposition velocities which follows from the analysis was demonstrated for some simple examples. A report on this part of the work is currently in press /8/.

The way in which the model must be extended in order to deal with layers having a non-uniform turbulent velocity scale has been discovered, paving the way for application of the model to a wider range of problems. As an example the model has been applied to an idealised vegetative canopy and, thereby, has provided some insights into the limitations of using the eddy-diffusivity approach in this context. A report of this work is in preparation /9/.

Discussion

(a) Dry deposition to vegetated surfaces

In past sensitivity studies of PCA models, it has been demonstrated that deposition is an important factor. Conventionally, many of the potential dependencies of the dry deposition velocity have not been included in PCA, for ex-

ample the dependence on windspeed and the dependence on characteristics of the underlying surface. With regard to the latter, data bases on land use - which have a number of applications in PCA - open the possibility of recognising differing deposition to regions of differnt surface cover (eg built, forest, crops, water). The present work would assist in the implementation of this categorisation.

Also, information on the physico-chemical characteristics of the released material is increasing in response to source-term research in recent years, which, in turn, makes it feasible to account for the initial size distribution of particulate matter and its subsequent modification during travel downwind. The present work would assist in enabling this finer detail to be taken advantage of in calculating deposition fluxes.

The modelling exercise also indicated that the partitioning of deposited material between the substrate and the elements of the vegetative canopy (which appears in PCA via the 'initial interception factor') is likely to be much more strongly dependent on particle size and windspeed that currently accounted for.

(b) Dry deposition to the urban complex

Much of the population in Europe lives in built-up areas and there has been a growing interest in recent years in a more realistic quantification of the doses to people in cities resulting from accidental releases of radioactivity. Calculation of external dose is complicated by the shielding effect of structures, and there has been a drive to evaluate these more rigorously. However, the dose calculation is dependent on a knowledge of the total amount deposited for a given bulk air concentration in the city and on the relative amounts deposited on various categories of surface (roofs, lawns, vertical walls).

The present work has shown how far we are at present from being able to estimate these quantities in terms of specified characteristics of the urban configuration or to generalise with confidence from the measurements that have been taken in a relatively few particular situations. More modelling effort (mathematical and, perhaps, physical also) is required to support the field compaigns that have been undertaken.

(c) Interpretation of puff-trajectory modelling

The use of puff-trajectory models in the PCA context leads to increased computational demands and, usually, increased data requirements. It is thus important to be clear as to what are the precise benefits of the approach in this context compared to the straight-line Gaussian, particularly bearing in mind the uses to which PCA results are put and the uncertainties in other phases of the risk calculation. The present work helps to bring this question into focus - for the particular issue of how to handle long-duration releases - by discussing them in terms of spatial and temporal resolution.

Atmospheric dispersion models cannot give information on the spatial structure of the concentration field from a particular release at greater resolution than that set by the turbulent nature of the atmosphere and by the limitations of the wind-field data. The key questions then concern how this resolution compares with the length scales set by other inputs to the calculation - such as the population data, land-use data or rainfall data - and how significant is the impact of unresolved concentrations fluctuations on consequence end-points. To answer these questions requires a substantial amount of further work.

(d) Deposition in foggy conditions

In PCA calculations, the meteorological conditions at any instant are characterised by only a few variables, typically wind direction, wind speed, atmospheric stability, mixing layer height and the intensity of rainfall (if any). In such schemes, foggy conditions would be 'squeezed' into one of the available meteorological categories (for example, for advection fog, typically D Pasquill category).

The work carried out here, however, shows that, at the upper limit, the magnitude of the consequences in foggy conditions could be very different from those in the non-foggy weather conditions included in the same category. Although intense rainfall can produce similar consequences, the frequency of incidence of fog at any point may well exceed the corresponding frequency of intense rain. This points to the need to treat fog separately if distortion of the frequency-consequence spectrum is to be avoided.

Nevertheless, there are significant problems in dealing with fog explicitly, both concerning the meteorological data and the quantification of the effect, as discussed in the 'Results' section above, and further investigation is required.

(e) Deposition of large particles

The work undertaken here has confirmed that, for neutral atmospheric stability at least, dry deposition for quite a wide range of particle size can be adequately handled by one or other of the simple extensions to the Gaussian plume model. The work has indicated the ranges over which each of the two principal extensions is preferable. Furthermore, it has quantified the discrepancies expected as particle size increases beyond these ranges. The results obtained from the reference model could form the basis for a convenient parameterisation in the regime where the simple models fail.

A similar picture is expected to emerge for non-neutral conditions but some further work is required to confirm this; the groundwork for this continuation has been laid within the current project.

Lagrangian-particle techniques (often called Monte-Carlo methods) are being increasingly considered both for assessment work directly or as a reference against which simpler models can be tested. A rigorously-defined method for dealing with realistic deposition at the ground has so far not been given, and the present work breaks new ground in establishing the correct approach for the particular type of Lagrangian-particle model utilized here. This opens the door to the model finding use in a wider variety of situations.

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

None

V. Publications:

1) B Y Underwood (1987). Dry deposition to a uniform canopy: evaluation of a first-order-closure mathematical model. Atmospheric Environment **21**,1573-1585.

2) B Y Underwood (1987). Dry deposition to vegetated surfaces: parametric dependencies. SRD R 442. Safety and Reliability Directorate, AEA Technology.

3) B Y Underwood (1987). Dry deposition to an urban complex. SRD R 423. Safety and Reliability Directorate, AEA Technology.

4) B Y Underwood (1987). Dry deposition to an urban complex. Radiation Protection Dosimetry **21**, 21-32.

5) B Y Underwood (1989). On the interpretation of puff-trajectory modelling. SRD R 483. Safety and Reliability Directorate, AEA Technology.

6) B Y Underwood (1988). Deposition in foggy conditions. SRD R 487. Safety and Reliability Directorate, AEA Technology.

7) B Y Underwood (1988). Gravitational settling of particles dispersing from an elevated point source in the neutral surface layer of the atmosphere. Contract report to the CEC.

8) B Y Underwood (1990) Deposition velocity and the 'collision' model of atmospheric dispersion. I. Framework and application to cases with turbulent velocity scale independent of height. To be published.

9) B Y Underwood (1990) Deposition velocity and the 'collision' model of atmospheric dispersion. II. Extension to cases with turbulent velocity dependent on height. In preparation.

RADIATION PROTECTION PROGRAMME
Final Report

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.: BI6-F-209-UK

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:

Mr A C Ponting
Dr S Wilmott

I. Objectives of the project:

The primary objective is to determine the uncertainty in ingestion dose estimates arising from uncertainties in the foodchain transfer and in the internal (human) dosimetry.

The secondary objective is to estimate the sensitivity of these uncertainties to the statistical sampling method chosen.

II. Objectives for the reporting period:

First, develop coupled foodchain/metabolic models for estimating (i) the thyroid dose from intake of ^{131}I in green vegetables, (ii) the effective dose from intake of ^{137}Cs in milk, and (iii) the effective dose from intake of ^{137}Cs in green vegetables. (NB. A model for estimating thyroid dose from intake of ^{131}I in milk was developed over the previous reporting period.)

Second, perform an uncertainty analysis on the thyroid and effective doses using the models that have been developed.

Third, study the sensitivity of the assessed uncertainties to the statistical methods used and to parameter correlations.

III. Progress achieved:

1. Methodology

(a) In the previous progress statement, it was reported that an analytical expression had been derived for the ^{131}I ingestion thyroid dose-intake factor. A similar expression has been derived from the ^{137}Cs ingestion effective dose-intake factor. The results of an uncertainty analysis on this factor are reported below.

(b) Separate dynamic foodchain models were developed to simulate the time variation of radionuclide concentrations in milk and in green vegetables following a discrete deposition from atmosphere of ^{131}I and ^{137}Cs. These models were directly coupled with the derived expressions for ^{131}I and ^{137}Cs ingestion dose-intake factors.

(c) The results of uncertainty analyses on the coupled models for a deposition in the growing/grazing season are described below. The sensitivity of the derived quantitative estimates of uncertainty to specified parameter correlations was also studied. Results are summarised below. All data used in the uncertainty analysis will be specified in a CEGB report [1]: some of these data have already been specified in [2] and [3].

2. Results

(a) Results of an uncertainty analysis of the ^{137}Cs dose-intake factor were as follows:

(i) Expected value = 1.08×10^{-8} Sv/Bq (This compares well with the ICRP30 value of 1.3×10^{-8} Sv/Bq.)

(ii) Uncertainty band based on 5th and 95th percentiles = A factor times/divide of 2.

(b) The uncertainty analysis of the coupled models was carried out on a dose expressed in Sv per Bq/km^2 initial deposition. Results were as follows:

Case	Expected value $\left(\frac{Sv}{Bq/km^2}\right)$	Uncertainty band (5th & 95th percentiles)
^{131}I/milk	8.57×10^{-4}	x3/÷8
^{137}Cs/milk	8.59×10^{-15}	x3/÷8
^{131}I/green vegetables	2.45×10^{-13}	x3/÷8
^{137}Cs/green vegatables	1.22×10^{-13}	x3/÷14

(c) The following parameter correlations were included in the sensitivity studies:

(i) correlation between thyroid mass and fractional uptake of iodine to thyroid,

(ii) correlation between body mass and biological half life of ^{137}Cs in the body,

(iii) correlation between soil fixation of Cs and transfer of soil incorporated Cs to milk, and

(iv) correlation between soil fixation of Cs and root uptake.

Results were as follows:

^{131}I/milk: Small decrease in expected value and a small reduction in the uncertainty band

^{137}Cs/milk: No effect on any of the results

^{131}I/green vegetables: Small decrease in expected value and a small reduction in the uncertainty band.

^{137}Cs/green vegetables: No effect on any of the results.

3. Discussion

Detailed results of the project will be presented in a CEGB report to be published [1]. A paper will also be presented covering the highlights of the project at a forthcoming CEC Seminar in Rome. All of the objectives for this reporting period were achieved. In particular it has been possible, possibly for the first time, to couple directly time dependent foodchain and internal dosimetry models, the resulting software package being sufficiently economical so as to allow detailed uncertainty analyses to be carried out. In addition, also possibly for the first time, the results of the uncertainty analyses were subjected to a sensitivity analysis both to specified parameter correlations and to the statistical method employed (Latin Hypercube method versus a technique described in [2]).

The overall conclusions from the work are as follows:

(a) The expected values for the ingestion dose-intake factors for ^{131}I/thyroid and ^{137}Cs/effective dose compare well with but are somewhat less than the ICRP30 values. The uncertainty was estimated to be a geometric factor of 2, based on the 5th and 95th percentiles, ie there is a 90% probability of the dose-intake factor being within a factor 2 of the expected value.

(b) The uncertainty, as defined above, is in the approximate range x3/÷10 on the expected value of dose, expressed in Sv per Bq/km^2 initial deposition for all the cases studied. This result is applicable only for the case of deposition in the growing/grazing season; however, the time of year chosen for the study is one which maximises the significance of the ingestion pathway.

(c) The effect on the results of correlations between specific metabolic parameters and between soil fixation rate of ^{137}Cs and its uptake into milk and green vegetables was studied. The studies indicate that these correlations have only a small impact on the results.

(d) The Latin Hypercube method for carrying out uncertainties was tested against an alternative technique and the minimum number of LHS trials needed to obtain acceptable resutls was established for all cases.

References

[1] S Wilmott, S Nair, A C Ponting, 1989, "An uncertainty analysis of the ingestion dose following a discrete deposition from atmosphere", CEGB/Nuclear Electric report to be published.

[2] S Nair, A C Ponting, 1988, "Seasonality and uncertainty aspects of ingestion dose: Some preliminary results", Proc. of a NEA/CEC Seminar on Recent Advances in Reactor Accident Consequence Assessment, Rome, January 1988.

[3] S Nair, A C Ponting, 1985, "An analysis of uncertainty and of dependence on season of year of ingestion population dose arising from design basis accidents in advanced thermal reactors", CEGB report TPRD/B/0628/N85.

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

Contact was maintained with Studsvik on their PRISM method for carrying out uncertainty analyses. This extended an existing collaboration involving a joint analysis of Chernobyl data.

V. Publications:

S.Wilmott, S Nair, A.C. Ponting, 1989, "An uncertainty analysis of the ingestion dose following a discrete deposition from atmosphere", CEGB/Nuclear Electric report to be published.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Institut National de la Santé et
de la Recherche Médicale, INSERM
101 rue de Tolbiac
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Contract no.: BI6-F-126-F

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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.: BI6-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
Ms C. GUIHEUNNEC and V. LASSERRE

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:

. Compare the results of the modified tests to those of a multiple regression with spatially parametrised variance-covariance matrix of errors.
. Investigate the feasability of defining non parametric permutation tests of association where the permutations would preserve some aspects of the spatial structure.
. Update the mortality data file.

III. Progress achieved:

This report presents a synthesis of the progress achieved during the first five years of this contract. It is not a final report since the contract has been extended until the end of 1991.

1. Aims

During the period 1985-1989, statistical methods suited to the analysis of models of association between spatially defined variables were developped along the following lines :

- design modified tests of simple and partial correlations between sets of spatial variables which take into account the existence of spatial autocorrelation,
- investigate the performance of these statistics in terms of type I error and power and compare them to standard tests,
- implement these tests on French geographical data with particular reference to the link between lung cancer and industrial exposure,
- implement multiple regressions at a geographical level with different spatial parametrisations of the variance-covariance error matrix,
- compare on some French geographical data the results given by the modified tests to those obtained by these multiple regressions,
- investigate the feasability of defining non parametric tests of association based on permutations.

Further the mortality file was recently updated to include mortality rates for specific cancer sites for the period 1984-1986 and in the last part of the project this new mortality data will be analysed along with industrial pollution and low dose radiation with the help of the new statistical methods which have been developped during this project.

2. Modified tests of simple and partial correlations

The product moment correlation coefficient r is a widespread measure of association between two variables X and Y and the partial correlation between X and Y conditionnal on a set of variables measures the residual link between X and Y after adjustment on these variables.

Examples abound of geographical, medical or ecological applications in which the variables are observed at a variety of spatial locations and where correlation coefficients are calculated and tested in the standard way. This standard procedure discounts the existence of spatial autocorrelation for both the variables X and Y.

2.1 - Methodology

We have first devised a modified test of association based on r_{XY} : the empirical correlation between pairs of observations (X_α, Y_α), $\alpha \in A$ where A is a set of N locations [1]. We shall use the notations : $\bar{X} = N^{-1}(\Sigma X_\alpha)$; $s_{XY} = N^{-1} \Sigma (X_\alpha - \bar{X})(Y_\alpha - \bar{Y})$, $s_X^2 = N^{-1} \Sigma (X_\alpha - \bar{X})^2$ (and similarly for $\bar{Y}$ and s_Y^2) and $r_{XY} = s_{XY}/s_X s_Y$.

Suppose that X and Y are independent but that both X and Y are multivariate normal vectors with constant means and variance-covariance matrix Σ_X and Σ_Y respectively. Imposing a stratified structure for Σ_X and Σ_Y so that pairs in AxA are

divided into strata S_0, S_1, S_2, ...such that the covariances within strata are constant, ie cov $(X_\alpha, X_\beta) = C_X(k)$ if $(\alpha,\beta) \in S_k$, i.e. we have derived an estimate of the variance of s_{XY} :

$$N^{-2} \Sigma N_k \hat{C}_X(k) \hat{C}_Y(k) \qquad (1)$$

where N_k is the numbers of pairs of AxA in strata S_k and $\hat{C}_Y(k)$ (respectively $\hat{C}_Y(k)$) is the estimated autocovariance : $\hat{C}_X(k) = \Sigma_{S_k} (X_\alpha - \bar{X})(X_\beta - \bar{X}) / N_k$.

Thus the estimate takes into account the autocorrelation of both X and Y. Further it can be shown that, to the first order, the variance of r_{XY}, σ^2_r is :

$$\sigma^2_r = \frac{Var(s_{XY})}{E(s_X^2)\, E(s_Y^2)} \qquad (2)$$

which leads to the following estimate of $\hat{\sigma}^2_r$: $\hat{\sigma}^2_r = \dfrac{\Sigma N_k \hat{C}_X(k) \hat{C}_Y(k)}{N^2 s_X^2 s_Y^2}$. (3)

In particular cases (for instance when either Σ_X or $\Sigma_Y = I$) it can be shown that the approximation given by (2) is exact and that r follows a t-distribution, t_{N-2}, (N-2 d.f) with the relationship : $N = 1+1 / \sigma^2_r$. In general we shall define an effective sample size $\hat{M}$ with the relationship : $\hat{M} = 1 + (\hat{\sigma}^2_r)^{-1}$ where $\hat{\sigma}^2_r$ is given by (3) and consider a modified t-test : $t_{\hat{M}-2}$ by which we reject the null hypothesis of no association when : $|(\hat{M}-2)^{1/2}\ r(1 - r^2)^{-1/2}| > t^\alpha_{\hat{M}-2}$

where $t^\alpha_{\hat{M}-2}$ is critical value of the t-statistic with $\hat{M}-2$ d.f.

We then extended this method for testing the association between two variables (Y_α, Z_α) adjusted on a third one, X_α, $\alpha \in A$ [2]. Its generalisation to any number of adjustment variables is straightforward.

We suppose that the 3N vector (X,Y,Z) follows a multivariate normal distribution. Then the joint distribution of (Y,Z) conditionnal on X is also a multivariate normal. We can therefore test the hypothesis : $\rho_{YZ.X} = 0$, where $\rho_{ZY.X}$ is the partial correlation between Y and Z conditional on X, by testing that the correlation between the residuals of the regression of Y on X and of Z on X is zero. Hence, the method previously outlined can be extended to test the partial correlation.

In practice, this implies using the modified $t_{\hat{M}-2}$ statistic on the residuals of the linear regression of Y on X and of Z on X respectively. These residuals need therefore to be estimated. We are proposing to do this by ordinary least squares (O.L.S.), thus ignoring the autocorrelation, since the O.L.S regression estimates are unbiased.

2.2 - Results

Simulation models

The performance of these tests was assessed by Monte Carlo simulation of X and Y for two models :

a) X and Y were generated on 3 lattice sizes (12x12,16x16,20x20) as nearest neighbour isotropic autoregressive gaussian processes,

b) X and Y were generated on the grid of the administrative centres of the French départements as gaussian variables with a disc model for their autocovariance and autocorrelation parameter $\rho(1)$. Several levels of autocorrelation in X or Y are chosen and 500 trials were executed either under the null hypothesis to assess type I errors, or under an alternate hypothesis to study the power.

Type I errors

With a nominal rejection level of 5 %, the observed type I errors of the $t_{\hat{M}-2}$ statistic for testing the simple correlation coefficient r_{XY}, ranged from 4.1% to 5.9% in the lattice case and from 2.8 % to 7.2 % in the irregular grid case (Fig.1). In contrast the standard t-test procedure based on N-2 d.f. leads to type I errors as high as 55 % instead of 5 % in the lattice case and as high as 52 % in the irregular grid case, with highly autocorrelated X and Y (Fig. 2).

Results on type I errors of the modified test of partial correlation are shown in Table 1.The performance of the modified $t_{\hat{M}-2}$ statistic is satisfactory as the value 5 % belongs to all the confidence intervals and there is no systematic variation with increasing autocorrelation. The empirical variance of $r_{YZ.X}$ is also shown to increase significantly with the autocorrelation. As expected, the type I errors of the non adjusted standard test of partial autocorrelation are greatly inflated for high values of the autocorrelation.

These results clearly demonstrate the importance of using a modified test when the presence of spatial autocorrelation is suspected. Further the modified statistics developed performed well under the null hypothesis for a wide range of parameters and for two different models of the spatial variations of X and Y.

Power

The power of the modified statistics was investigated under an alternative hypothesis of a linear model between X and Y, [2][3]. The process Y was defined as Y=aX + W. Five hundred 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 the modified tests, we also calculated the power of the classical test of r_{XY} in a case which would be compatible with the observed empirical variance of r_{XY}, v_e, estimated by the Monte Carlo simulations. For non autocorrelated variables X and Y, the variance of r_{XY} for a sample of N observations is approximately equal to $(1 - \rho^2_{XY})^2 / N\text{-}1$ when N is large. 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 π of the classical test of r_{XY} based on N* observations. Similars steps were followed in studying the power of the modified tests of partial correlation. The equivalent sample size, N* for the computation of reference value π for the power of the modified $t_{\hat{M}-2}$ statistic of partial correlations is given by :

$$N^* = 2 + (1 - \rho^2_{YZ.X})^2 / v_e$$

A summary of the results is shown in Tables 2 and 3. On the first line one can see that v_e increases noticeably with increasing ρ_X and ρ_W. N* and π are given respectively on the 2nd and 3rd lines. The observed power of the test $t_{\hat{M}-2}$ is given on the 4th line. Clearly the modified tests perform satisfactorily as their power are comparable to π.

Implementation of these tests to French geographical data

Computer programmes were developed which carried out these testing procedures on French data collected by départements. The testing method was applied to study the association between lung cancer and industrial factors after adjustments on cigarette consumption and a linear gradient (Table 4). Interesting results come to light on significant links with the metal industry and general engineering which agree with results of individual epidemiological studies. This confirmed the suitability of the methods developped for studying problems in geographical epidemiology. Using the modified tests substantially diminished the d.f. in some cases. As a consequence the association of lung cancer with the textile industry becomes non significant. This points out again the importance of using the modified tests in the case of geographically autocorrelated data.

In the next paragraph we describe the alternative regression approach which was developed during the year 1989 and compare on the same examples the results from the modified tests and those from the regression.

3. Regression with spatially parametrised variance-covariance error matrix

In the regression models involving spatially distributed variables, the spatial structure can be taken into account by allowing spatial autocorrelation in the error variable, i.e. considering models of the form

$$\underset{(nx1)}{Y} = \underset{(nxp)}{X}\ \underset{(px1)}{\beta} + \underset{(nx1)}{U} \qquad (4)$$

where U follows a multinormal distribution $N(0, \Sigma_U)$.

In the classical framework of independent errors distributed as $N(0, \sigma^2 I)$, tests of a regression coefficient β_i, $1 \leq i \leq p$, and tests of the partial correlation between Y and X_i conditional on $\{X_j, j \neq i\}$ are equivalent. In the more general framework of (4), tests of the coefficients β_i are done *conditionally* on an estimated structure for Σ_U since the matrix Σ_U is only known in theoretical cases.

Different approaches to the modelling of Σ_U have been suggested. They basically follow two lines :

(i) assuming a specific parametric model for U

(ii) assuming a known functional form for Σ_U

Once the model for Σ_U has been specified, estimation can be carried out in a Gaussian framework by maximum likelihood in cases (i) and (ii) when Σ_U is non-negative definite. When the data set contains a large number of points, maximum likelihood estimation becomes computationally very heavy and is fraught with difficulties.

3.1 Methodology

Three models for the variance-covariance error matrix Σ_U in the regression model : $Y = X\beta + U$ were chosen [4]. The first two involve only one shape parameter for the autocorrelation.

(a) $U = cWU + \varepsilon$, $\varepsilon \sim N(0, \sigma^2 I)$,

W is defined as an (82 by 82) 0-1 contiguity matrix on the French *départements* normalised such that each row sum is equal to unity, and with non-zero (i,j) element if *départements* i and j have a common border length.

(b) Σ_U follows a disc model.

Letting d_{ij} be the distance between locations of *départements* i and j, the (i,j) element of Σ_U is then given by $\sigma^2 f_a(d_{ij})$ with :

$$f_a(r) = \frac{2}{\pi}\left[\cos^{-1}\left(\frac{r}{2a}\right) - \frac{r}{2a}\left(1 - \frac{r^2}{4a^2}\right)^{1/2}\right],\ r \le 2a\ ;\ f_a(r) = 0\ ,\ r > 2a \quad .$$

This covariance function exhibits a fairly linear decrease with increasing distance similar to that observed for several cancer mortality rates in France.

(c) Σ_U follows an exponential model.

The (i,j) element of Σ_U is given by $\sigma^2\ \gamma\ e^{-\lambda d_{ij}}$.

All models were fitted by maximum likelihood.

3.2 Regression results

Metal industry and general engineering workers

There is broad agreement amongst the three models in finding a significant link between lung cancer rates and these two industries, with adjustment for cigarette sales (Table 5). Nevertheless, we observe some amount of variation among the models concerning the regression slopes or their significance levels, with closer similarity within the 1-parameter models. For both industries the disc model gives lower estimates of this residual autocorrelation and consequently higher t values.

Mining industry

A non-significant association between male lung cancer rates and the percent of workers employed by the mining industry is found in all three models (Table 5). There is close agreement of the values of the t-statistics between all three models.

Textile industry

There is a discrepancy between the results given by the disc model which finds a borderline association after adjustment for cigarette sales, and other models which find a non- significant association with the textile industry (Table 5).

Amongst the investigated associations with industrial exposure we thus found overall general agreement between the results given by different parametrisations of the variance-covariance error matrix. Nevertheless, significance levels did vary by a non-negligible ratio and in one case this could lead to a different interpretation of the results. Hence, it is important, when using this regression approach, to check the coherence of the results for at least two different models of Σ_U.

3.3 Comparison between the regression results and the modified $t_{\hat{M}-2}$ statistic results

For the mining industry and general engineering workers, the $t_{\hat{M}-2}$ test agrees overall with the results of the regressions by finding a statistically significant link. For the mining industry there is a divergence of results since the modified $t_{\hat{M}-2}$ statistic finds a significant link at the 5 % level whereas none of the three models do. This example was also investigated after removal of a linear trend component (Table 4), and after this further adjustment the $t_{\hat{M}-2}$ statistic becomes clearly non-significant. This points to the existence of a simple gradient-like structure for the residuals of mining, cigarette sales and lung cancer. For the textile industry, the $t_{\hat{M}-2}$ finds a non-significant link, agreeing qualitatively with the results of two of the models. Since metal industry and general engineering were finally the only two industries significantly linked to lung cancer, a final model was estimated including both these industries, cigarette sales and a linear gradient (Table 6).

For this final model there is very close agreement between the significance levels given by the three parametrisations and the modified $t_{\hat{M}-2}$ statistic.
In conclusion, except for mining, the results given by the modified $t_{\hat{M}-2}$ statistic would lead to the same conclusions as those given by the regression models. We note however that in cases of high autocorrelation the values of the $t_{\hat{M}-2}$ statistic tend to be more moderate in their adjustment for spatial autocorrelation than those obtained by the regressions with parametrised variance-covariance error matrix.This is probably due to the more flexible nature of the adjustment for spatial structure carried out by $t_{\hat{M}-2}$. It is also clearly apparent that the use of standard regressions with i.i.d. errors would lead to quite erroneous conclusions.

4. Feasability of defining tests of association based on permutations

All the models and tests developped so far are based on an underlying assumption of normality of the variables. When this assumption is felt to be too restrive, one usually turns to non parametric tests.

4.1 - Tjøstheim index of association

A non-parametric spatial index of association A was proposed by Tjøstheim (Biometrika 1978). It is based on the sum of the "distances" between locations of similar ranks for the variables X and Y, with a suitably chosen distance function. To evaluate the moments of A under the null distribution of no association between X and Y, the authors assumed that the N! permutations of the values of one of the variables, the other staying fixed, are equally likely. For autocorrelated variables X and Y, this is no longer true and the existing autocorrelations are perturbed by the permutations.This leads to over-significant tests in the case of positive autocorrelation. In a Monte Carlo study the type I error of A was shown to be around 14% instead of the nominal 5% in the most autocorrelated case. Further the power of A under a linear alternate hypothesis (cf 2.2), is very low. Even in the case $\rho_{XY}=0.4$ where the power of $t_{\hat{M}-2}$ is in most cases over 80%, that of the index A does not exceed its significance level. Indeed it is around 5% in most cases and only reaches 10 to 15% in cases of high autocorrelation in X, where it was shown that its significance level is also increased [3].

4.2 - Tests of r_{XY} based on permutations

Monte Carlo tests can be defined, which rank an observed statistic among a set of statistics simulated under the null hypothesis, defining in this way a Monte significance level for the statistic.

In the case of autocorrelated X and Y we first checked that by using all the permutations of the values of X and Y without restriction to simulate the set of statistics, as commented before, the autocorrelation structure is destroyed and Monte Carlo tests are over significant. Indeed for autocorrelations respectively of 0.4x0.4, 0.6x0.6 and 0.8x0.8, the observed type I errors of the Monte Carlo tests based on a 1000 permutations of the irregular network of 82 points, were respectively 10.8 %, 18% and 40 % (with a 5% nominal level).

Next we defined a restricted set of permutations by only allowing local permutations. The 82 points were split into 16 regions and only *within region* permutations were allowed. The type I errors became 6 %, 13.2% and 21.2% respectively. Hence this locally restricted set of permutations leads to some improvement which is nevertheless not sufficient in the highly autocorrelated cases.

Consequently we are now investigating a definition of a more restricted set of permutations. The selection criterion would be to choose those permutations which lead to realisations of X and Y "*close*" to the original ones ; "*close*" being defined with respect to the autocorrelation structure, as a distance measure to be chosen between the original autocorrelations of the observed X and Y and the autocorrelations of the simulated X and Y..

In the next part of this project, several criteria of "closeness" will be considered and the performance of such Monte Carlo statistics evaluated. Further the robustness of the modified statistics under departure from normality will be investigated and the performance of the parametric and non parametric tests compared in these situations.

Table 1

Type I errors (per cent) of the $t_{\hat{M}-2}$ statistic for testing the partial coefficient $r_{YZ.X}$ between $Z = aX + U$ and $Y = cX + W$ after adjusting on X.

500 simulations are carried out for several levels of autocorrelation in X,U and W ($\rho_X(1)=\rho_U(1)=\rho_W(1)$), with $a = 2c = 0.204$.

autocorrelation $\rho(1)$ for variables X,U and W	0.0	0.2	0.5	0.8
empirical variance of $r_{YZ.X}$	0.0117	0.0129	0.0171	0.0500
% type I errors for $t_{\hat{M}-2}$ [95 % CI]	4.2 % [2.4% - 6.0%]	3.8 % [2.1% - 5.5%]	4.8 % [2.9% - 6.7%]	3.8 % [2.1% - 5.5%]
% type I errors for the t test * of $r_{YZ.X}$ based on N observations [95 % CI]	5 % [3.1% - 6.9%]	5.6 % [3.6% - 7.6%]	10.60 % [7.9% -13.3%]	38.60 % [34.3% - 42.9%]

* test of $\sqrt{N-3}\ r_{YZ.X} / (1 - r^2_{YZ.X})^{1/2}$ as a t_{N-3} distribution

Table 2

Power of the $t_{\hat{M}-2}$ statistic for testing the correlation r_{XY} 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 takes the value 0.2 or 0.4.

$\rho_W(1)$	$\rho_X(1)$	$\rho_{XY} = 0.2$ 0.0	0.4	0.8	$\rho_{XY} = 0.4$ 0	0.4	0.8
0	v_e	0.01227	0.01193	0.01345	0.0093	0.00801	0.01059
	N*	N*=76	N*=78	N*=70	N*=77	N*=89	N*=68
	π	41%	42%	38%	95%	98%	93%
	power of $t_{\hat{M}-2}$	44%	42%	36%	96%	97%	90%
0.4	v_e	0.01166	0.01485	0.01924	0.00862	0.01188	0.01539
	N*	N*=80	N*=63	N*=49	N*=83	N*=60	N*=47
	π	43%	36%	28%	97%	90%	81%
	power of $t_{\hat{M}-2}$	43%	35%	22%	97%	92%	75%
0.8	v_e	0.01134	0.01742	0.05252	0.01005	0.01689	0.03622
	N*	N*=82	N*=54	N*=19	N*=71	N*=43	N*=20
	π	44%	31%	13%	94%	77%	43%
	power of $t_{\hat{M}-2}$	52%	37%	13%	96%	87%	44%

Table 3

Power of the modified $t_{\hat{M}-2}$ statistic for testing the partial correlation coefficient $r_{YZ.X}$ between $Z = aX + U$ and $Y = cX + dZ + W$ after adjusting on X.

500 simulations are carried out for several levels of autocorrelation in X,U and W ($\rho_{X(1)} = \rho_{U(1)} = \rho_{W(1)}$), with $a = 2c = 0.204$.

$\rho_{YZ.X}$	$\rho(1)$	0.0	0.2	0.5	0.8
0.2	v_e	0.0111	0.0121	0.0156	0.0465
	N*	85	78	61	22
	π	44. 8 %	40.4 %	33.3 %	10.8 %
	power of $t_{\hat{M}-2}$	43.4 %	41.7 %	34.6 %	13.6 %
0.4	v_e	0.0087	0.0094	0.0120	0.0372
	N*	83	77	61	21
	π	96.4 %	95 %	89.1 %	41.7 %
	power of $t_{\hat{M}-2}$	96 %	95.4 %	91 %	46.8 %

Table 4

Comparison of the significance levels for tests of simple and partial correlations between male lung cancer mortality rates and industrial risk factors (adjusted on cigarette sales or on cigarette sales and a linear gradient) given by standard tests and $t_{\hat{M}-2}$ tests.

	r	standard test (N = 82) (values of the t_{N-2} statistic and significance level)	Modified $t_{\hat{M}-2}$ test (values of the $t_{\hat{M}-2}$ statistic and significance level)	$\hat{M}$
Metal industry workers				
no adjustment	0.63	7.16 $p < 10^{-9}$	3.00 $p = 0.01$	16
adjustment on cigarette sales	0.52	5.46 $p < 10^{-6}$	3.46 $p = 0.002$	34
adjustment on cigarettes sales and a linear gradient	0.35	3.34 $p = 0.002$	2.72 $p = 0.01$	55
General engineering workers				
no adjustment	0.43	4.23 $p < 10^{-4}$	2.72 $p = 0.01$	35
adjustment on cigarette sales	0.37	3.58 $p < 10^{-3}$	2.86 $p = 0.007$	53
adjustment on cigarettes sales and a linear gradient	0.26	2.41 $p = 0.02$	2.32 $p = 0.02$	76
Mine workers				
no adjustment	0.33	3.16 $p = 0.003$	2.37 $p = 0.02$	47
adjustment on cigarette sales	0.24	2.26 $p = 0.03$	2.42 $p = 0.02$	94
adjustment on cigarettes sales and a linear gradient	0.14	1.27 NS	1.26 NS	81
Textile industry workers				
no adjustment	0.28	2.57 $p = 0.01$	1.52 $p = 0.14$	30
adjustment on cigarette sales	0.26	2.40 $p = 0.02$	1.91 $p = 0.07$	53
adjustment on cigarettes sales and a linear gradient	0.11	1.02 NS	0.96 NS	73

Table 5

Results from the regression between lung cancer mortality rates and industrial factors under different parametrisations of Σ and comparison with the results of the modified $t_{\hat{M}-2}$ test.

	Standart test*	Autoregressive model	Disc model	Exponential model	$t_{\hat{M}-2}$
Metal industry+ (significance level)	t = 5.3 ($<10^{-6}$)	t = 3.7 (3×10^{-4})	t = 4.69 (10^{-5})	t = 2.9 (5×10^{-3})	3.48 (2×10^{-3}) $\hat{M} = 37$
General engineering+	t = 3.5 (7×10^{-4})	t = 3.67 (4×10^{-4})	t = 3.96 (2×10^{-4})	t = 3.24 (2×10^{-3})	2.88 (6×10^{-3}) $\hat{M} = 55$
Mining+	t = 2.1 (3×10^{-2})	t = 0.78 NS	t = 0.55 NS	t = 0.66 NS	2.23 (3×10^{-2}) $\hat{M} = 89$
Textile+	t = 2.3 (2.5×10^{-2})	t = 0.96 NS	t = 2.06 (4×10^{-2})	t = 0.8 NS	1.89 (7×10^{-2}) $\hat{M} = 57$

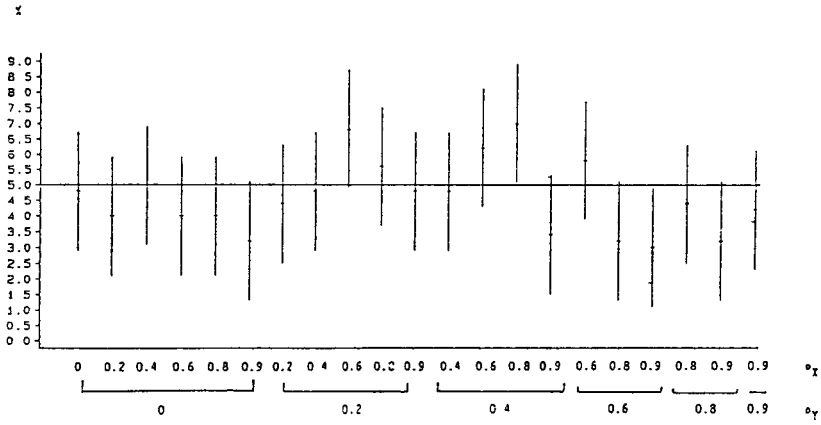
+ all the regressions are carried out after adjustment on cigarette sales.
* test of the regression parameters when $\Sigma = \sigma^2 I$.

Table 6

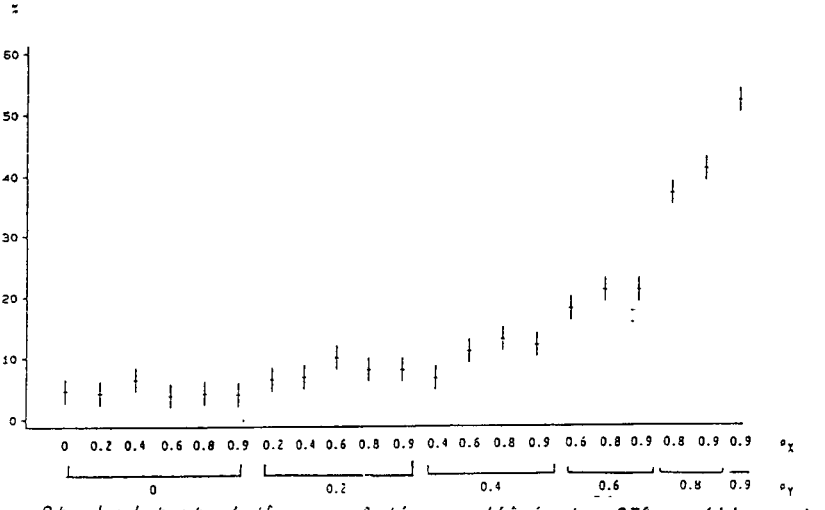
Comparison of the significance levels of partial correlations between male lung cancer mortality rates and industrial factors after respective adjustments on metal industry and/or general engineering given by $t_{\hat{M}-2}$ tests and by regressions with spatially autocorrelated errors.

	Model	value of the t statistic and significance level	Modified $t_{\hat{M}-2}$ test (values of the $t_{\hat{M}-2}$ statistic and significance level)	$\hat{M}$
Metal industry workers				
adjustment on cigarette sales, general engineering and a linear gradient	disc autocor. exp.	2.48 p=0.02 2.33 p=0.02 2.24 p=0.03	3.32 p = 0.02	53
General engineering workers				
adjustment on cigarette sales, metal industry and a linear gradient	disc autocor. exp.	2.69 p=0.009 2.70 p=0.009 2.56 p=0.01	2.20 p = 0.004	73

Figure 1



Test based on the modified t_{M-2} statistic : 95% confidence intervals for the proportion of type I errors for a 5% nominal test in the case of two mutually independent process generated by a disc model on a network of 82 points.



Standard test of the correlation coefficient : 95% confidence intervals for the proportion of type I errors for a 5% nominal test in the case of two mutually independent process generated by a disc model on a network of 82 points.

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

Dr. P. CLIFFORD, Mathematics Institute, University of Oxford.

V. Publications:

P. CLIFFORD, S.T. RICHARDSON, D. HEMON
Assessing the significance of the correlation between two spatial processes. Biometrics, 45(1), 123-134 (1989)

S.T. RICHARDSON
A method for testing the significance of geographical correlations with application to industrial lung cancer in France.
Accepted for publication in Statistics in Medecine.

S.T. RICHARDSON
Some remarks on the testing of association between spatial processes
Syracuse Symposium on Spatial Statistics, April 1989
to be published in the Institute of Mathematical Geography monograph series, under the title of Spatial Statistics : Past, Present, and Future.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-110-UK

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

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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

Issues in establishing authorised limits for effluent releases

Head(s) of project:

Ms M D Hill, Assessments Department, NRPB, Chilton, Didcot, Oxon OX11 ORQ, UK

Scientific staff:

S Haywood, C Robinson, M Morrey, N McColl

I. Objectives of the project:

The objectives of this project were 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 would be useful to the authorities responsible for setting these limits. The issues to be considered included: 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:

As a result of the 1985 ICRP Paris statement on dose limits for members of the public, work initially focused on the implications of using a limit on annual average dose over a lifetime when setting authorised limits, rather than a limit on the dose in a single year. Attention then turned to various aspects of the definition of critical groups in an exposed population and the calculation of doses to these groups. In the final stages of the project consideration was given to the source upper bound concept, and discharge/disposal comparisons, in the context of the ALARA requirement (optimization of protection).

III. Progress achieved:

Limit on Annual Average Dose over a Lifetime

1. Methodology

The approach used for this study was to calculate derived limits for a few radionuclides and environmental materials, based on both a dose limit for a single year and a limit on the annual average dose over an individual's lifetime. These derived limits are in terms of radionuclide concentrations in environmental materials and thus differences in them will reflect the differences which would occur in effluent release limits. The dose limits used were i) 5 mSv per single year (effective dose); ii) 50 mSv per single year to a single organ; iii) 1 mSv per single year (effective dose); and iv) 1 mSv lifetime average annual effective dose. In each case, the lifetime of the individual was taken to be 70 years, and account was taken of variations in dose per unit intake, and in diet, with age.

2. Results

The following table shows the results of the calculations of derived limits for ^{131}I and ^{239}Pu in milk and offal.

Dose Limit	Derived Limit	
	^{131}I	^{239}Pu
	Milk (Bq l^{-1})	
5 mSv	175	1.6
50 mSv	52	0.88
1 mSv (single year)	35	0.32
1 mSv (average)	170	6.2
	Offal (Bq l^{-1})	
5 mSv	9300	440
50 mSv	2800	240
1 mSv (single year)	1860	88
1 mSv (average)	3500	150

3. Discussion

The results of the calculations of derived limits showed that the most restrictive procedure will always be to use a limit of 1 mSv on the effective dose in a single year. However, for the radionuclides and foodstuffs considered, derived limits calculated on this basis do not differ greatly (a factor of 3 at most) from those obtained on the basis of the 50 mSv dose limit to a single organ. It could also be seen that use of a 1 mSv limit on average annual dose over a lifetime will generally be more restrictive than using a 5 mSv limit for a single year, but exceptions occur when dietary intakes and doses per unit intake are higher in childhood than adulthood (eg ^{239}Pu in milk). The conclusions drawn from this work were that while it is possible to use a limit on average annual doses over a lifetime as a basis for setting effluent release limits, it is simpler and more conservative to use a limit on the dose in a single year.

Subsequent to this study, the NRPB published revised generalised derived limits for a number of radionuclides, based on a limit of 1 mSv on the effective dose in a single year[1].

Critical Groups

1. Methodology

The starting point for this work was a review of the ICRP recommendations on the choice of critical groups and the calculation of doses to these groups (see ICRP Publication 43). These recommendations are intended to be applied in the context of establishing authorised limits and for other purposes, in particular the retrospective demonstration of compliance with dose limits and source upper bounds. In the review, however, only their application in setting authorised limits for effluent releases was considered. Following the review it was decided to undertake a statistical investigation of the possible variation in critical group doses due to variations in food consumption rates and in doses per unit intake of radionuclides by ingestion.

2. Results

The review of the ICRP recommendations on critical groups indicated that, although they work well in practice, there were a number of conceptual problems in their formulation and in their application to the establishment of authorised limits. Firstly, ICRP state that their dose limit for members of the public, and hence also any source upper bound, applies to the mean dose in a reasonably homogeneous group ie that the dose to be calculated is that to a single, hypothetical person. However, they also state that the critical group would not usually consist of one individual, and that only in an extreme case, such as when dealing with conditions well into the future, would it be appropriate to consider a single hypothetical person. These recommendations can be seen as contradictory. Secondly, ICRP state that, in calculating doses to critical groups, it is important to select appropriate mean values for factors such as food consumption rates, but that metabolic parameters (and hence dose per unit intakes of radionuclides) should be chosen to be typical of the relevant age group in the normal population rather than extreme values. Again there is an apparent inconsistency. Thirdly, ICRP give criteria for homogeneity in a critical group which vary with proximity of the mean dose to the relevant source upper bound; such criteria are difficult to apply in prospective situations where source upper bounds may not have been finalised. Fourthly, ICRP recommend that the results of a habit survey at a particular point in time should be regarded as an indicator of an underlying distribution, but no guidance is given on how to determine this distribution, or how to make allowance for possible changes in the distribution if the period for which authorised limits are intended to be in force is fairly long.

As a way of resolving some of these problems, it has been suggested that, at least for some major foodstuffs, it would be preferable to use a particular percentile of an entire frequency distribution of consumption rates in a population as the mean for a critical group, rather than to define the group and then calculate the mean. The potential implications of such an approach are illustrated in the following table, which shows the rates of consumption of terrestrial foodstuffs assumed by NRPB when calculating doses to a critical group of one year old children near a typical UK nuclear installation, and two percentiles of the distribution

of consumption rates obtained from a large UK Department of Health (DH) survey of pre-school age children. Similar data can be obtained for

Food Consumption Rates for One Year Old Children
(kg a^{-1} unless otherwise stated)

Food	NRPB critical group value	DH Survey	
		90th percentile	95th percentile
Milk (l a^{-1})	260	230	256
milk products	16	29	41
Eggs	18	17	22
Beef + veal	7	7	8
Mutton + lamb	3	3	4
Pig meat	7	4	7
Offals	2	2.5	3.3
Chicken	3	3	4
Root crops	50	33	39
Green veg	30	7	9
Fruit	20	26	31
Grain products	50	29	34
Oat products	6	12.4	16.8

other age groups and foodstuffs, one of the principal sources for the UK being the National Food Survey carried out by the Ministry of Agriculture, Fisheries and Food (MAFF).

While the percentile approach has attractions, it gives the impression of statistical precision and would not be worth pursuing if variations in doses per unit intake of radionuclides were greater than likely variations in critical group food consumption rates. To discover whether this may be the case, it was decided to use Latin Hypercube Sampling techniques to produce probability distributions of critical group doses from distributions of consumption rates of key foods and distributions of doses per unit intake of important radionuclides, and to use available statistical programs to identify the main source of variability in the results. Much of the necessary input data was collated and the computer programs to be used were tested. However, although some information on variability in dose per unit intake became available during 1989[2], the

full data set was not produced in time for the calculations to be performed and the results analysed during the contract period. Also, some of the MAFF consumption rate data are not yet available.

3. Discussion

At present, the identification of critical groups and the calculation of the mean doses to these groups relies to a large extent on expert judgement guided by some empirical recommendations. This approach has the disadvantage that varying degrees of conservatism can be introduced when establishing and explaining authorised limits for effluent discharges, and there is thus the possibility that the real degree of protection achieved in each case will not be clear. The eventual aim of the work described above is to produce practical recommendations to avoid such situations, not by removing the element of judgement in critical group identification and dose calculation, but by making it more explicit and quantitative.

Source Upper Bounds

1. Methodology

The concept of upper bounds was introduced by ICRP in Publication 37. The purpose of the source upper bound is to act as a constraint on optimization for that source, so the exposure of any individual will remain below the dose limit, even if the individual is exposed to several sources. Thus source upper bounds have to be set to be some fraction of the dose limit. The methods for establishing authorised limits for effluent discharges in various countries were reviewed, with the aim of determining whether the source upper bound concept is utilised in practice.

2. Results

From published information on methods for establishing authorised limits in EC countries, the US, Japan and Sweden, it appears that the concept of source upper bounds has not been formally incorporated in legislation, regulations or government policies. Nevertheless, most of these countries employ dose standards which are a fraction of their overall

dose limits for members of the public when setting authorised discharge limits for nuclear installations. These standards effectively act as source upper bounds, although they are more frequently described as targets, or as site or discharge specific limits. The following table shows the values used in a number of countries.

Country	Dose "Upper Bound" for Discharges from Nuclear Installations (effective/whole body dose, mSv)
Belgium) Spain) USA)	0.03 liquid) 0.05 gaseous) LWRs
Japan	0.05
Italy) Sweden)	0.1
FRG) Luxembourg) Netherlands)	0.3
UK	0.5
France	(upper bounds in terms of activity discharged)

3. Discussion

It was concluded from the review of published information that regulatory authorities in most countries find it useful to have a dose standard lower than the overall dose limit for members of the public in mind when setting authorised discharge limits. From the differences in values used it is apparent that, while these standards effectively act as source upper bounds, they have been derived in different ways. This could be one of the reasons why the concept of source upper bounds has not been formally adopted by regulatory authorities. Another possible reason is that some authorities may prefer to use the concept of dose targets which can be exceeded if it is optimum to do so, rather than imposing formal constraints other than the dose limit at the start of the optimization process, because in principle the former approach allows more flexibility.

Discharge/Disposal Comparisons

1. Methodology

The objective of this work was to highlight some of the difficulties which can occur when, in order to fulfil the ALARA requirement, discharge of radioactive material to atmosphere or to the aquatic environment has to be compared to trapping, immobilisation and disposal of the material as a solid. The study focused on those parts of comparisons concerned with radiological impact on the public. Since no examples of comprehensive comparisons appear to have been published, the work necessarily dealt with difficulties which would be encountered if such comparisons were made rather than difficulites which had actually arisen.

2. Results

The radiological impact of solid waste disposal is influenced by probabilistic events and processes, and thus the results of impact calculations are in the form of doses to individuals and populations, together with estimates of the probabilities that these doses will be received. It is also becoming conventional to estimate the uncertainties in doses and probabilities, and present results as distributions rather than single values. This is not yet the case in assessments of the radiological impact of discharges, where results are usually given as single values. Hence, discharge/disposal comparisons could involve the use of disparate pieces of information. Even in the apparently straightforward case of comparing the maximum risk to an individual from discharge as an effluent to the maximum individual risk from disposal as a solid, problems could arise because of differing bases for the choice of the habits, location and other characteristics of the individuals considered in each case. With doses and risks to individuals and populations in the long term, there is the further complication that potential environmental changes are being given increasing emphasis in solid waste disposal assessments, while in discharge assessments such changes are normally ignored. Thus, again it may prove impossible to compare discharge with disposal on the basis of consistent measures of radiological impact on the public.

3. Discussion

The main conclusion drawn from the work was that there is a need to improve the consistency between methods for calculating the radiological impacts on the public of solid waste disposal and effluent discharges. Differences appear to have arisen because, on the whole, those involved in solid waste disposal assessments are not concerned with discharges, and vice versa. Even if no comprehensive discharge/disposal comparisons are made as an input to setting authorised limits for discharges, as new solid waste disposal facilities are developed questions are bound to arise about how their potential radiological impact compares with that of existing nuclear installations. Hence it would be preferable if effluent discharge and solid waste disposal impact assessment methods were consistent with each other (and with accident consequence assessment methods). This seems more important at present than questions such as those about the weight to be attached to long term doses and risks when waste management methods are compared[3].

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

V. Publications:

1. NRPB, Revised generalised derived limits for radioisotopes of strontium, iodine, caesium, plutonium, americium and curium. NRPB-GS8 (1987) (London, HMSO).

2. Kendall, G M et al, Methods for the calculation of doses to members of the public from intakes of radionuclides. Chilton, NRPB-M163 (1989).

3. Hill, M D, Safety objectives and criteria for waste disposal. Proc. CEC/IAEA/NEA Symposium on Safety Assessment of Waste Repositories, Paris, 9-13 October 1989 (to be published by NEA/OECD).

[Note: these publications are relevant to the work carried out under this contract but were not produced as part of this work.]

Title of project no.: 2

Methods for the practical implementation of the ALARA principle - Part II of Contract No. BI6-F-110-UK.

Head(s) of project:

Ms M D Hill, Assessments Department, NRPN, Chilton, Didcot, Oxon OX11 0RQ, UK.

Scientific Staff:

M J Crick
J R Croft
A B Fleishman
A P Hudson
R McGeary
P J Saunders
A D Wrixon

I. Objectives of the project:

These are formally stated as follows:

(1) To review the difficulties that have arisen in the practical implementation of ALARA;

(2) To suggest methods whereby these difficulties may be resolved;

(3) To develop a simple structural framework for future ALARA studies; and

(4) To demonstrate the use of this framework by applying it in example studies.

II. Objectives for the reporting period:

Since this is the final contract report, these are identical to the objectives of the project itself, listed above.

III. Progress achieved:

1. METHODOLOGY

The second European Scientific Seminar on the Optimisation of Radiological Protection was held in Luxembourg on 8th and 9th November 1983 and explicitly identified a need for clearer guidance on the implementation of the ALARA principle in practical radiation protection programmes. As a result this report was instigated as a joint undertaking of two organisations, namely the National Radiological Protection Board (NRPB, UK) and the Centre d'étude sur l'évaluation de la protection dans le domaine nucléaire (CEPN, France), and had the formal objectives listed above.

It was clear from the beginning of the project that, in order for the objectives to be met, it would be necessary to combine the expertise of staff working on the theoretical aspects of optimisation techniques with the experience of staff working with practical radiation protection problems. At NRPB, appropriate staff were identified within the organisation to participate in the work of the project.

One of the first steps was then to compare the practical experience in optimisation of radiation protection of the two organisations, NRPB and CEPN. It became clear that NRPB had practical experience in the industrial and medical fields, whilst CEPN had closer links to the nuclear industry. Together they covered the range of activities of radiation protection.

By means of a series of meetings and mutual correspondence between technical staff at NRPB/CEPN, a general framework for performing optimisation studies was to be developed. At the same time each organisation was to identify radiation protection problems that could be used to test the applicability of the general framework and use the experience gained to refine this framework. NRPB, because of the background of the staff involved, concentrated on applications in the non-nuclear sector. In addition, it was agreed to review other published ALARA studies to check that they, too, could have been approached from the perspective of applying the general framework.

It was appreciated that to assist the implementation of the ALARA concept into radiation protection programmes a document was required that explained, in language appropriate to the practitioner, the ideas behind the ALARA principle and described the practical measures for implementing

it together with examples from real situations. It was agreed to write such a document based on the work performed in the project.

2. RESULTS (Achievements)

Following a review of the concepts underlying ALARA and from detailed consideration of the role that ALARA studies should play in aiding decisions on radiation protection matters, a general framework for applying the ALARA principle to practical radiation protection problems was developed jointly by NRPB and CEPN. The framework was given the name the "ALARA Procedure" and a schematic diagram (Figure 1) illustrates its composition. It provides a simple structured approach that can be applied to any problem involving optimisation of radiation protection.

NRPB staff have applied the ALARA procedure to several case studies addressing practical radiation protection problems, including the design of an industrial radiography facility and optimising doses to patients during diagnostic radiology. The structured approach, in all cases, provided a way of thinking that assisted and clarified the decision-making process.

From this work, some twelve scientific papers were published, which are listed at the end of this report. They provide detailed accounts of the case studies and further information on the application of the ALARA procedure to optimisation problems.

The most visible result of this project will be the document that explains the ideas behind the ALARA principle, the ALARA procedure, and measures for implementing them into radiation protection programmes. Entitled "ALARA - from theory to practice", the document is written in language that makes it accessible to radiation protection practitioners and is supplemented with practical examples to illustrate the points being made; many of these examples are from the case studies performed in the course of the project. A copy of the structure of this document is attached in order to facilitate an appreciation of its content. It is hoped that this will be a useful addition to the literature and, more importantly, be read extensively.

From 12th to 14th September, 1988, the CEC held its Third European Scientific Seminar on Radiation Protection Optimisation, which provided a forum for the presentation of the work carried out under this project. Staff from NRPB and CEPN were heavily involved in the organisation of this

seminar and were responsible for some 30% of the papers. Papers that were presented and the subsequent discussions were taken into account in producing the final version of the document on ALARA described above.

3. DISCUSSION

Several general comments can be made about the state of development of the work on optimisation of protection. It is now clear that the ALARA principle is a workable concept and that tools for structuring optimisation problems and aiding decisions, such as the ALARA Procedure, exist and have been shown to work in practice. It is also clear that a structured approach can be applied to all levels of radiation protection problems, but that the resources allocated to each problem need to be commensurate with the level of decision involved. For example, in many cases of low-level decision, intuitive thinking by experts coupled with ALARA awareness may be all that is necessary. At high levels of investment, an extensive desk-top ALARA study involving several man-months of effort may be needed. It is also clear that methods for incorporating these ideas into radiation protection programmes (e.g. ALARA audits and ALARA predictive plans) exist, and again have been demonstrated to work in practice.

What is required now is that these ideas, principles, tools, and methods be actually used widely in radiation protection programmes. The document produced on ALARA is an important step in this, but there is additionally a need to provide manuals of good practice, training on the implementation of these methods, and simple computer tools to aid decision-making as well as guidance on the application of these methods to more complex problems. It has also been recognised that there is a lack of relevant data on dose-saving techniques and associated costs for detailed application of quantitative methods to operations warranting them. Future work is needed to address these specific areas.

Finally, it should be noted that the developments made during the course of this project have fed into the work of the ICRP Task Group set up in 1984, which produced ICRP Publication 55 - *Optimisation and Decision-making in Radiological Protection.*

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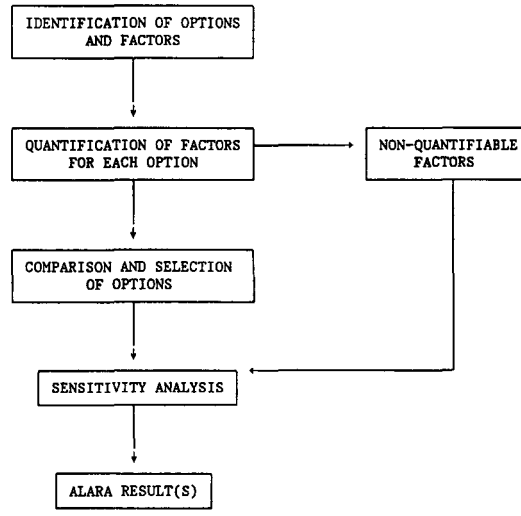

Figure 1 - The steps of the ALARA procedure

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V. Publications:

Webb, G A M *et al.* Development of a general framework for the practical implementation of ALARA. *Proceedings of an International Symposium on the Optimisation of Radiation Protection, Vienna 10-14 March, 1986.* IAEA Vienna, pp. 123-136.

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-127-UK

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

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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:

Ms M D Hill, Assessments Department, NRPB, Chilton, Didcot, Oxon, OX11 ORQ, UK

Scientific staff:

J A Jones, M 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).

A further objective of the project is to develop a flexible computer program system (COSYMA - COde SYstem of MARIA) to allow people within the Community who are not experts in ACA to carry out their own studies and consequence assessments.

II. Objectives for the reporting period:

The main objective for the final year of the programme was to finalise the COSYMA program system. A secondary objective was to carry out a series of sensitivity studies to enable advice to be given on the choice of model or parameter values for running the program in a range of conditions, including running it on small computers.

III. Progress achieved:

The work carried out under this topic is described in four sections:

- the modelling of atmospheric dispersion and deposition;
- meteorological sampling;
- the importance of and modelling of deposition to skin;
- the production of COSYMA.

1. The Modelling of Atmospheric Dispersion and Deposition

The main objective of this work was to decide which sorts of atmospheric dispersion model are required in accident consequence assessments, and whether the models in general use at present are adequate. The work focussed on models for dispersion in conditions with low wind speeds, on different methods of modelling the spatial and temporal variation of rain, and the need to include trajectory models in accident consequence assessments (ACA).

The Gaussian plume model is widely used in ACA programs. However the basic equation for calculating air concentration contains the reciprocal of the wind speed, and hence the model is not applicable in calm (zero wind speed) conditions. This problem is usually bypassed by assigning a minimum value of wind speed which is used whenever the value of the meteorological data file is lower than this minimum. The choice of this value is partly arbitrary. The NRPB's computer program for accident consequence assessments, MARC-1 [1], has been used to investigate the sensitivity of the results of probabilistic risk assessments to the value selected for the minimum wind speed. The calculations were carried out for a hypothetical large release from a PWR in the UK. Some of the results for early deaths, fatal cancers and people evacuated obtained from 2 runs of MARC-1 for each of the values of minimum wind speed adopted (0.1, 0.5 and 1.0 m/s) are shown in Table 1. The results given there show that the variation with minimum wind speed is small, less than the uncertainty in the predictions of the MARC-1 program (see the report for Project 4). This shows that the predicted numbers of these effects are not sensitive to the choice of value for the minimum wind speed, and that the widely adopted value of 0.5 m/s is adequate. This work has been written up for publication.

Table 1

Consequences Obtained with Different Values of the Minimum Wind Speed

	Results for Minimum Wind Speed					
	0.1 m s^{-1}		0.5 m s^{-1}		1.0 m s^{-1}	
Early Death						
Mean value	$1.2\ 10^2$	$1.4\ 10^2$	$1.1\ 10^2$	$1.5\ 10^2$	$1.5\ 10^2$	$8.3\ 10^1$
90th percentile	$3.0\ 10^2$	$3.6\ 10^2$	$2.9\ 10^2$	$3.3\ 10^2$	$4.4\ 10^2$	$2.5\ 10^2$
99th percentile	$1.5\ 10^3$	$2.1\ 10^3$	$1.4\ 10^3$	$2.8\ 10^3$	$1.8\ 10^3$	$1.0\ 10^3$
People evacuated						
Mean value	$1.2\ 10^6$	$1.3\ 10^6$	$1.2\ 10^6$	$1.2\ 10^6$	$1.2\ 10^6$	$1.2\ 10^6$
90th percentile	$2.7\ 10^6$	$3.2\ 10^6$	$2.7\ 10^6$	$2.7\ 10^6$	$2.8\ 10^6$	$2.8\ 10^6$
99th percentile	$7.5\ 10^6$	$9.4\ 10^6$	$7.5\ 10^6$	$7.8\ 10^6$	$8.1\ 10^6$	$7.7\ 10^6$
Fatal Cancer						
Mean value	$3.7\ 10^3$	$3.7\ 10^3$	$3.7\ 10^3$	$3.6\ 10^3$	$3.9\ 10^3$	$3.6\ 10^3$
90th percentile	$9.2\ 10^3$	$8.8\ 10^3$	$8.9\ 10^3$	$8.9\ 10^3$	$9.8\ 10^3$	$9.0\ 10^3$
99th percentile	$2.0\ 10^4$	$2.0\ 10^4$	$2.1\ 10^4$	$1.8\ 10^4$	$2.1\ 10^4$	$1.9\ 10^4$

There are many models available for describing the dispersion and deposition of material released to atmosphere. These models are based on different assumptions and approximations and may require very different amounts of computer time for their application. Some of the work carried out in this area was intended to give guidance as to which of the available models should be used for particular applications, partly for providing guidance to users of COSYMA. The work concentrated on two aspects, namely the difference between models which allow for changes of wind direction during plume travel (trajectory models) and those which do not, and the differences between puff and plume models and in particular the way in which they describe the pattern of wet deposition.

This part of the work was carried out in two stages. In some cases conclusions were drawn solely from qualitative considerations of what the likely differences between the model predictions will be. In others quantitative results were obtained using computer programs implementing the models under consideration.

A version of the Risø puff model RIMPUFF [2] was obtained from Kernforschungszentrum Karlsruhe (KfK). The program was modified to make it compatible with the MARC program and simplified slightly to reduce the amount of computer time it required. RIMPUFF describes a release as a series of puffs emitted at a uniform rate over the period of the release. The computer time required by the program is almost proportional to the number of puffs used to describe the release, and tests were carried out to determine the minimum number which gives an adequate description of the dispersing plume [3]. The model can be used with meteorological data from a single location, or can derive the atmospheric conditions at the plume by interpolating between data from several locations in the area of interest. Studies showed that the differences between the results obtained for these two sources of meteorological data are fairly small [3].

The need to consider trajectory models has been examined both qualitatively and quantitatively. The pattern of contamination predicted by trajectory models depends on the method adopted to describe the effect of changes in wind direction on the dispersing material.

The segmented plume model describes the changes as if they occur at the same point for all dispersing material. There is a well defined plume centre line, and the variation of concentration or dose with distance along this line is the same as that predicted along the centre line of the plume in a linear model. There is therefore no reason to suppose that the consequences predicted using segmented plume models or linear models will be significantly different if the population distributions under the different plume trajectories are similar. There is no reason to suppose that wind direction changes will carry material systematically over or away from populated areas (at least in much of Europe where there is no strong channelling of the wind). Therefore one can conclude that there is not likely to be a significant difference between the predictions of an ACA program using a segmented plume model and one using a linear trajectory model.

However the pattern produced by a model in which the release is described as a series of puffs each following its own trajectory is not like that predicted by a linear model. In this case, there may not be a well defined plume centre-line. The differences between the predictions of this type of model and those of a linear model cannot be derived

qualitatively, and calculations with a trajectory model and a linear model were carried out. To minimise the differences between the models used, the RIMPUFF model was used with and without allowing for changes of wind direction. To reduce the amount of computer time required by RIMPUFF the study was restricted to those consequences within 200 km of the site. Two runs were carried out where RIMPUFF was used as a trajectory model, and one where it was used as a linear model. The predicted numbers of early effects for these runs are shown in Table 2. The two sets of results from the trajectory model have expectation values differing by about a factor of 2, which is smaller than the difference between the results from the trajectory and linear models. This work has been written up for publication.

Table 2

Numbers of Early Deaths Obtained with a Puff Model in a Linear and a Trajectory Mode

	Mean	50th percentile	90th percentile
Linear model	$1.3\ 10^2$	$1.7\ 10^1$	$3.3\ 10^2$
Trajectory model	$1.1\ 10^2$	6.0	$2.2\ 10^2$
	$6.3\ 10^1$	2.8	$2.0\ 10^2$

Note: The 2 sets of results from the trajectory model are for different sets of atmospheric conditions.

A qualitative comparison of the differences between plume and puff models for wet deposition had been carried out as part of the first MARIA programme from 1983 to 1985 [4], but no definite conclusions could be reached. This work was extended in the second phase of MARIA (1985 to 1989) with a quantitative comparison. The numbers of early deaths and of people evacuated for a hypothetical release were calculated using the normal MARC dispersion model and using RIMPUFF, both for preselected single sequences of atmospheric conditions and for the probability distribution of all conditions. The results for single sequences showed that in some cases, particularly when the rain occurred over a town, there were some large differences between the model results. The plume model predictions were sensitive to the exact overlap between the rain band and the populated area, while the puff model predictions were much less sensitive. The probabilistic results of the two models are however

much more similar. The expectation value and 90th and 99th percentiles obtained with the puff model were 110, 300 and 1300 respectively. These results can be compared with the ones given in Table 1 for the standard version of MARC using the standard wind speed of 0.5 m/s. This suggests that, while the different models may give very different results in particular sequences of atmospheric conditions, the overall results of a probabilistic analysis for total numbers of effects in a population are not sensitive to the choice of model for wet deposition.

These studies show that, for short releases, the predicted numbers of effects in the population are not very sensitive to the type of dispersion model used. Therefore, the use of a relatively simple model for atmospheric dispersion should be adequate in many accident consequence assessments.

2. Meteorological Sampling

The work done in MARIA-1 on meteorological sampling was carried out to determine an adequate sampling scheme for use with the MARC program. This used a Gaussian plume dispersion model with no allowance for changes of wind direction during plume travel. The work showed that the identification of "rain" sequences was important. This work has been continued, and a more refined system of identifying these sequences developed.

The method used to define the sequences should reflect some features of the atmospheric dispersion model being used. The schemes for use with MARC for characterising the rain sequences were defined in terms of the fraction of the plume deposited within a short distance band. This type of scheme is not necessarily applicable with other dispersion models in which the overlap between the dispersing material and the area covered by rain may be described differently. Therefore alternative schemes have been developed in which the rain sequences can be identified in terms of the washout coefficient and the wind speed.

ACA programs using linear dispersion models generally calculate the consequences for selected sequences of conditions assuming that the same pattern of dispersion and deposition can occur in all wind directions with an appropriate frequency. This procedure is not possible with trajectory models, and the meteorological sampling scheme must take

explicit account of the wind direction both at the time of the release and during the plume's travel. Versions of the meteorological sampling program have been developed for use in COSYMA in which the wind direction and its changes are explicitly considered.

3. The Importance of and Modelling of Deposition to Skin

Accident consequence programs consider early death from irradiation of several organs, including the skin and the bone marrow. The major contribution to skin doses comes from material deposited on the skin, while a major contributor to bone marrow doses is gamma exposure from material deposited on the ground. Recent changes to model parameter values (an increase in the shielding from deposited gamma dose assumed for people indoors, and a reduction of the LD50 for death from skin exposure) have increased the relative importance of skin exposure compared to the exposure of other organs in terms of predicted numbers of early deaths. However there is very little information on the value to adopt for deposition velocity to skin.

Scoping calculations were carried out using the MARC program, where the deposition density on skin is taken to be a fraction of the total wet and dry deposition to the ground outdoors. Different values were used for this fraction for people inside or outside at the time of plume passage. These calculations showed that, for some values of the ratio of deposition on skin to that on ground, skin exposure could be a major contributor to early deaths.

These calculations were based on the simple assumption that the relative deposition was the same for wet and dry deposition, as ACA programs generally only calculate the total deposition. It is reasonable to assume that the ratio will be different for wet and dry deposition, and in particular that there will be no wet deposition to people indoors. Therefore a version of MARC was written in which wet and dry deposition were calculated separately, and skin deposition calculated allowing for different relative depositions to skin and ground for people inside and outside and for wet and dry deposition. Some of the results are given in Table 3. They show that skin deposition could be important for some values of the ratio of deposition to skin and ground. However using

Table 3

Early Deaths Predicted for Different Ratios of Deposition to Skin and to Ground

Skin/ Ground Deposition						
	Dry	Wet	Mean	Percentiles		
				50%	90%	99%
Indoors	0.0	0.0	24	0	70	390
Outdoors	0.0	0.0				
Indoors	0.01	0.01	25	0	75	400
Outdoors	0.1	0.1				
Indoors	0.03	0.0	44	2	140	560
Outdoors	0.3	0.1				
Indoors	0.03	0.03	52	3	160	680
Outdoors	0.3	0.3				
Indoors	0.1	0.0	210	79	550	1840
Outdoors	1.0	0.1				
Indoors	0.1	0.1	280	88	680	2500
Outdoors	1.0	1.0				

different ratios for wet and dry deposition does not make a significant difference to the results. A summary of this work is being prepared for presentation at the MARIA seminar in 1990.

This work showed that deposition to skin and the resulting skin doses are important, and should be considered in accident consequence assessments. However the current practice of calculating only the total deposition rather than the wet and dry components separately is adequate. The work also showed the need for research into radionuclide deposition onto skin and clothing.

4. The Production of COSYMA

One of the objectives of the MARIA programme is to develop a computer program system for assessing the off-site consequences of accidental releases of radioactive material to atmosphere. The new program system, COSYMA (COde SYstem from MAria), was developed jointly by NRPB and Kernforschungszentrum Karlsruhe. It is intended for use generally within the EC by people who are not specialists in the area of accident consequence modelling but wish to use such a program package. The system is intended to provide a flexible package which will allow users to investigate special problems by appropriate choice of models, parameter values, and data sets. COSYMA incorporates ideas from the NRPB MARC programs, the KfK program UFOMOD, new developments, and contributions from other MARIA contractors.

The general structure of the COSYMA system was agreed with KfK. The system includes a version of the atmospheric dispersion module of MARC which has been modified to make it compatible with the other parts of the system. Work was carried out to provide this modified program. The system also includes a flexible program for meteorological sampling, based on that in MARC and the additional work described above. A new economics module is being prepared for inclusion to the system; work towards this is described under Project 3 in this report. COSYMA has the structure of the KfK program UFOMOD, and a number of agreed modifications have been made to UFOMOD so that it can include models which were included in parts of MARC but were not in UFOMOD.

Two reports are being prepared with KfK to accompany the new computer system. The first of these describes the package of computer programs

and data libraries which are to be provided to other people. It describes those models which are included in the computer progams and those which were adopted to derive the data libraries for the package. It describes the applications for which the COSYMA system is intended, and indicates which program should be used for particular applications. The second report is the user guide to the programs in the package, and describes in detail how the program system is used.

The work involved in extending the UFOMOD program is described in detail in the KfK report.

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

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Federal Republic of Germany.

V. Publications:

Jones J A. Special topics in atmospheric dispersion modelling - wet deposition and coastal effects. In Proc. CEC Workshop on Methods for Assessing the Off-Site Radiological Consequences of Nuclear Accidents, Luxembourg, 15-19 April 1985. EUR-10397 (1986).

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Title of the project no.: 2

The assessment of exposure due to deposited material

Head(s) of project:

Ms M D Hill, Assessments Department, NRPB, Chilton, Didcot, Oxon, OX11 ORQ, UK

Scientific staff:

J R Simmonds, J Brown, A Walmsley, S M Haywood

I. Objectives of the project:

The aim of this project is to develop or improve models for predicting doses to people following deposition of radioactive material. The exposure pathways considered include: external irradiation from material deposited on the ground and on buildings, transfer of radionuclides through terrestrial foodchains, radionuclide transfer in freshwater bodies and in the marine environment, and inhalation of resuspended material. The work forms part of the MARIA-2 Programme (Methods for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

The objectives for the final reporting period centred on the need to finalise the models for COSYMA. Supporting work was to be completed on the collection of relevent data for use with the models.

III. Progress achieved:

1. External Exposure following Deposition on the Ground and Other Surfaces

External irradiation of people from radioactive material deposited onto the ground and surfaces following an accidental release to atmosphere is frequently an important exposure pathway. In the past, models for estimating the radiation doses from this pathway were based on rather simple assumptions because of the relatively limited understanding of the behaviour of radionuclides deposited on urban surfaces. However, in recent years a significant body of research work, much of it within the MARIA programme, has been carried out. This has enabled NRPB to develop a more detailed and realistic model for calculating individual and population doses from gamma-emitting material deposited in an urban environment. This model, given the acronym EXPURT (EXPosure from Urban Radionuclide Transfer), simulates the movement of activity deposited on various surfaces in the urban environment. By taking into account the shielding properties of buildings and the habits of the population EXPURT then evaluates the external doses to people living in such urban environments as a function of time after deposition.

EXPURT is a linear compartmental model employing first-order kinetics to calculate the transfer of activity from one urban surface to another. The model is illustrated schematically in Figure 1. EXPURT has seventeen compartments each representing various environmental pools in which activity may reside. Five main surface types have been considered in the model: roofs, walls and indoor surfaces of buildings, impermeable ground surfaces (including roads, pavements, etc) and permeable surfaces (including grassland, parks, soil, gardens). The three compartments termed, 'ground water', 'loss' and 'sewers' have been introduced to hold activity lost from the soil column, from indoor surfaces of buildings and from the drains, respectively. This enables the potential for other exposure pathways to be identified and included at a later date if necessary. An additional compartment labelled 'decontamination' has been included into which activity removed following decontamination of a surface may be transferred. This enables the amount of activity removed from the urban area by decontamination processes to be monitored and again allows for the possibility of studying other exposure pathways in the future, eg, doses to decontamination workers, or doses from the disposal of

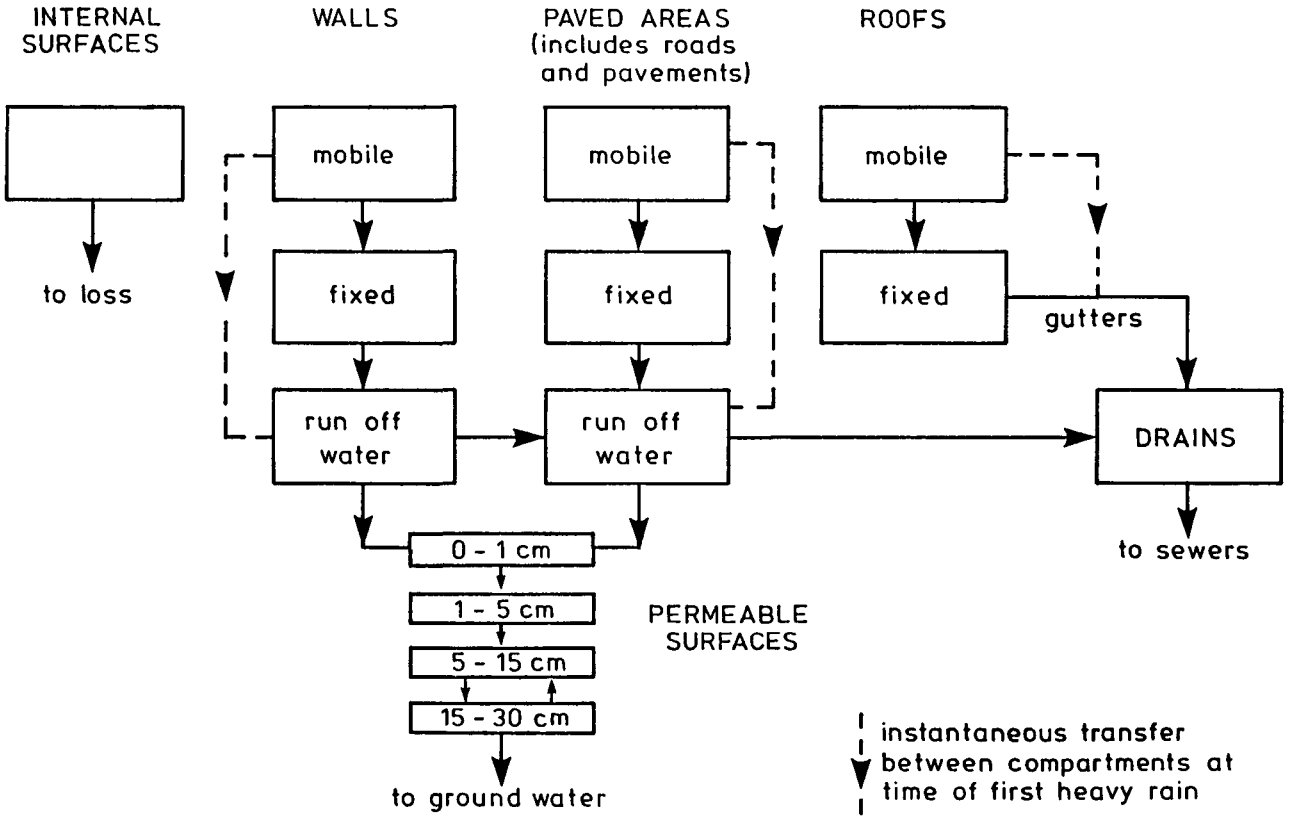

Figure 1 EXPURT – the urban dose model

radioactive waste arising from decontamination. Full details of the EXPURT model are given in an NRPB report in press (see list of publications).

The calculation of external gamma doses to people comprises two distinct parts. In the first part the variation of activity levels both in time and spatially within the environment are predicted using the model shown in Figure 1. In the second part a dosimetric calculation is performed summing the dose contributions from the various sources of activity in the urban environment. Account is taken of the location of individuals both inside and outside buildings and of the protection offered by various types of buildings against penetrating gamma radiation. The dose rates in various building types are calculated according to the methodology used by the NRPB GRINDS program [1]. The default locations and building types assumed in EXPURT are described in the full report on the model.

Following development of the EXPURT model a preliminary sensitivity analysis was performed with the following objectives:

- To identify those parameters in the model whose uncertainty contributes most to the uncertainty in the model predictions and which would therefore warrant more detailed study.
- To identify those parameters whose uncertainty does not greatly affect the model predictions, for which more detailed study is not required.
- To compare the NRPB's existing model for calculating external gamma doses from deposited activity with the range of values that might reasonably be predicted using the EXPURT model.

The sensitivity analysis is reported in detail in the full report and a few of the findings are given here.

For the model outputs considered in the study the more important parameter uncertainties were found to be those for the fraction of ground that is paved, the fraction of time spent indoors, the migration of activity through soil and the ratio of dry deposition on smooth urban surfaces to that on grass. The importance of the uncertainty in these parameters reflects the relative importance of doses received whilst outdoors compared to those received whilst indoors.

The results of the uncertainty analysis indicated that the estimate of the uncertainty associated with the assessment of dose rates and integrated doses in the urban environment following unit deposition is about a factor of ten. The results of EXPURT were also compared with those predicted by the relatively simple model used in the NRPB's accident consequence code, MARC [2]. In the MARC model account is taken of radionuclide migration through soil, radioactive decay and attenuation by soil. An average indoor occupancy of 90% and a shielding factor of 0.5 for buildings are assumed. The predictions of the EXPURT model are systematically lower at all times than those of the MARC model by a factor of between 5 and 50. However, the curves run parallel to each other which suggests that simple correction factors could be applied to the MARC model predictions to harmonise them with the range of urban doses predicted by EXPURT. For example, revising the shielding factor from 0.5 to 0.1 brings the MARC model prediction down to within the range of EXPURT values.

EXPURT has also been used to assess the effect of decontamination in reducing external doses to populations living in urban environments. Decontamination of the various surfaces modelled is simulated by the transfer of activity from one compartment to another, or by the removal of activity from a particular surface. The decontamination techniques considered so far using EXPURT are given in Table 1, together with the range of times at which they were assumed to be applied in a particular study. The effectiveness of various decontamination techniques for reducing external gamma doses was analysed in this study, where the term 'effectiveness of decontamination' describes the reduction in dose or dose rate that a particular technique may produce. In the study an uncertainty analysis was carried out and EXPURT was run 100 times for each decontamination option. Also best estimates of the effectiveness of the same techniques were calculated using the default values of the parameters.

Table 2 shows the results of the uncertainty analysis giving the effectiveness of various decontamination techniques in reducing the external gamma dose. The study showed that the removal of all radioactive material from impermeable surfaces (ie, roof, walls, interior surfaces and paved surfaces) is on the whole, not very effective. One of the more effective of the methods considered for reducing doses, if not

Table 1 Decontamination techniques considered in a study using EXPURT

Decontamination technique		Times at which technique applied following deposition
Hosing of external impermeable surfaces		2d 7d 14d
Surface removal/removal of 100% of contamination		
	roofs, walls, paved areas	30d 6m 1y
	internal surfaces	2d 7d 14d
Soil removal to depth, cm		
	1, 5, 15	30d 6m 1y
	30	
Soil ploughing to depth, cm	5, 15, 30	14d 30d 6m 1y
'Cheap' decontamination-hosing of all external impermeable surfaces and ploughing of soil to a depth of 15 cm		2d 7d 14d
'Expensive' decontamination-complete removal of building surfaces, (roofs, walls, internal surfaces and paved areas) and the removal of the top 1 cm of soil		30d 6m
'Effective' decontamination-complete removal of building surfaces (walls and internal surfaces) and the ploughing of soil to a depth of 15 cm		14d 30d 6m

Table 2

Effectiveness of various decontamination techniques in reducing external gamma dose

Decontamination technique		Decontamination in period[1]	Range of percentage reduction in dose[2]
Hosing		2 - 14 days	neg[3] - 5
Surface removal -	roofs	30 days - 1 year	neg - 5
	walls	30 days - 1 year	neg - 40
	paved areas	30 days - 1 year	neg - 65
	internal surfaces	2 -14 days	neg - 25
Soil removal depth, cm	1	30 days - 1 year	5 - 90
	5	30 days - 1 year	20 - 90
	15	30 days - 1 year	20 - 90
	30	30 days - 1 year	20 - 90
Soil ploughing to depth, cm	5	14 days - 1 year	5 - 30
	15	14 days - 1 year	10 - 55
	30	14 days - 1 year	15 - 70

Notes:

1. These were the time periods during which it was thought that this type of decontamination technique may be used.
2. This is the range of the results given by 100 simulation runs.
3. Neg represents negligible reduction in dose.

the most practical, is the complete removal of external wall contamination. This leads to a maximum reduction in integrated dose of about 50%, although the 'best estimate' of reduction is only about 15%. Decontamination techniques such as steam cleaning and scrubbing would not remove all of the external wall contamination and so would lead to a smaller reduction in dose. The removal of soil and vegetation or ploughing of soil appear to be more effective, especially removal, although this is likely to be expensive both in carrying out the decontamination and disposal of the soil and vegetation. Ploughing of soil to a depth of 15cm in an urban area can reduce doses by up to 55% and can probably be termed a relatively 'cheap' option and one that is practicable. This work is currently being extended to look at the cost effectiveness of various decontamination techniques and to consider the potential reductions in dose to different groups in the population.

2. The Transfer of Radionuclides through Terrestrial Foodchains

During the period of this contract the NRPB's set of foodchain models, now called FARMLAND, has been further developed and various model validation studies carried out. In addition, work has been carried out to look at the patterns of production and consumption of terrestrial foods in the European Community and the distribution of foods between different regions.

An improved root crop model has been developed as part of FARMLAND. The processes modelled include the interception and retention of material deposited on the plant's surface and its translocation from the plant's surface to the edible roots. Also modelled is the uptake of radionuclides from the soil to the edible parts of the plant. The model can predict the concentration of activity in the root crop as a function of time following deposition from atmosphere at any time of the year. Account is taken of the times of planting and harvesting of the crop and the subsequent pattern of consumption. The model is based on transfers and agricultural practices applicable to potatoes, as this is the main root crop consumed in the UK. It could easily be adapted to be more applicable for other types of root vegetables. For potatoes, a distinction is made between early and late varieties, where the early varieties are consumed in a short period after harvesting while a proportion of late varieties are stored and consumed over a longer period.

The revised root crop model predicts higher concentrations of most radionuclides in root crops than the previous model used at NRPB. This is particularly the case for radioisotopes of elements such as caesium, which are readily translocated within plants. For radionuclides such as those of strontium or the actinides, where translocation is less important, the differences between the previous and revised root crop model are smaller. However, using the results of the revised root crop model does not significantly affect the predicted agricultural consequences of accidental releases. This is due to the importance of the transfer of radionuclides to other foods, in particular milk and green vegetables, together with grain for releases at certain times of the year.

Following the Chernobyl nuclear reactor accident in April 1986, a variety of environmental data became available. Some of these data were suitable for validating parts of the NRPB's model for the transfer of radionuclides through terrestrial foodchains. Initially, the FARMLAND predictions were compared with measurement data averaged over large areas of the UK and for two specific farms. The aim was to test the ability of FARMLAND to represent the general conditions for which it was developed, and also to test its ability to simulate site-specific conditions. The comparisons were carried out for iodine-131, caesium-134 and caesium-137. For the general, large area case, the foods considered were milk, green vegetables and lamb. The two specific farms were dairy farms in Cumbria and Berkshire and detailed measurement data were available for the deposition to ground and activity concentrations in animal fodder and milk.

Comparisons of FARMLAMD results with measurement data applicable to large regions of the UK have shown that the models predict the time dependence of radionuclide concentrations well. In using the post-Chernobyl monitoring data there are considerable uncertainties in the compatability of the deposition measurements with those in food. It is therefore difficult to draw any real conclusions from a comparison with these data on the ability of the model to predict the scale of transfer to a particular food. From this point of view many of the environmental monitoring information collected after Chernobyl were disappointing for detailed model validation.

Figure 2 shows the concentrations of iodine-131 and caesium-137 in milk at the two farms in Cumbria and Berkshire as a function of time after deposition. Also shown are the model predictions. For iodine-131 the model overestimates the transfer to milk by about a factor of five. These and other post-Chernobyl results indicate that the transfer factor from feed to milk for iodine used in FARMLAND is too high. This is true for a number of sites in Europe and regardless of whether the deposition occurred under wet or dry conditions. However, it is felt that more work is needed to look again at all the past experimental work on the transfer of iodine to milk and that the transfer factor should not necessarily be changed on the basis of one event. For both farms and radionuclides the model gave a reasonable representation of the time dependence of the transfer to milk. For caesium-137 the model results are closer to the measured data for the Cumbrian farm than to those for the Berkshire farm. For the Berkshire farm dry deposition led to the contamination following Chernobyl while for Cumbria the deposition was wet. It is thought that the dry deposited material may have been less available for transfer across the cow's gut and hence to milk, at least in the first month or so following the accident.

Further model validation studies were carried out using FARMLAND under the CEC post-Chernobyl research programme on underlying data for Derived Emergency Reference Levels and are described in the final report for this programme.

Calculation of the distribution of doses arising from the contamination of foodstuffs following an accidental release requires information on the distribution of food from the places of production to those of consumption. There has been a review of available data on food distribution in the UK, for the food categories of milk, milk products, beef, sheep meat, offals, grain, green vegetables and root crops. The information obtained from this review, together with data on the distribution of foodstuff production and population in the UK, has been used to estimate distribution patterns for these food categories, between nine regions in the UK. The data are appropriate for use in ACA programs. Table 3 provides an illustration of the results obtained. It contains data on the distribution of beef and veal from the point of production to the point of consumption. The data are presented in the form of percentage movements from a source region to a target region and are for the UK only.

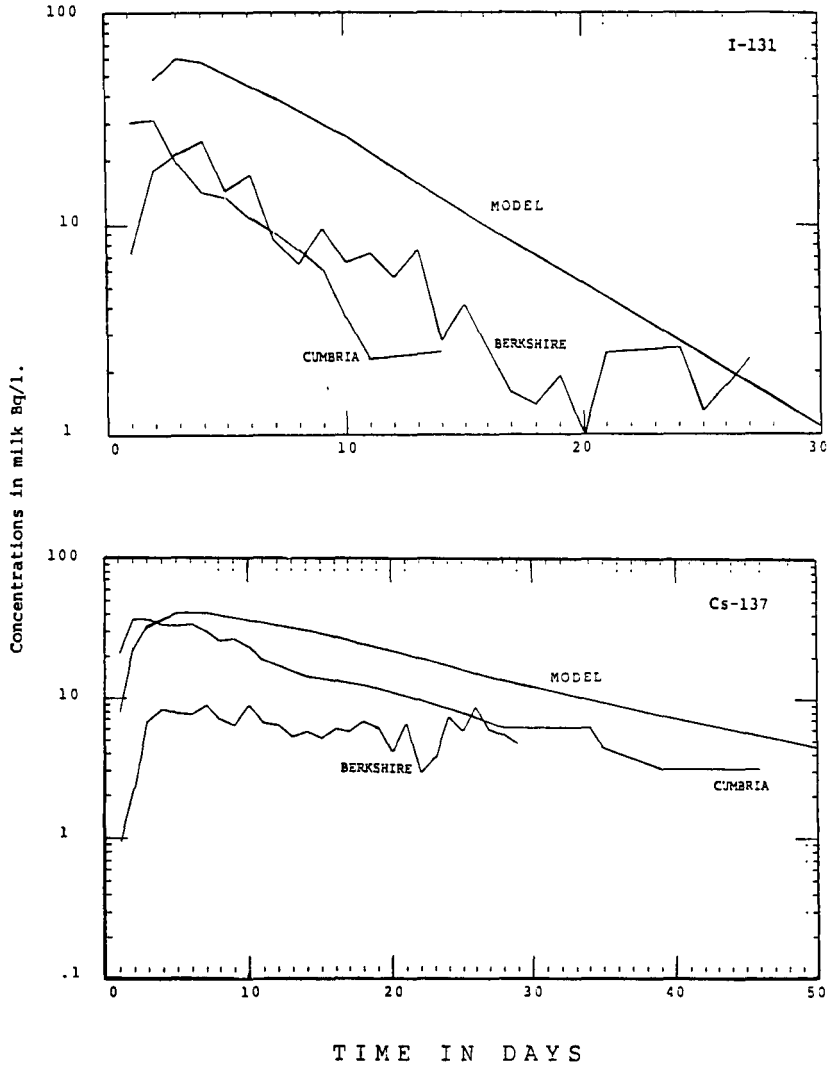

Figure 2. Iodine-131 and caesium-137 concentrations in cows milk normalised to 100 Bq/m² deposition on grass.

Table 3

Percentage of Beef and Veal from Source Region Consumed in Target Region

Source Region		Target Region 1	2	3	4	5	6	7	8	9
Scotland	1	38	-	19	6	6	6	6	13	6
N Ireland	2	18	27	37	-	-	-	-	18	
North	3	5	-	75	-	5	5	-	5	5
Wales	4	-	-	27	27	10	-	9	27	-
W Midlands	5	-	-	12	-	63	13	-	12	-
E Midlands	6	-	-	17	-	-	66	-	17	-
South West	7	-	-	-	5	5	-	27	63	-
South East	8	-	-	-	-	-	-	12	75	13
East Anglia	9	-	-	-	-	-	50	-	-	50

As part of the CEC post-Chernobyl research programme on underlying data for Derived Emergency Reference Levels NRPB and the Gesellschaft für Strahlen und Umweltforschung (GSF) have carried out a programme of work on terrestrial foodchain models. One of the aims of this work was to make recommendations on a general model suitable for use in the EC. As a first step to recommending a model, predictions of the GSF foodchain model ECOSYS and FARMLAND were compared with sets of environmental measurements. Secondly, the predictions of the two models were compared for a range of situations. Based on this work NRPB and GSF were able to recommend a general model for use in the EC in the absence of site specific information. This general model will be used to generate data sets for foodchain concentration for a number of radionuclides for use with the COSYMA package (see progress report for project 1).

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[1] Crick, M J and Dimbylow, P J, GRINDS - A computer program for evaluating the shielding provided by buildings from gamma radiation emitted from radionuclide deposition on ground and urban surfaces. Chilton, NRPB-M119 (1985).

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Simmonds, J R, Review of foodchain transfer in relation to accident consequence assessments. In Proc. CEC Workshop on Methods for Assessing the Off-site Radiological Consequences of Nuclear Accidents, Luxembourg, 15-19 April 1985, EUR 10397 (1986).

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Brown, J, Crick, M J and Hill, M D, The effectiveness of various decontamination techniques for reducing external radiation doses to people living in an urban environment. In Proc. Joint CEC/OECD(NEA) Workshop on recent advances in reactor accident consequence assessment, Rome. January 1988. CEC EUR 11408 (1988).

Crick, M J and Brown, J, EXPURT - a model for evaluating exposure from radioactive material deposition in the urban environment. NRPB Report R235 (In press).

Title of the project no.: 3

Countermeasures to reduce the impact of accidental releases of radioactive material

Head(s) of project:

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Scientific staff:

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I. Objectives of the project:

The objective of the project is to enable improved representations of the effects of countermeasures to be included in ACA computer programs, and thus to allow these programs to be used to provide a greater input into emergency response planning. The work forms part of the MARIA-2 Programme (Methods for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

The main objective of the work in this reporting period was to finalise the models for COSYMA. The coding required to implement an economics model in COSYMA was to be constructed jointly with staff at KfK, together with the addition of any features necessary to assess economic consequences in the FRG, and more broadly in the rest of the EC. The remainder of the data required by the model was to be collected. Once coding was completed the module was to be tested and example applications produced. Reports were to be prepared on the model, and input made to the COSYMA user guide.

III. Progress achieved:

At NRPB, work in this period centred around the continuing development of an improved model for calculating the economic consequences of accidents, and plans for the incorporation of this model into COSYMA. Work on the latter project was undertaken at both NRPB and KfK, and there were a number of meetings through 1989 at which collaborative aspects of the study were discussed. The work described in this progress report is limited to the parts of the study that have been undertaken at NRPB.

Several models for predicting the economic impact of accidents have been developed, both in Europe and in the US. These models have, however, either been incomplete in the economic aspects considered, or have been inappropriate for application in the UK because of national economic differences. Under this contract, a new model, COCO-1 (Cost Of Consequences Off-site) has been developed, for estimating the impact of an accident in monetary terms. COCO-1 is intended for application in studies of the off-site impact of accidents at nuclear installations. It excludes consideration of the cost of on-site consequences of accidents, which are beyond the scope of the applications planned for the model.

The new model is applicable to the UK and other countries, particularly to those Western European countries where the structure of the economy is similar to that in the UK. However, the data derived at NRPB is in general specific to the UK and may not be appropriate elsewhere. Work in progress at KfK will indicate data suitable for use in the COCO-1 model, for assessments of accidental consequences in FRG.

In the period covered by this report, the COCO-1 model was finalised. All the necessary input data for the prediction of economic consequences in the UK have now been obtained. Limited input data for the EC have also been obtained. A report on the model and its data has been prepared (Publication 2 below). Throughout this year's work, Dr C Heady of University College, London has acted as a consultant to NRPB.

Most of the consequences of an accident can, at least theoretically, be associated with an economic cost. These may be broadly summarised as resulting from:

- the application of countermeasures to reduce doses,
- radiation induced health effects in the exposed population,

- impact on the activity with which the installation is associated, for example the power programme,
- long-term social and political impact,
- ecological impact.

Ideally, a model for estimating the economic impact of an accident should consider the cost of all benefit foregone as a result of the accident, and should therefore include consideration of all of the above categories. However, because of the difficulties in calculating the economic impact of intangible effects, the approach generally adopted in the COCO-1 model is to calculate the tangible costs that have a direct and measurable effect on the economy. In one or two areas, the calculation is broadened to include less tangible costs, to represent lost benefits. The COCO-1 approach is thought to offer a broadly applicable and robust technique for estimating the economic impact of most accidents.

The principal application of the COCO-1 model is likely to be in the general areas of emergency planning, where information on the costs and effectiveness of remedial actions will provide input into decisions on the type and extent of countermeasures to be imposed after an accident, and into studies of siting policy and plant safety design features. To enable the model to be used for such applications, it has been constructed in a form appropriate for use in standard accident consequence codes.

The categories of economic cost considered in this version of the model are:

i. Countermeasures to reduce dose

The countermeasures considered are those which affect individuals' economic activity because of restrictions on movement or enforced movement away from the affected area, namely sheltering, evacuation or relocation; food restrictions; decontamination of land and buildings.

ii. Radiation-induced health effects in the exposed population

The health effects to be considered are: fatal cancers; other fatal injuries; non-fatal cancers; other non-fatal effects; genetic effects.

Certain consequences of an accident, such as social disruption and anxiety, are not included in the model; these would require separate assessment.

The treatment of the economic impact of evacuation and relocation in the model includes the cost of transport, accommodation and the costs arising from the inability of the moved population to work. Also included are the costs of the longer term loss of utility of the land and its assets, and the depreciation of these in value. For these longer term costs the ability to discount the costs occurring, to present day value, is included. The basic economic quantity used in the calculation of loss of income and capital costs is Gross Domestic Product (GDP).

In calculating the economic impact of food bans the model includes the cost of the lost production on the economy (including the cost of replacement supplies), the cost of disposal of the food, and the lost capital value of the affected land. Again, the ability to discount the longer term costs, to present day value, is included.

Data on the costs of decontamination of land, buildings etc, have been derived following a review of the literature. The COCO-1 model includes the cost of three levels of decontamination for three different types of environment (urban, semi-urban and rural) and also average values for applications which do not distinguish between types of land. Aspects included are the cost of the cleaning process in terms of equipment, materials, disposal, transport and labour.

The costs included in the model for calculating the economic impact of health effects include the costs of medical treatment and individuals' production potential. These may be regarded as 'direct' costs, ie, those which have a directly measurable effect on the economy. The model also has the capacity to include non-pecuniary costs through the 'value of life' approach. Data on the costs of medical treatment of radiation induced effects have been obtained.

Illustrative results of the COCO-1 model were obtained for a postulated accidental release from a PWR at an assumed location in south west England. The extent and variation with time of countermeasures and

health effects resulting from this accident sequence, were estimated using results from the MARC-1 (Methodology for Assessing Radiological Consequences) program. The results used were probabilistic, taking into account a range of possible weather conditions and their probability of occurrence. Specific countermeasure scenarios were assumed which are described in Publication 2 below. Standard COCO-1 default assumptions were used.

The release considered is typical of small PWR degraded core accidents.

The costs of the release are shown in Table 1. The total cost is of the order of £0.5 billion (£5.10^8). The great majority of these costs arise in the first 2 years after the accident. For this release, the majority of the cost comes from food restrictions. Of the total cost of food bans, about £0.4 billion, around £0.3 billion comes from a livestock ban and the remainder is contributed equally by milk and crop restrictions. Although most costs occur in the first 2 years there are small continuing costs from the capital value of the restricted land 50 years after the accident. Figure 1 shows the population movement costs for the release broken down by category and time. These are dominated by the lost income costs, and the cost of the lost accommodation is relatively small. The cost of transport is insignificant. There are no significant capital costs for this release.

These costs are illustrative, and are included to provide an example application of the COCO-1 model. A different economic impact would occur for accidents of different scale, and in different locations. In particular, the relative distribution of the cost of countermeasures and health effects would be affected by varying the criteria for the introduction of countermeasures.

Consideration continued to be given to the incorporation of this model into COSYMA, and the coding and interfaces that are required. The development of the coding of the economics module of the COSYMA program is being undertaken largely at KfK, together with the construction of the relevant parts of the COSYMA user guide.

Table 1

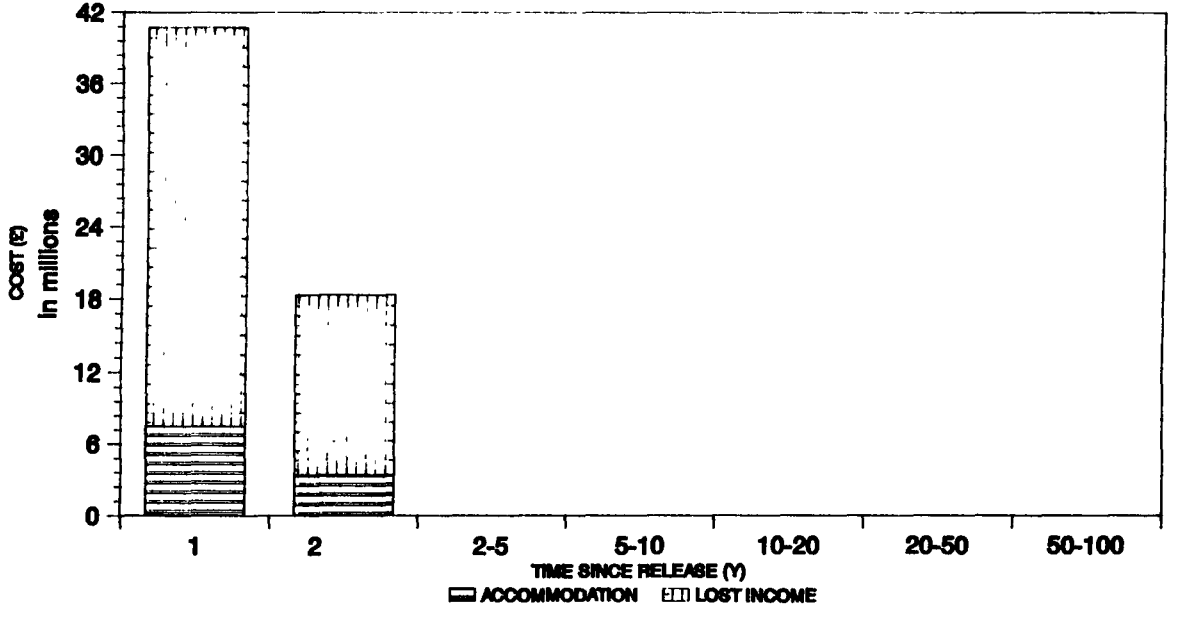
Summary of Economic Costs by Category and Time

TIME (years)	POPULATION MOVEMENT (£)	FOOD BANS (£)	DECONTAMINATION (£)	TOTAL COUNTERMEASURE COSTS (£)	HEALTH EFFECTS METHOD (i) (£)	HEREDITARY EFFECTS METHOD (i) (£)	HEALTH EFFECTS METHOD (ii) (£)	HEREDITARY EFFECTS METHOD (ii) (£)
					(2)	(1)	(2)	(1)
0-2	5.9E+07	3.8E+08	7.9E+06	4.4E+08	6.5E+05	7.6E+06	1.7E+06	2.2E+07
2-5	0.0E+00	4.8E+06	0.0E+00	4.8E+06				
5-10	0.0E+00	4.9E+06	0.0E+00	4.9E+06				
10-50	0.0E+00	3.8E+04	0.0E+00	3.8E+04	5.1E+06		2.4E+07	
50-100	0.0E+00	1.6E+01	0.0E+00	1.6E+01	1.5E+06		1.1E+07	
TOTALS	5.9E+07	3.8E+08	7.9E+06	4.5E+08	7.2E+06	7.6E+06	3.7E+07	2.2E+07

Notes:

1. Total costs of hereditary effects occurring in the first two generations.
2. The first value given is for the period 0-10 years.

(Fig .1.) CONTRIBUTIONS TO POPULATION MOVEMENT COSTS



ACCOMMODATION COSTS AND INCOME LOSSES ARE CALCULATED FROM TIME 0 TO RECOVERY 2 YEARS.(Transport cost=21.75e4 in 1st yr)

In addition to the development of a detailed economic consequence model, work was also carried out under this project to improve the countermeasures models in the NRPB's accident consequence code, MARC. The models for evacuation, sheltering and relocation were made more flexible and general and the code was modified so that the effect of introducing food restrictions at one dose criterion and withdrawing them at another could be assessed. An illustrative analysis was undertaken to demonstrate the use of the method to determine the optimum dose criterion for withdrawing food restrictions.

A version of MARC was developed in which the criteria for banning food is if the contamination exceeds a given level, rather than being related to the annual dose. The effect of introducing restrictions using the two methods is illustrated in Table 2, which gives the results of a separate study carried out using MARC[1]. This shows that, for the criteria considered, restrictions based on a contamination level are more extensive than those based on an annual individual dose of 5mSv used in earlier studies.

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Table 2

DB1 - characteristics of the distributions of agricultural consequences

Consequences	Criteria	Expectation value, E	Value at the pth percentile				% probability	
			p = 1	p = 50	p = 90	p = 99	P(N = 0)	P(N > E)
Milk restricted at 7 days (litres)	5 mSv[a]	$1.81\ 10^{2}$	0	0	$3.06\ 10^{2}$	$2.17\ 10^{3}$	57	8
	EC[b]	$7.67\ 10^{4}$	$3.46\ 10^{2}$	$4.63\ 10^{4}$	$2.17\ 10^{5}$	$4.63\ 10^{5}$	0	31
Total milk restricted (litres)	5 mSv	$4.05\ 10^{2}$	0	0	$8.18\ 10^{2}$	$3.88\ 10^{3}$	57	13
	EC	$3.32\ 10^{5}$	$5.71\ 10^{3}$	$2.0\ 10^{5}$	$9.37\ 10^{5}$	$1.98\ 10^{6}$	0	31
Crop area restricted at 1 year (km^2)	5 mSv	$9.38\ 10^{-5}$	0	0	0	0	99	$2.7\ 10^{-1}$
	EC	$1.01\ 10^{-3}$	0	0	0	$3.25\ 10^{-3}$	98	2
Time integral of area of crop restrictions (km^2 y)	5 mSv	$1.26\ 10^{-4}$	0	0	0	0	99	$6.6\ 10^{-1}$
	EC	$4.12\ 10^{-2}$	$9.10\ 10^{-4}$	$1.79\ 10^{-2}$	$1.10\ 10^{-1}$	$2.73\ 10^{-1}$	0	22
Initial number of livestock restricted	5 mSv	$4.88\ 10^{-1}$	0	0	0	$8.76\ 10^{-1}$	98	1
	EC	$1.90\ 10^{2}$	$1.29\ 10^{-1}$	$3.56\ 10^{1}$	$4.65\ 10^{2}$	$1.76\ 10^{3}$	0	18
Time integral of livestock restricted (livestock y)	5 mSv	$1.76\ 10^{-1}$	0	0	0	$1.87\ 10^{-2}$	98	$5\ 10^{-1}$
	EC	$3.60\ 10^{1}$	$1.55\ 10^{-2}$	$3.62\ 10^{0}$	$8.26\ 10^{1}$	$4.18\ 10^{2}$	0	15
Collective dose from food (man Sv)	5 mSv	6.31	$5.30\ 10^{-1}$	$4.24\ 10^{0}$	$1.41\ 10^{1}$	$2.57\ 10^{1}$	0	34
	EC	5.19	$4.33\ 10^{-1}$	$3.45\ 10^{0}$	$1.08\ 10^{1}$	$2.21\ 10^{1}$	0	33

Notes:

(a) The 5 mSv values represent the distribution of consequences arising from food restrictions imposed on an effective dose equivalent level to an adult of 5 mSv from an annual intake of the foodstuff.

(b) The EC values represent the distribution of consequences arising from food restrictions imposed on the basis of the EC intervention levels for foodstuffs.

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V. Publications:

1. Hill, M D, Methods for using the results of probabalistic accident consequence assessments in decisions on the siting, design and operation of nuclear installations. IN proceedings of the joint CEC/OECD(NEA) workshop on recent advances in reactor accident consequence assessment, Rome, January 1988. Report EUR 11408 (1988).

2. Haywood, S M, Robinson, C A and Heady, C, COCO-1: A Model for Assessing the Cost of Off-Site Consequences of Accidental Releases of Radioactivity. NRPB report, to be published.

Title of the project no.: 4

Uncertainty analysis

Head(s) of project:

M D Hill, Assessments Department, NRPB, Chilton, Didcot, Oxon, OX11 ORQ, UK

Scientific staff:

M J Crick, S M Haywood, J A Jones, 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 accident consequence assessments. The techniques were then to be used to identify the major contributors to uncertainty in such assessments. The work forms part of the MARIA-2 Programme (Methods for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

The objectives for the final year of this programme were concerned with the need to finalise the models for COSYMA and with an uncertainty analysis of the whole of MARC, including the uncertainty on those items in data libraries which would be directly applicable to the COSYMA package.

III. Progress achieved:

Programs written at the SANDIA National Laboratories for carrying out uncertainty analyses have been obtained and implemented on the NRPB VAX computer. The programs were modified slightly to make them more easily usable at NRPB.

All the work on uncertainty analysis at NRPB has been carried out using Monte Carlo methods, in which values are assigned to each of the parameters considered to be uncertain and results of the ACA programs for those values of the input variables obtained. There are two ways of sampling values for the input variables from the distributions specified for each, and the relative merits of the two systems have been investigated. The variables can be selected at random when particular quantitative statements are to be made about the probability of the output quantity exceeding particular values (the Statistical Tolerance Limit Approach). This method has the disadvantage that the random samples obtained do not necessarily cover the full range of the input distributions unless a large number of input variables are considered. The alternative method is to use the Latin Hypercube Sampling method, in which the values selected for the input variables must cover the whole of the parameter value distribution. This method, therefore, has the advantage of covering the whole of the parameter space but the disadvantage that quantitative statements about the probability of the output quantity exceeding particular values cannot be made. However the identification of important variables is easier with the Latin Hypercube Sampling method than with the Statistical Tolerance Limit Approach. Both methods have been used in studies of the uncertainty in predictions of modules of the MARC computer program.

An analysis of the uncertainty in the food chain modules of MARC-1 using the Statistical Tolerance Limit Approach was carried out as part of MARIA-1 for a very large hypothetical accident (that designated UK1) at a PWR in the UK. A similar analysis was carried out as part of MARIA-2 for a source term equal to 1% of that for UK1 [1]. The same random sample for the input variables was used in the two sets of analyses. The analysis showed that the range of the output values for the small accident was slightly larger than that for the large accident, both in terms of the range at the higher percentiles of the distribution and in terms of the probability of exceeding relatively low values of the

consequences. This is illustrated in Figure 1, which shows probability distributions of the numbers of animal years lost for the two accidents. The variables contributing most to the uncertainty, identifed by partial rank correlation coefficients (PRCC) between input and output, also differed for the two cases. This is illustrated in Table 1, where some of the PRCCs for the expectation value of number of animal years lost are shown. The initial resuspension factor has the fourth greatest PRCC for the large accident, but is much less important for the small accident.

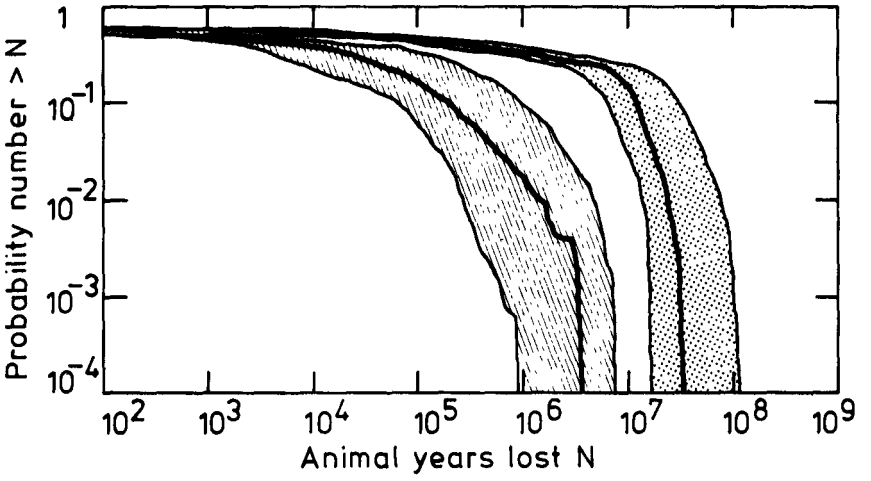

Figure 1 Uncertainty bounds and default curves for the ccfds of animal years lost following a large and a small accident

Table 1 PRCCs for the Expectation Value of Animal Years Lost

	large accident	small accident
Cs transfer to beef	0.88	0.93
Cs-137 dose factor	0.77	0.57
Cs-134 dose factor	0.74	0.89
Initial resuspension factor	0.62	0.26
Interception factor for hay	0.59	0.71
Biological half life of Cs in a cow	0.49	0.57

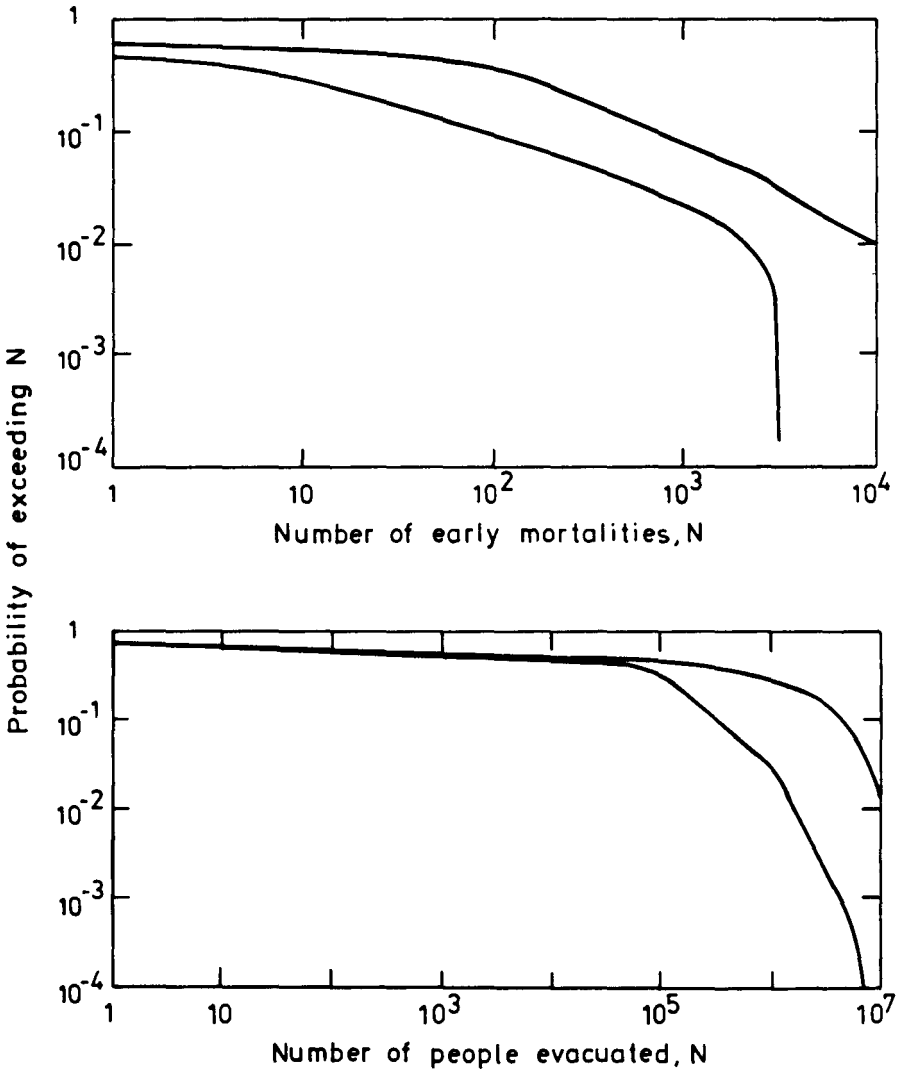

Figure 2 Subjective uncertainty bands for the complementary cumulative frequency distributions for numbers of early mortalities and people evacuated

This study demonstrates the difficulty of drawing conclusions about the uncertainty in one situation from analyses of other similar situations.

An analysis was carried out as part of the MARIA-1 programme into the uncertainty in air concentration and deposition of selected nuclides at specific points. This work was extended in the MARIA-2 programme by continuing the analysis to obtain the uncertainty on the consequences of a large hypothetical release (that designated UK1) [2]. 59 runs of MARC-1 were carried out with input parameter values selected at random from their distribution. The range of uncertainty for the numbers of health effects and countermeasures was typically about a factor of 8, as indicated in Figure 2 which shows the upper and lower envelopes of the complimentary cumulative distribution functions (ccdfs) obtained in the runs. The uncertainty on the amounts of food banned resulting from the uncertainty on the atmospheric dispersion model parameters is comparable to that found in the earlier analyses of the uncertainty in the food chain parameter values. The parameters contributing most to the uncertainty were identified by considering PRCCs between the input and output values. Unfortunately the results were not clear, with some parameters having a very high PRCC for one end-point but a very low value for other end-points which would be expected to have similar results (eg number of early deaths and number of people evacuated).

An analysis of the uncertainty in the predictions of the whole of MARC was started during the period, but has not yet been completed. The method of carrying out the analysis has been finalised, and ranges assigned for the 98 parameters considered in the atmospheric dispersion model, the food chain module, the dose and risk calculations and the economic costs module. The criteria assumed for, and timings of, the countermeasures are not considered in this analysis.

Experience gained in the earlier study of the food chain module has been used to reduce the number of uncertain parameters considered. Values for each of the parameters considered were obtained from the ranges specified by Latin Hypercube Sampling.

The amount of computer time required for this analysis is considerable, and so simplified source terms are being adopted. Two source terms, representing a severe degraded core accident and a smaller but more

frequent accident at a PWR, have been derived. Each considers 8 nuclides, some have their releases increased in order to more closely match the results from the desired source terms. This simplification also reduces the number of parameters required to describe the uncertainty on the dose per unit intake, which is considered separately for each nuclide.

Some of the parameters which are considered to be uncertain are read into MARC from data libraries. There are two ways in which the uncertainty on these parameters can be included, and both methods are used in this analysis. A data library of dose per unit intake was created for each MARC run from their standard value and an uncertain multiplying factor. However the food chain data library was calculated for each run of the MARC program from values for each of the basic transfer coefficients describing transfer of material along food chains. This was necessary to describe adequately the correlations between concentrations in the same food at different times or in different foods.

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[1] Crick M J, Uncertainty analyses of a methodology for assessing the radiological consequences of accidents at nuclear facilities. Proc Conf on Modelling under Uncertainty, Fulmer, 1989, Inst Phys Conf Ser no 80.

[2] Jones J A and Hill M D, Uncertainty analysis of the atmospheric dispersion module of MARC. Proc Joint CEC/OECD(NEA) Workshop on Recent Advances in Reactor Accident Consequence Assessment. Rome, EUR 11408 (1988).

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

Kernforschungszentrum Karlsruhe
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Postfach 3640
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Gesellschaft für Reaktorsicherheit
Garching
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V. Publications:

Hofer, E et al, Uncertainty and sensitivity analysis of accident consequence sub-models. IN Proc. ANS/ENS Topical Meeting on Probabilistic Safety Methods and Applications, San Francisco, 24 February - 1 March 1985.

Crick, M J and Jones, J A, Uncertainty of selected models of the MARC suite. IN Proc. CEC Workshop on Methods for Assessing the Off Site Radiological Consequences of Nuclear Accidents, Luxembourg, 15-19 April 1985. EUR-10397 (1986).

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Crick, M J, Uncertainty of a methodology for assessing the radiological consequences of accidents at nuclear facilities. IN Proc. International Conference on Modelling under Uncertainty, April 1986, at Fulmer UK. Inst. Phys. Conf. Ser. 80.

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-R184 (1988).

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).

Jones, J A and Hill, M D, Uncertainty analysis of the atmospheric dispersion module of MARC. IN proceedings of the joint CEC/OECD(NEA) workshop on recent advances in reactor accident consequence assessment, Rome, January 1988. CEC, EUR 11408 (1988).

Jones, J A, Brown, J and Hill, M D, Uncertainty analysis of reactor accident consequence assessments. In Transactions of the American Nuclear Society, Vol. 56, p358 (1988).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-F-111-D

Gesellschaft für Strahlen-
und Umweltforschung mbH
GSF
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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.: 1a

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:

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.

II. Objectives for the reporting period:

- final analysis of the experiments performed by Lafuma et al. (in cooperation with J. Broerse et al.).
- analysis of the incidences of various neoplasms in Sprague-Dawley rats at low γ- and n-doses; derivation of RBE-values.
- further analysis of new data from RERF, Hiroshima, to clarify the actual effect of the new dosimetry on derived risk factors.
- evaluation of radiation carcinogenesis models regarding the dose rate reduction factors for various biological end points.

III. Progress achieved:

1. Methodology

- Numerous epidemiological studies on different populations exposed to ionizing radiation were reviewed to derive exposure-time-risk surfaces for radiation induced cancer risks at low doses and dose rates;
- quantitative statistical methods were tested for their operation characteristics in the presence of confounding variables by means of the simulation codes SIRIS. This Monte Carlo code was developed during the proceeding contract period; it generates synthetic epidemiological data which then were evaluated using the Cox proportional hazards model and the contingency table analysis based on Mantel-Haenszel techniques;
- the data of the follow-up study with patients exposed to Radium Ra-224 for therapeutic purposes were quantitatively evaluated by means of non-parametric methods and model fits to derive risk estimates for bone sarcomas and lens opacifications;
- various mathematical models that predict exposure-time-risk surfaces based on biological assumptions for radiation induced tumors were critically reviewed.

2. Results

- New estimates of radiation induced cancer risks are now available for bone marrow, breast, GI-tract, lung, skeleton, skin, thyroid and for the "remaining tissues". These estimates were calculated as functions of dose, age at exposure and time since exposure. Table 1 gives a summary of these results as life time risks for the tissues mentioned for a standard population. The epidemiological observations do not allow a definitive decision concerning the dose-effect relationship. So, our results in this table are given for both the linear and the linear-quadratic model. Based on the observations among the atomic bomb survivors the risks were derived by means of an absolute risk model for leukemias and bone sarcomas and of a relative risk model for all other radiation induced solid cancers; these models form the basis for the extrapolation of observed cancer

frequencies to life-time risks.

These results do not take into account the revision of the dosimetry for the atomic bomb survivors which is not yet finished. This revision is expected to increase our risk estimates by not more than 40%.

Table 1: Somatic lifetime risks (mortality) per 10^4 persons per Gy for linear (L) resp. linear-quadratic (LQ) dose-response relationship

organ	LQ	L
Bone marrow	21	52
Bone	1	1
Breast *	80	80
Lungs	36	90
GI-tract	90	224
Thyroid **	17	17
Others	15	38
Total	260	502

* including men

** exclusively linear dose-response relationship

- The Monte Carlo simulation code SIRIS was used to generate synthetic epidemiological data for radiation induced leukemia risks. These data were evaluated by means of the Cox proportional hazards model and the contingency table analysis. The calculation of the statistical power of these methods to detect the radiation effect indicated a superiority of the classical contingency table method. This was due to the fact that the actual shape of the time dependence of the excess leukemia risk cannot be described sufficiently well by the proportional hazards model. This result is more pronounced at low doses than at higher doses. Concerning validity both analytical methods are acceptable because they both use internal controls.

- The large beagle study performed at the University of Utah was evaluated to investigate the health effects of the injection of long-lived Radium Ra-226. One of the most important results was the finding, that osteosarcomas at low injected activities appear later in animal life than those induced by higher activities.

- The follow-up study of patients treated with Ra-224 injections was evaluated. Spiess, Mays and Stefani had early recognized that the radium 224 treatment had caused an increased incidence of lens opacifications. They were able in a few cases to observe chemically the characteristic features of radiation induced cataracts.

  For most of the cataracts it was however not possible to distinguish between a spontaneous and a radiation etiology. The analysis was then based on severe cataracts i.e. with impaired vision, the derivation of the dependence on dosage and on time after treatment as well as on other factors such as age at treatment was performed with statistical methods. Scattered diagrams were made which permitted a direct judgement of the data. A non parametric analysis was performed, i.e. an analysis without assumptions on the shape of the dependencies and in a final step the analysis was completed in terms of suitable analytical models.

  Most important in the results from this analysis were the quasi threshold dose dependence with a threshold around 500 MBq/kg injected activity and the independence on age at treatment of the radiation induced cataracts. The study showed also that the risk of radiation induced cataract did not decrease until now i.e. at more than 40 years after injection. It was therefore decided to start a systematical ophthalmologic examination of the younger patients in the hope to diagnose radiation induced cataracts at an early stage. This might permit to extend the first study i.e. to infere for early cataracts the dependence between dosage and time after treatment and to follow the evolution in time of the radiation cataracts.

- The data for bone sarcomas in the same collective of patients are probably the most complete among the radiation epidemiological stu-

dies, it has permitted a detailled analysis of various factors involved. The original analysis of Mays and Spiess had been based on the assumption of linearity in dose of the bone sarcomas. In a first non-parametric analysis based on a proportional hazards model, it was shown that the data depart significantly from linearity. An important result was the derivation of a risk at low doses which was roughly half of the earlier estimated risk.

In a next step it was shown that the time dependence for the bone sarcoma appearance after treatment could very well be fitted with a log normal distribution, this agrees with the assumption used in computation of the NIH epidemiologic tables.

More recently an effort was done to clarify the question of a possible protection effect. Spiess and Mays had years ago concluded to such an effect, i.e. to an increase incidence of bone sarcomas at equal doses for longer injection times. In the present work non-parametric procedures were deviced to list such an effect while correcting for the correlation between dose and injection duration or between age at injection and injection duration. It was shown that at equal mean skeletal dose and after correction for the differences in time at risk the risk for bone sarcoma is increased for the longer injection periods. It was possible to quantify the effect and it was shown that for equal injection periods the dose effect relation is linear, but that in situations where the injection periods are proportional to the injected activity the dose relation becomes linear-quadratic. This agrees with the earlier results which concluded to a linear quadratic dose relation. This earlier conclusion has been based on an analysis which did not account for the different treatment times of the patients. Since in this collective the patients were on average given the radium injection over times proportional to the total injected activites it was then right to conclude to a linear-quadratic dose relation. The effect of prolonged injection periods is not neglegible since the risk is doubled when the same activity is injected over a period of one year instead of two months.

- An analysis of experiments performed at CENFAR (Dr. Lafuma et al.)

has been performed in collaboration with the group at CENFAR and with the Inst. für Med. Strahlenkunde (Würzburg). It dealt with the comparison of the efficiency of radon inhalation, neutron or γ-irradiation in producing lung carcinomas in male Sprague-Dawley rats.

This work was the continuation of earlier studies in which statistical methods applicable to incidental tumors had been developed. The same methods were used in the present analysis and further improved. Since the radon dosimetry for earlier experiments had proven to be faulty the present work included experiments the evaluation of which had to be corrected. Equivalence ratios between radon inhalation and neutron irradiation given in an earlier publication had to be corrected.

The present analysis based on a corrected dosimetry gave an equivalence ratio of 15 WLM for 1 mGy neutron. The equivalent ratio is the ratio of the radon exposure and the neutron dose which lead to the same increase in lung carcinoma incidence. The study has also confirmed high RBE values for neutrons relative to γ-rays. The estimated RBE for lung carcinomas in the SD rats is 40 at a gamma dose of 1 Gy.

The analysis of the other neoplasms in the experiments with low doses of neutrons or of gammas will be completed in the frame of a collaboration with the group at TND which had performed similar experiments.

3. Discussion

The derivation of risk estimates on the basis of epidemiological observation requires various extrapolations

- from medium/high doses (and dose rates) to low doses
- from limited observation periods to life-time
- from the observed population to the population of interest for the risk assessment.

The functional dependencies needed for these extrapolations can not definitively derived from epidemiological studies. This stresses the importance of the need to clarify the physical and biological mechanisms underlying the induction of cancer by ionizing radiation. In this context the modelling of primary physical interactions and effects seems to be a promising way in this direction.

IV. 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).

V. Publications:

1. Chmelevsky, D., Kellerer, A.M., Spiess, H., Mays, C.W.
A Proportional Hazards Analysis of Bone Sarcoma Rates in German 224-Radium Patients. In: "The Radiobiology of Radium and Thorotrast, (W. Gössner et al., Eds.), 32-37, Urban & Schwarzenberg, München, 1986.

2. Mays, C.W., Spiess, H., Chmelevsky, D., Kellerer,A.M.
Bone Sarcoma Cumulative Tumor Rates in Patients Injected with 224-Ra. In: 'Radiobiology of Radium and Thorotrast' (W. Gössner et al., Eds.), 27-31, Urban & Schwarzenberg, München, 1986.

3. 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. Environ Biophys 27, 103-114, 1988.

4. Chmelevsky, D., Kellerer, A.M., Land, C.E., Mays, C.W., Spiess, H.
Time and Dose Dependency of Bone-Sarcomas in Patients Injected with Radium-224.
Radiat. Environ. Biophys 27 ,103-114, 1988.

5. Jacobi, W.
Types and risks of radiation effects. In: Radiation Protection - 40, Proc. of Standing Conference on Health and Safety in the Nuclear Age (Ed.: Commission of the European Communities), ISBN 92-825-8898-X, Report EUR 11608 EN (1988).

6. Chmelevsky, D., Mays, C.W., Spiess, H., Stefani, F.H., Kellerer, A.M.
The cataract response in radium 224 patients. BIR-Report 21, 21-25 (1989)

7. Chmelevsky, D., Gerken, M., Henrichs, K., Schindel, F.
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8. Jacobi, W.
Lungendosis und mögliches Lungenkrebsrisiko durch Radon in Häusern. In: Radon und Strahlenbiologie der Lunge (Hsg.: R. Crameri, W. Burkhart), Würenlingen und Villingen: Paul Scherrer Institut, PSI Bericht 22, 31-40 (1989).

9. Lafuma, J., Chmelevsky, D., Chameaud, J., Masse, R., Morin, M., Kellerer, A.M.
Pulmonary Carcinomas in Sprague-Dawley rats after exposure to low doses of radon-daughters, fission-neutrons or gamma rays. Radiat. Res., 118, 230-245 (1989).

10. Nikjoo, H., Goodhead, D.T., Charton, D.E., Paretzke, H.G. (Eds.)
Energy Deposition of Ultrasoft X-Rays in Cylindrical Volumes. Med. Res. Council, Monography 89/1. Chilton, England (1989).

11. Paretzke, H.G.
Extrapolation from radiogenic cell transformation to human cancer risks. In: Cell Transformation and Radiation-Induced Cancer (Eds.: K.H. Chadwick, C. Seymour, B. Barnhart). Bristol, New York: Adam Hilger, 387-399 (1989).

12. Spiess, H., Mays, C.W., Chmelevsky, D.
Malignancies in patients injected with radium 224. BIR-Report 21, 7-12 (1989).

Title of the project no.: 1b

SOMATIC RADIATION RISKS AND OPTIMIZATION-PROCEDURES

Head(s) of project: W. Jacobi and H.G. Paretzke

Scientific staff: W. Bock-Werthmann, J. Godt
H.G. Paretzke, W. Jacobi

I. Objectives of the project:

Analysis and evaluation of quantitative and qualitative methods of decision making for the optimization of radiation protection of workers and the general public.

II. Objectives for the reporting period:

Final Report on possibilities and limitations of quantitative methods for the optimization of radiation protection and on risks of the daily life in various European countries as a yardstick.

III. Progress achieved:

This sub-project had a very low weight relative to projects 1a and 2. Therefore only few results could be obtained, which, in addition, were thematically influenced by the Chernobyl reactor accident occurring during the period of this project.

After large environmental contaminations, the introduction of various activity limits for agricultural products used for human nutrition was considered in countries of the EC. We analysed the applicability and efficacies of various strategies for the usage of agricultural products with contaminations above these limits as animal feed in such a way that the final contamination of the respective animal end product (e.g. milk, meat, eggs) are below those limits for human nutrition. Here the nuclides Sr-90, I-131, Pu-239, Am-241, and Cs-137 were considered, and various feeding procedures could be identified were agricultural products with contaminations more than ten times the limits for human consumptions could safely be used as feed. The results of this work formed also the base of a respective recommendation of the German Strahlenschutzkommission (published in Bundesanzeiger No. 208, 5.11.88, p. 4758-60).

This experience has clearly shown, that it remains doubtful whether single- or multi-criteria decision making in the optimization of radiation protection measures will lead to a higher acceptability of the minimized total detriment strategy. However, the assumptions and weight-setting underlying a decision process will become more transparent. It is important to assess also the actual feasability of an optimum strategy. In the context of contaminated agricultural products, it must be expected that in a given situation the public market and political considerations might lead to different settings of weights and thus to the realization of different decisions.

Numerous papers and books published in the open literature on cost-benefit analyses in the public domain where evaluated. However, interviews with decision makers in administrations and in industry has clearly shown, that actual decisions are mainly influenced by subjectively perceived "opinions", which cannot be quantified for mathematical procedures in decision theory.

Cost/benefit-analyses are actually used in industry only to compare the "expected utilities" from various alternatives. These "utilities" for a company can sometimes be expressed in monetary values in most cases. The net effects on the environment and on public health, however, cannot be reduced easily to financial units. Thus, multi-criteria decision methods must be used by those responsible for permitting the realisation of an alternative chosen as optimum by a company. The actual importance for the whole system of those parameters which cannot be expressed in monetary values make quantitative and comprehensive optimization studies rather difficult and open to discussion. However, they essentially contribute to the transparency of a decision process and the subjective preferences expressed.

A prerequisite for optimization strategies is the knowledge of comparable risks (or of risks attributable to alternative decisions). Therefore a great number of common risks were quantified. Also a number of technological risks were included to allow comparisons with risks of new technologies. In this way they can be put into perspective with those already accepted.

Risks attributed to the construction of energy systems have been assessed thoroughly for nuclear power, hard coal and hydropower plants. The total investment figures were broken down into branches of the construction activities and the corresponding manpower was then calculated using productivity numbers. The German workmen's compensation insurance statistics were used to evaluate the anticipated construction risks. For a model power plant with an annual electricity production of 1 GW(e) yr the following construction risks have been calculated:

Power plant	Fatalities MDL / cases		Injuries MDL / cases	
Nuclear	1200	0.2	1250	120
Hard coal	1540	0.09	625	59
Hydro	1860	0.3	1100	130

These figures were compared with those of other authors and some of the discrepancies were discussed. All those assessments had to deal with uncertainties which had been identified carefully and taken into account.

The risk figures obtained demonstrate that construction risks of power plants form a substantial share of the total risks from all steps of fuel cycles.

A worldwide standardisation of methods and systems would be very desirable.

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

IAEA Vienna

V. Publications:

1. Jacobi, W., Paretzke, H. G.:
   Strahlenbedingte Risken
   In: Strahlenschutz. wiss. Grundlagen, rechtl. Regelungen, prakt. Anwendungen, Ed. J. Hacke, Verlag H. Hoffmann, Berlin (1985)

2. Paretzke, H. G.:
   Impact of the Chernobyl Accident on Radiation Protection
   Health Physics 55, 139 - 143 (1988)

3. Bock-Werthmann, W.:
   Construction Risks of Nuclear Power Plants for Use in Cost/Effectiveness Considerations
   GSF-Bericht 28/86, Neuherberg (1986)

Title of the project no.: 2

a) Assessment of external exposures of members of the public after accidental releases of gamma emitters from industrial facilities

b) Assessment of internal exposures due to incorporated radionuclides for members of the public.

Head(s) of project:

W. Jacobi and H.G. Paretzke

Scientific staff:

P. Jacob, K. Henrichs, H. G. Paretzke,
R. Meckbach, W. Jacobi

I. Objectives of the project:

a)- Calculation of organ doses in certain homes and in open air for various relevant gamma emitters in the soil and sitting on walls of constructions by means of Monte-Carlo methods,
- Check of the accuracy and reliability of simplified calculation methods,
- Comparison of computed results with experimental data.

b) - Calculation of organ-specific exposure rates for the internal exposure due to consumption of contaminated foodstuffs (elements: Sr, Tc, Cs, I, U, Pu, Am, Np, Cm) by members of the public,
- Assessment of the reliability and variability of these dose-factors.

II. Objectives for the reporting period:

a) The results obtained will be summarized in a model for the assessment of external exposures due to radionuclide depositions in urban environments.

b) In a final report the calculated dose conversion factors for the nuclides mentioned above, and, as far as possible, their variability will be summarized as functions of age and time since incorporation. Radionuclides for which further research is needed will be identified.

III. Progress achieved:

1. Methodology

a) The photon transport in different environments was simulated by the Monte Carlo method. In a first step the gamma dose rate in air due to finite and infinite disk sources at different depths in the soil was calculated. The model of a plane source in the ground, shielded by a soil layer of 0.5 $g \cdot cm^{-2}$, was chosen for the calculation of organ doses of anthropomorphical phantoms in an unshielded location after a wet deposition of radionuclides. The results of the environmental transport for this source geometry and of the photon transport in the phantoms were then coupled in such a way that all characteristics of the radiation field (dependence on angle and photon energy) were taken into account. The calculation was first performed for monoenergetic sources. Then organ dose rate factors for radionuclides have been calculated, including the contributions of daughter nuclides.

Five urban environments (a house of prefabricated parts, a semidetached house and a row of terrace houses in suburban environments, a house block with another house block or a park across the street) have been simulated in a computer. Fig. 1 shows as an example the geometry 'house block with park' and the floor plan of the house block. The photon transport in these geometries has been simulated by the Monte Carlo method for three different source energies (0.3, 0.662, 3.0 MeV). From these results the gamma exposure at several locations in the simulated environments has been calculated for different contamination scenarios.

In collaboration with the Commissariat à l'Energie Atomique, Paris (CEA) a shielding experiment was performed in Cadarache (France). A house was exposed to a ^{60}Co source and the radiation field inside the house was detected by a transportable high-purity Germanium detector. The photon transport inside the detector was simulated by the Monte Carlo method to obtain from the detected impulse height distribution the photon spectrum. For comparison the geometry and the photon transport in the environment were simulated by the same method used for the calculations mentioned above.

b) The calculation of dose conversion factors for incorporated radionuclides requires the knowledge of two factors: the specific effective energy (SEE), which describes the transport of radiation in the body, and the number of disintegrations in various body tissues, which depends on the distribution and turnover of the incorporated compound.

SEE-values were calculated on the basis of simplified anthropomorphic phantoms by using Monte Carlo techniques for the simulation of radiation transport. The phantoms make use of those published by M. Cristy (ORNL).

Biokinetic data were derived by evaluating numerous publications for the relevant radionculides. SEE-values, the numbers of disintegrations in various body tissues and the resulting doses were calculated by means of ICRP computer codes modified to take into account the age-dependence of SEE and, if available, of the biokinetic data.

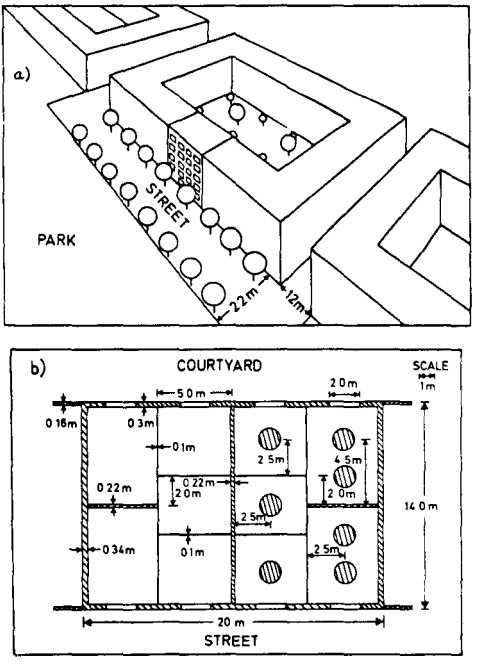

Figure 1a) Birds-eye view of the house block with a park across the street and trees.
b) Floor plan of the house block. One half of the symmetrically distributed detection regions is indicated by the shaded areas.

2. Results

a) As shown in Fig. 2 the conversion factor from the kerma rate in air to the effective dose equivalent was found to be 0.8 • 0.08 for plane sources in the range of 70 keV to 3 MeV. This factor is by 15 % higher than for volume sources in air. In the range of 150 keV to 3 MeV the conversion factors for the other organs (with the exception of the skeleton) differ by less than 20 % from the effective dose equivalent.

Dose equivalent and dose equivalent-rates in 22 organs have been tabulated for several times after the deposition of 159 radionuclides. For most of the radionuclides the contributions of daughter nuclides can be taken into account just by adding dose factors. For other radionuclides as ^{95}Zr, ^{129m}Te and ^{140}Ba the time dependence of the gamma dose rate after the deposition is nontrivial. Due to the production and decay of the daughter nuclides the maximum of the gamma dose rate is reached several days or even later after the deposition of these radionuclides.

The contribution of the various deposition areas to the kerma at several locations inside and outside different house types have been tabulated for 5 environments and 3 source energies, as shown exemplarily in Table 1. The buildup-factor method turned out to give results which are wrong by up to several orders of magnitude for locations in these geometries, which are well shielded against the direct irradiation from the source but have only a poor shielding against the scattered radiation (example: upper storys in a house block and a ground source, scattered radiation enters through the windows).

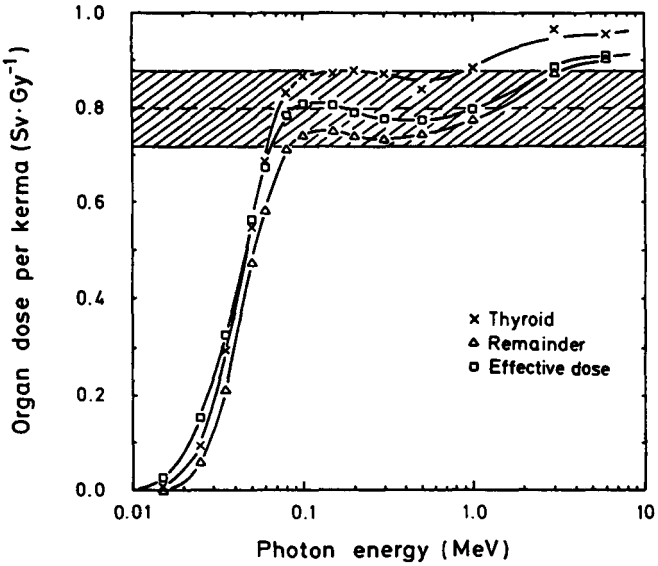

Fig. 2. Dose equivalents per kerma free in air at a height of 1 m above- ground for thyroid, remainder and for the effective dose equivalent. Infinite plane source shielded by a slab of 0.5 g·cm^{-2} to simulate surface roughness and initial migration.

Kerma (pGy per g·mm^{-2}) - Detector locations.

Deposition area	Basement	Ground floor	First floor	Attic	Outside, front
On the house:					
Windows	0.1	14	15	0.1	10
Walls and doors	0.08	13	11	5.1	73
Roof	0.004	0.25	5.5	214	5
Basement windows	1.2	0.02	-	-	0.02
Light shafts	0.51	0.2	0.02	0.005	0.5
Street	0.008	7.3	2.6	1.5	340
Gardens	0.03	26	10	7.7	155
Ground beyond neighbouring buildings	0.004	4	4	30	36
Walls and windows of neighbouring buildings	0.01	6.2	4.8	7.4	57
Roofs of neighbouring buildings	-	0.6	0.9	14	7
Trees	0.025	11	6.0	5.4	44

Kerma 1 m above an infinite smooth air-ground interface: 825 pGy per g per mm^{-2}

Table 1. Contribution of the various deposition areas to the kerma at several locations inside and outside the row of four large terrace houses, for a source energy of 0.662 MeV.

The study of different contamination patterns for the urban surfaces showed, that the kerma rate at most of the locations divided by the kerma rate over a large lawn is more or less constant in the time after the deposition. This ratio is in many cases by about 30 % lower for wet depositions than for dry depositions. For dry depositions due to the potentially high deposition on trees the external exposure may be doubled compared to an open lawn.

The dose rates in the houses are in general lower than assumed in previous studies, partly since these have used the lighter constructions of American houses (compared to European) and partly since the relatively low deposition on paved surfaces and the shielding by the urban environment has been taken into account in this study. Dose rates in single family houses are found to be reduced by about a factor of 10 compared to an open lawn, in large buildings by about a factor of 100.
The comparison of the results of a measurement of the photon spectrum in a house, which was exposed to a ^{60}Co source, and the Monte Carlo calculation of the photon transport in the simulated geometry showed a very good agreement (see Fig. 3)

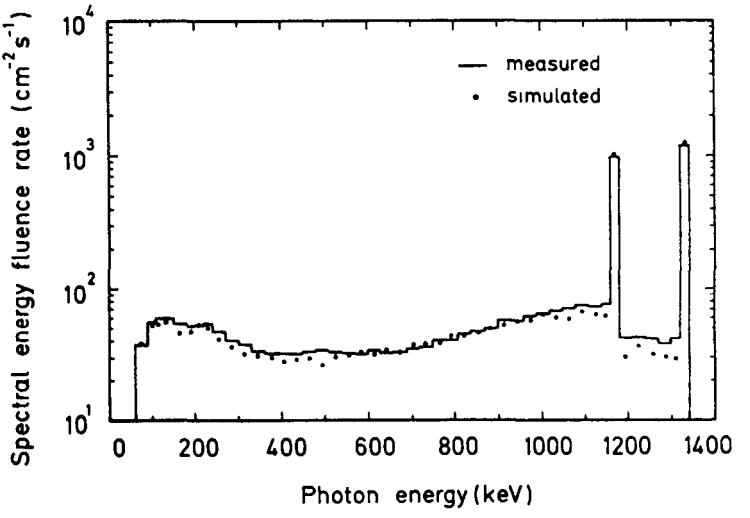

Fig. 3 The spectral photon fluence in a house, which was exposed to a ^{60}Co source. Results of a measurement and a Monte Carlo simulation.

The method used by the GSF has been compared with calculations of the CEA for a semi-infinite cloud source exposing a single house with five floors. The CEA results appear to be systematically higher (by 50-120 %) for radiation coming through the roof, and systematically lower (by about a factor of 0.6) for radiation coming through the walls and the windows.

b) **Specific effective energies**

A prerequisite for the calculation of dose conversion factors applicable for the general public is the availability of SEE-values for the relevant radionuclides as functions of age (at time of incorporation). As the announced data calculated at ORNL were not available in time, specific absorbed fractions and based on these values, specific effective energies were calculated for six different ages: newborn, 1, 5, 10 and 15 years and adult. The basis for this calculation were simplifications of the anthropomorphic phantoms published by M. Cristy (ORNL). These SEE-values are available now for more than 100 radionuclides of interest in the context of releases from nuclear facilities.

Biokinetic data

The biokinetic data needed for the quantification of the numbers of disintegrations of radionuclides (and their progenies in different body regions were derived by a review of the relevant literature. During this review, which began already in 1978, several thousand publications were evaluated with special emphasis on age dependencies of the important parameters.

The published data were interpreted by means of (first order) compartiment models in combination with the ICRP models for the respiratory tract and the GI-tract. Only for few elements sufficiently reliable biokinetic data are available which were derived from studies with humans, examples are Cesium and Iodine. For the majority of the elements the data needed are based on animal studies extrapolated to humans. In some cases experimental data are not available at all and they have to be estimated by means of analogies to chemically similar substances.

The most comprehensive compilation of biokinetic parameters are publications by the ICRP (ICRP 30, ICRP 48, ICRP 53, ICRP 54). But these tabulations are restricted to occupationally exposed adults, they do not give any information on age dependencies or on the influence of incorporation pathways typical for the general public (e.g. nutritional status). Only about 4% of the reviewed publications give age dependent biokinetic data which can be used for dose calculations.

The calculations performed for this project were mainly based on the data given by the ICRP publications 30 and 48. For few elements new, partly age dependent data are available: Hydrogen, Strontium, Iodine, Cesium, Plutonium and Americium. For the bone seeking substances age dependent models and data were published by ORNL.

Dose conversions factors

On the basis of the formalism described in ICRP publication 30 the dose conversion factors were calculated for the inhalation and ingestion pathways. They were compiled for the ages listed above as functions of time since incorporation. For the bone seeking substances some of these factors (for the 50-years comitted dose equivalent) could be taken from a publication of M. Cristy (ORNL).

As an example figure 4 shows the results for the inhalation of $^{239}PuO_2$, which is typical for

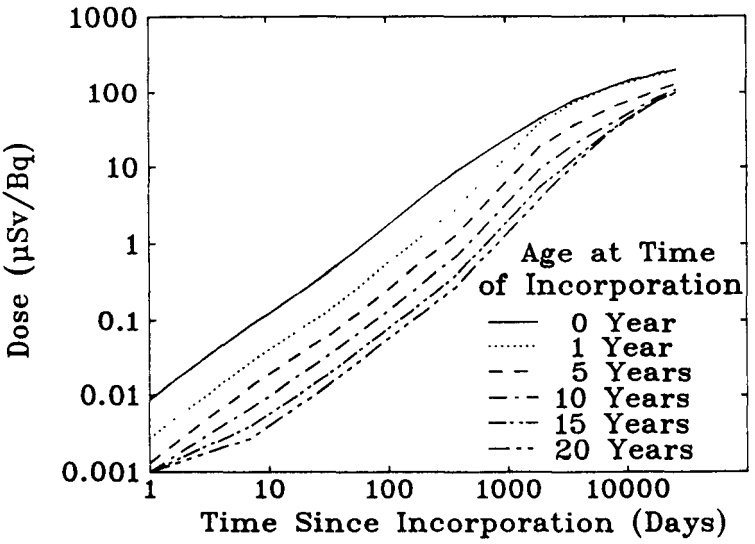

Figure 4: Effective dose equivalent for the inhalation of $^{239}PuO_2$ as a function of age at inhalation and of time since inhalation

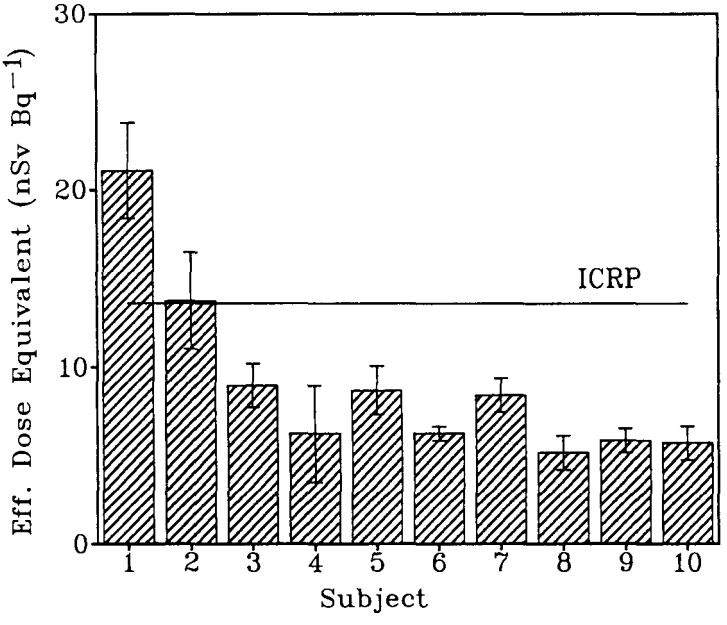

Figure 5: Effective dose equivalent for ^{137}Cs (ingestion) : measured results for ten volunteers in comparison to the value given by the ICRP.

radionuclides with a long residence time in the body.
The complete results will be available on request on a magnetic tape which is presently in preparation.

Reliability of dose conversion factors
Two sources of uncertainties determine the reliability of dose conversion factors

- the use of phantoms for the simulation of radiation transport in the organism and
- uncertainties in the biokinetic data.

General statements quantifying the resulting uncertainties are not possible due to the complex influences and interactions of the parameters and models. Especially, the frequency distributions of individual biokinetic parameters in a population are not available.

The only exceptions are Iodine and Cesium isotopes. For shortlived iodine isotopes the 95% percentiles of the thyroid dose are about twice as high as the best estimates, for ^{129}I about 3.5 times higher. The corresponding factor for Cesium isotopes is about 1.6; this estimate takes into account the correlation of body mass and biological half time.

For other nuclides only the contributions of some parameters to the total uncertainty can be quantified. The statistical errors the radiation transport simulations vary between 5% and 30%. The individual variability of organ masses (within one age group) contribute about 15% to the total uncertainty.

A limited possibility to quantify the reliability of calculated dose conversion factors is given by the comparison to results of other scientists. One such comparison was performed by G. Kendall (NRPB). For 19 selected radionuclides the (ingestion) dose conversion factors (for the adult) calculated by NRPB differed by 5% on the average, the maximum deviation was 40%.

The reactor accident in Chernobyl made it possible to check experimentally with a small group of 10 volunteers the biokinetics of Cesium incorporated via foodchains. The results are shown in Fig.5, demonstrating an acceptable agreement of the measured dose conversion factors with the corresponding ICRP-values.

Identification of radionuclides for further research
For many elements the biokinetic data available are scarce, little reliable and often extrapolated from animal studies. Some of these elements are of high importance in the context of possible releases from nuclear installations. The results of a literature review was published in a report of Eurados committee 6 indicating a strong need for further research for a large number of elements.

3. Discussion

a) Results obtained in this study allow the assessment of external exposures in urban environments for various contamination scenarios. Besides different deposition patterns the effectiveness of decontamination measures can be studied. A model for the external exposure of the public after a deposition of radionuclides has been derived from these results. Due to several reasons the exposure over an open lawn has turned out to be a favourable location for the assessment of the

external exposure in unshielded locations: most is known on the deposition on lawns, in case of accidents measurements on the deposition on lawns are performed routinely and the ratio of the exposure at other locations to the exposure over a lawn (location factor) turned out to be constant in time or to decrease smoothly. Therefore a realistic, but smoothly overestimating method for the external exposure at longer times after the deposition is obtained by multiplying the exposure over a lawn with location factors for the time of deposition.

Simple calculation methods as the buildup factor has not been proven to be generally applicable to the calculation of external exposure in urban environments. It has become evident that it is not worth-while to develop calculation schemes which improve such a method. It is more favourable to use more exact methods like the Monte Carlo simulation, since the computers have become much faster in the past five years and complex problems can now be solved by these methods.

Although large progress has been achieved in the past five years in the knowledge and modelling of the external exposure, there are several gaps or uncertainties to be clarified in the future. First, deposition and weathering of radionuclides on trees in urban environments, in light shafts for basements and indoors have to be known with a much better accuracy than it is the case today. These deposition areas can influence the external exposure outside, in basements and in living rooms in large buildings considerably. Second, the external exposure from indoor photon sources has not been calculated in a satisfactory manner up to now. Third, the dependence of the external exposure in basements on various parameters and the effect of decontamination measures on the external exposure could be understood much better. The methods developed up to now could be used for the support of the management of emergency situations.

b) The need of anthropomorphic phantoms for the simulation of radiation transport in the human body rises the question how representative are the currently used mathematical phantoms in comparison to real persons. The development of so called voxed phantoms on the basis of CT- or NMR- tomographies for external exposures promises a possibility to answer this question also for internal emitters. For the extrapolation of biokinetic data derived from adults for the use for members of the general public suffers especially from a lack of knowledge of age dependencies. The only possibility to overcome this difficulty is the inforced development and use of physiologically based models for the relevant elements.

Another shortcome of the present situation is that the available models were developed exclusively for dose factor calculations but are in general not well suited for the assessment of actual incorporation events on the basis of measured body burdens. Here, models for short term retentions, especially in the lungs, and excretion rates are urgently needed for incorporation surveillance.

The results of an evaluation of the available literature indicated that the quality of the data base for the derivation of dose conversion factors varies largely among the various nuclides. Expecially the extrapolation of data derived from animal studies is highly questionable in many cases. A good chance for an improvement of this situation is the use of stable isotopes in connection with studies with humans. The identification of research needs during this project helped to define and to plan a new CEC-project starting at present in cooperation with various European research laboratories.

IV. Other research group(s) collaborating actively on this project:

NRPB-Chilton (Drs. Dennis, Adams, Fry), AEC- Harwell (Dr. Gibson),
ORNL-Oak Ridge (Dr. Eckerman)

V. Publications:

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3. P.Jacob and R.Meckbach : Shielding Factors and External Dose Evaluation,
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4. P.Jacob and H.G.Paretzke : Dose-Rate Conversion Factors for External Gamma Exposure, Nucl. Instrum. Methods Phys. Res. A255, 156-159 (1987).

5. R.Meckbach, P.Jacob, H.G.Paretzke : Shielding of Gamma Radiation by Typical European Houses,
Nucl. Instrum. Methods Phys. Res. A255, 160-164 (1987).

6. P.Jacob and H.G.Paretzke : Neue Berechnungsverfahren für externe Strahlenexposition, Proc. IVth European Congress/XIIIth Regional Congress of the International Radiation Protection Association (IRPA), Salzburg, Österreich, pp. 148-152 (1988).

7. R.Meckbach, P.Jacob, H.G.Paretzke : Abschirmung von Gammastrahlung durch Gebäude, Proc. IVth European Congress/XIIIth Regional Congress of the International Radiation Protection Association (IRPA), Salzburg, Österreich, pp. 153-157 (1988).

8. P.Jacob, H.G.Paretzke, H.Rosenbaum, M.Zankl : Organ Doses from Radionuclides on the Ground. Part I: Simple Time Dependences, Health Phys. 54, 617-633 (1988).

9. P.Jacob and H.G.Paretzke : Organ Doses from Radionuclides on the Ground. Part II: Non-Trivial Time Dependences,
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Radiat. Prot. Dosim. 25, 167-179 (1988).

11. R.Meckbach and P.Jacob : Gamma Exposures Due to Radionuclides Deposited in Urban Environments. Part II: Location Factors for Different Deposition Patterns,
Radiat. Prot. Dosim. 25, 181-190 (1988).

12. P.Jacob : External Exposure from Radionuclides Deposited in Rural and Urban Environments,
Proc. XVth Regional Congress of the International Radiation Protection Association (IRPA), "The Radioecology of Natural and Artificial Radionuclides", Visby, Sweden, pp. 359-366. Ed. W. Feldt, Verlag TÜV Rheinland, Köln (1989).

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14. K. Henrichs, H. G. Paretzke, G. Voigt, D. Berg: Measurements of Cs Absorption and Retention in Man,
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15. K. Henrichs, D. Berg, L. Bogner: Whole body measurements since Chernobyl,
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Title of the project no.: B16-F-111-D

QUANTIFICATION OF RADIATION RISKS, OPTIMIZATION PROCEDURES AND ANALYSIS OF OCCUPATIONAL EXPOSURE

Head(s) of project:

Dr. G. Drexler

Scientific staff:

Dr. D.F. Regulla
Dipl.-Phys. J. David
Dipl.-Ing. (FH) H.-N. Brand
Dipl.-Phys. H. Eckerl (1983-1988)

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:

III. Progress achieved:

1 Interpretation of measured doses in terms of risk relevant quantities

The research activities during 1985/1989 covered aspects of modelling of exposure situations for external radiation. Physical and mathematical exposure models were sucessfully used to generate necessary links between measurement and interpretation of doses. The elaborated relationships between radiometric, dosimetric and radiation protection quantities in the entire field of x and gamma radiation are to a large extent incorporated in basic publications of ICRP and ICRU. Especially ICRP 51 (1987) reflects the relevant activities in detail covering the last two CEC research periods. Achieved progress can mainly be related to better modelling of the radiation source and the human body; further improvements were made concerning calculations of the radiation transport and the investigations on the influence of the quality factor definition.

1.1 Modelling of the radiation sources

Radiation source modelling was extended to the occupational situation of irradiation from plane sources, e.g., contaminations of the ground following accidents. A ground source code was developed, starting with a simple one and later the presently used more detailed and sophisticated code. The simple version assumed a uniform distribution of radioactive material on the surface of a plane ground and neglected any absorption of the radiation in the air. The improved code allows not only for consideration of air absorption but also for absorption in the soil, if the active material is not on the surface but in the depth. The radioactive material can either be located as a thin homogeneous layer in a certain effective depth accounting for the surface roughness or it can be distributed uniformly throughout the soil as this is the case for the natural radionuclides (28).

Fig. 1 shows dose conversion factors for the mean total body dose equivalent of the adult male phantom, for the two different models of a plane ground source. The latter model leads to lower conversion factors because photons lose part of their initial energy in the environment before they enter the phantom.

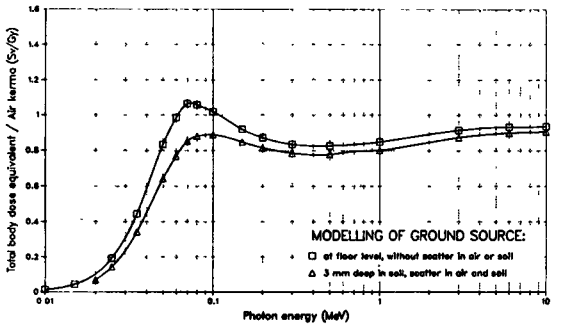

Fig. 1:
Total body dose equivalent per air kerma 1 m above the ground (male adult phantom)

1.2 Models of the human body

Most of the heterogeneous mathematical phantoms in use for dose calculations are based on the ICRP Reference Man data (2). In 1982, two sex-specific adult phantoms ADAM and EVA were introduced (1), based on the design characteristics of the MIRD-5 phantom (3). According to the definition of the effective dose equivalent for the specification of numerical values of H_E, sex-specific phantoms are needed. This is, for example, illustrated in Fig. 2 which shows a comparison of lung dose conversion factors for a parallel a.p. whole body irradiation for three different phantoms, a hermaphrodite and the two sex-specific GSF phantoms (25). Here two effects can be studied: The difference of the lung dose values for the male and the hermaphrodite phantom is due to shielding of the lungs by the breasts for a.p. projections; and the higher dose values for the female compared to those for the hermaphrodite phantom result from the smaller body size of the female phantom.

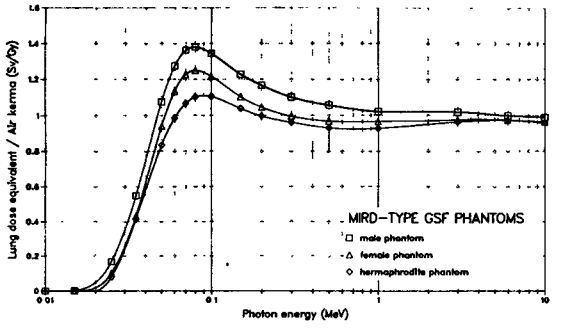

Fig. 2:
Lung dose per air kerma for parallel a.p. whole body irradiation

Another set of dose calculations using the phantoms with and without arms is demonstrated in Fig. 3: Here the ratio of the effective dose equivalent H_E for a parallel left-lateral whole body irradiation of the different phantoms is shown for various photon energies. The values of H_E are lower for the phantoms with arms, especially in the low energy range, again due to the shielding of relevant organs by the arms (25).

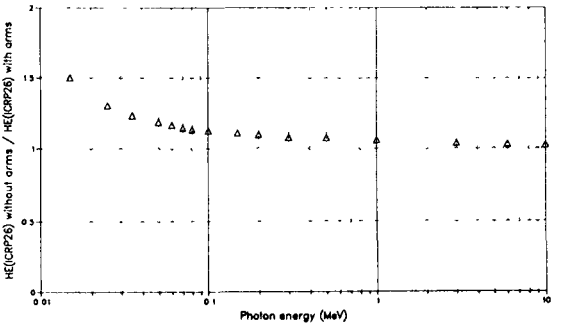

Fig. 3:
Ratio of effective dose for parallel lateral whole body irradiation. Phantoms with and without arms

Although the body characteristics of the MIRD-type phantoms are in good agreement with those of the reference man and woman, they have some disadvantages related to the location and shape of organs and the form of the whole body. Furthermore, the phantoms ADAM and EVA represent adults, which limits their applicability in calculation of radiation exposures of the members of the public. In order to overcome these disadvantages and to obtain more realistic phantoms, a technique based on computer tomographic (CT) data was developed. This technique allows any physical phantom or real body to be converted into computer files which can be coupled to a code for organ dose calculations. Each organ and tissue of this "voxel" phantom consists of volume elements, derived from CT data. Therefore, the location and shape of the organs and tissues are accurately modelled. The following media are taken into account: hard bone, bone marrow, soft tissue, muscle tissue, lung tissue, skin and air. Special care is given to the modelling of the red bone marrow. The relative amount of bone marrow in each voxel within the skeleton can be estimated from the CT numbers of the respective bone pixels. Hence, the spatial distribution of the red bone marrow can be assessed with high resolution (22, 29).

So far three voxel models were constructed: one of an 8 week old baby, one of a 7 year old child and one of the Alderson Rando phantom which is a physical phantom used for dose measurements mainly in radiotherapy. The latter mathematical phantom was constructed in order to perform comparisons between measurements and calculations, and to compare calculations using the male MIRD-type phantom ADAM and the voxel model of an Alderson-Rando phantom (25, 27)).

Organ dose conversion factors for these different types of phantoms are shown in the following figures: Fig. 4 shows dose conversion factors for the male GSF phantom and for the voxel model of an Alderson-Rando phantom (25).

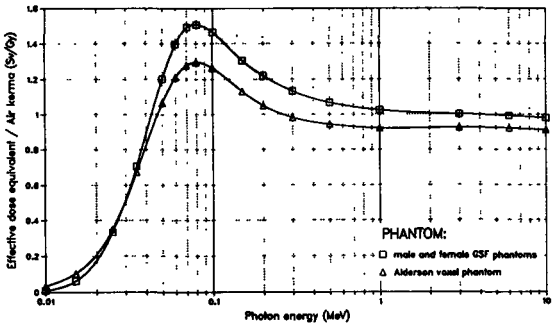

Fig. 4:
Effective dose equivalent per air kerma for parallel a.p. whole body irradiation

Fig. 5 presents red bone marrow dose conversion factors for the adult MIRD-type GSF phantoms and the voxel phantoms of a seven year old child and an eight week old baby (25). The geometry is a plane ground source, the normalization quantity is air kerma free in air 1 m above the ground. All phantoms are standing on the ground in an upright position.

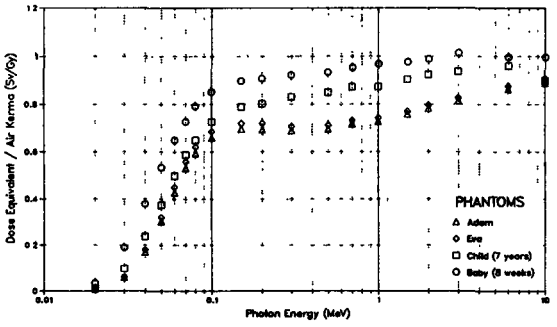

Fig. 5:
Red bone marrow dose per air kerma 1 m above ground for irradiation from a plane ground source

1.3 Models of the radiation transport in matter

The basis of the Monte Carlo (MC) method for photon transport is the computer simulation of individual photon histories and the averaging of these histories over many thousands of photons to provide the quantities of interest. At each stage in a photon history, random numbers are used to select the parameters of the photon according to known probability distributions. The energy transferred in each photon interaction is assigned to a certain organ or tissue and summed up for the respective tissues. The doses are finally obtained by dividing the total energy deposited in a tissue by the mass of this tissue. The first MC codes assumed secondary particle equilibrium and, consequently, all energy transferred in a photon interaction was deposited at the point of interaction without taking into account any energy transfer to secondary electrons ("kerma approximation"). Most of the codes used now are able to transfer the photon interaction energy to secondary electrons which are further pursued (25).

2 Development of strategies for optimization of radiation protection

2.1 Quality factor

In the past few years the concept of the quality factor Q in radiation protection has been widely discussed. Various proposals for Q as a function of LET (linear energy transfer) or y (lineal energy) have been made in order to consider results of radiobiological experiments indicating a higher RBE (radiobiological effectiveness) of neutrons than expressed by the quality factor for neutrons according to the present convention.

The consequences of the different proposals were, by Monte Carlo calculations, analyzed for photons (a) for the effective dose equivalent H_E in the anthropomorphic phantoms ADAM and EVA, and (b) the ambient dose equivalent H*(10) in the ICRU sphere (10, 30). Accordingly, each of the proposals leads to different functions of the effective quality factors for H_E (Fig. 6) and H*(10); none of them yielded an energy range with Q to be energy independent and equal to unity. A reasonable compromise was developed here, as part of the project's results, by proposing the following definition (1):

$$Q(L) = \begin{cases} 1 & \text{for} \quad L_\infty < 10 \ \text{keV}/\mu\text{m} \\ 0.32\ L - 2.2 & \text{for} \quad 10 \leq L_\infty < 100 \ \text{keV}/\mu\text{m} \\ 300/\sqrt{L} & \text{for} \quad L_\infty \geq 100 \ \text{keV}/\mu\text{m} \end{cases} \qquad (1)$$

From this, the maximum deviation in Q for photons from unity would be about 10%, while Q for neutrons follows, e.g., the ICRU 40 recommendations (Fig. 7).

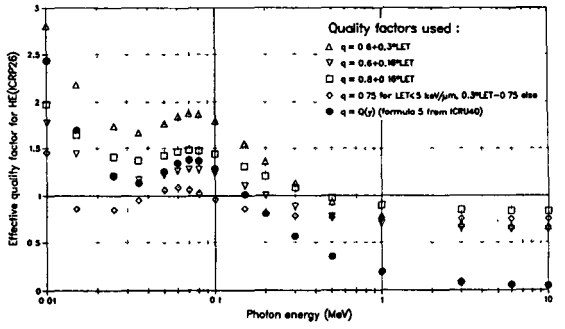

Fig. 6:
Impact of various proposals on the effective quality factor

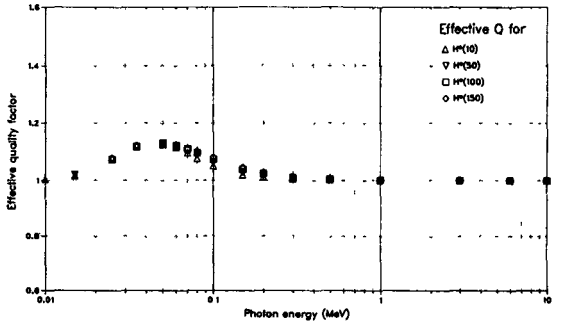

Fig. 7:
Effective quality factor when using formula (1)

2.2 Conservatism of H*(10) as compared with H_E

The ICRP has recommended a system of dose limitation based on the effective dose equivalent H_E. As H_E is a complex quantity and not measurable, ICRU recommended an estimation of H_E from the ambient dose equivalent H*(10). To ensure H*(10) to be a conservative estimate for H_E, H*(10) and H_E were compared on the basis of Monte Carlo calculations. It could be shown that H*(10) exceeds H_E in case of the anthropomorphic phantoms ADAM and EVA and external photon radiation under all exposure conditions considered (1). After the Chernobyl reactor accident intensive assessments of doses to members of the public were

performed. With the new realistic voxel-phantoms based on whole body computer tomographic (CT) data, effective dose equivalents for children were calculated. The results show that a dosemeter calibrated in terms of H*(10) is suitable for estimating H_E to the public in the entire photon energy range of interest (27).

3 Statistical analysis of occupational exposures and evaluation of trends

Statistical evaluation of the annual data from the GSF Personnel Dosimetry Service revealed trends of the total collective dose, the number of persons being monitored and the number of persons with an individual dose exceeding 0.1 mSv at least in one month of the calendar year. The analysis was made for the main protection areas, i.e., medicine, nuclear technology, conventional industry, research establishments etc. (12-16).

The results show that the mean annual doses for large groups are below one-tenth of the limits, even for the most exposed subgroups, but that there are individuals, especially involved in maintenance of nuclear power plants and in certain fields of medicine, with annual dose equivalents of 20 mSv and above for many years. These persons, although observing the annual dose limit in every year of their occupational life, are at a risk well above that implicitly attributed to the chosen value of the annual dose limit (4).

A compilation of the dosimetric data obtained since 1980 shows, that the collective dose resulting from medical applications remained almost constant at around 8 manSv. 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 afterwards and finally remained constant since 1984 (12, 14, 16).

In order to assess individual dose histories and life-time doses, the personal dose records of a subgroup of 18,300 occupationally exposed persons was analyzed, who were continuously monitored in the years 1980 through 1986. 191 of them received an annual dose equivalent higher than 10 mSv (medicine 26 individuals, research 2, industry and nuclear power production 163). A limitation of the life-time dose equivalent to 400 mSv, as discussed in the Federal Republic of Germany, would hence affect only few radiation workers.

4 Workplace analysis

4.1 X-ray diagnosis

In order to determine workplace-specific dose values for occupational exposed persons, measurements were performed at selected workplaces in diagnostic radiology, including digital substraction-angiography, cardiac catheterisation and conventional angiographic examinations (31). To this end physicians and assistants at the Herzzentrum München and Krankenhaus Kronach were provided with seven types of partial body dosemeters to be worn in addition to the usual personal dosemeter. This set of dosemeters, part of them specially designed for this study, consisted out of two TLD-finger rings for the right and left hand, two TLD-wristlets for the arms, a plastic button containing a TLD-chip to be fixed by adhesive tape near the eye, and two small plastic boxes, both filled with four TL-dosemeters, to be worn below and outside of the lead apron next to the usual personal dosemeter.

The results show that the personal dosemeters covered by the lead apron nearly always indicates monthly dose values below 0.1 mSv whereas the dosemeters outside, worn at the collar of the apron, are remarkably higher, ranging in general between 0.3 mSv and 3 mSv with extreme values up to 12 mSv. A significant correlation between personal dose (below apron), dose at the collar and dose at the eyes could not be detected. Special emphasis was given to the analysis of doses to the finger rings and the wristlets. Finger rings are disturbing, they reduce the finger sensation, complicate sterility and the handling causes inconveniences. There is a pronounced reluctance on the side of physicians to wear such devices. In order to avoid finger rings for partial body dosimetry it

might appear as a way out to substitute them by less disturbing wristlet dosemeters if there exists a reliable, constant relation between dose to the finger and dose to the wrist.

The results as presented in tab. 1 demonstrate that for the purpose of partial body dose monitoring there is a sufficiently constant relation. However, such a favourable finding has to be considered rather as an exception which holds only for highly standardised examinations in which the hands do not reach into or close to the useful beam. In addition, cardiac catheterisation in general is far from causing alarming doses to the hands and partial body dose monitoring is a less stringent problem. The situation, however, is worse for a radiologist performing a wide spectrum of conventional angiographic examinations and DSA at a county hospital in Bavaria (tab. 2). Besides that the results in tab. 2 demonstrate the necessity for partial body dose monitoring in such situations: The values for the first two control periods reveal that the physician would probably have exceeded the maximum dose of 500 mSv per year and that the results from the partial body dose measurement obviously caused him to receive his working method.

Table 1: Partial body dose equivalents (mSv) and dose equivalent ratios of four physicians performing cardiac catheterisation

Physician	No. of examin.	RH	RA	RH/RA	LH	LA	LH/LA
1	21	0.66	0.70	0.94	3.12	2.40	1.30
2	44	0.99	0.45	2.20	5.89	5.00	1.18
3	9	0.77	0.95	0.81	2.13	1.85	1.15
4	12	0.46	0.59	0.78	2.72	2.22	1.23

RH: Finger ring right hand
RA: Wristlet right hand
LH: Fingerring left hand
LA: Wristlet left hand

3.2 Beta radiation in power plants

The objective of this study was to (a) identify relevant beta fields and (b) to evaluate qualitative and quantitative figures on source and field parameters (18, 21). The figures have been considered to be the basis for requirements for a personal dosemeter capable to detect beta radiation

Table 2: Partial body doses (mSv) of a radiologist performing DSA and conventional angiography during 12 control periods

Control period	RH	RA	RH/RA	LH	LA	LH/LA
1*	66.6	13.9	4.8	8.7	12.7	0.7
2*	90.3	9.10	9.9	8.5	9.5	0.9
3***	4.1	13.5	0.3	2.3	5.9	0.4
4*	4.9	4.1	1.2	45.3	7.9	5.7
5*	3.3	4.3	0.8	7.5	5.4	1.4
6*	2.8	1.0	2.8	5.7	4.0	1.4
7**	1.3	1.3	1	1.7	1.9	0.9
8**	6.6	4.2	1.6	25.3	10.8	2.3
9**	3.8	5.5	0.7	8.1	6.1	1.3
10***	8.5	2.5	3.4	2.5	2.5	1
11*	7.4	4.4	1.7	38.2	11.7	3.3
12*	1.8	3.3	0.5	3.0	1.9	1.6

RH: Finger ring right hand LH: Finger ring left hand
RA: Wristlet right hand LA: Wristlet left hand
Control periods: 1 month*, 2 months**, 3 months***

and allow for correct dose assessment also in mixed photon-beta fields. Measurements were made at a BWR during both the operational and maintenance phase using survey meters with ion chamber technique, GM counter, film and TL dosimetry. Preliminary results revealed dose rates up to around 0.2 Sv/h, resulting from extended contaminations and beta energies of around 0.5 MeV. Similar experients were started in fuel element production applying different integrating dosemeter types for comparison. Results will be reported at a later stage.

5 Development of personal dosemeters

5.1 Partial body dosemeter

The development of a partial body TL dosemeter together with the prototype of a PC-assisted TL readout device was completed. The extremity type dosemeter is based on a plastic ring with a single beryllium oxide chip on a stainless steel support also used as a heating planchet. Each ring carries an identification code. The TL sample is hermetically sealed with a special foil for mechanical and optical protection equally resistant against the annealing procedure, chemical solvents and gaz sterilisation

The readout device provides automation for magazine transport (20 dosemeters), readout cycle, code reading and documentation. The TL signal is transmitted to the PM tube by a flexible fluid light-pipe to reduce thermal effects. The PC-assisted dose assessment is based on photon-counting and a digital signal treatment.

On the basis of about 3 500 test runs with more than 600 beryllium oxide detectors it was shown that, in partial body dosimetry, all specifications of a relevant DIN standard (DIN 6816) could be fulfilled. Further activities were addressed to the reduction of optical fading and system application in beta-ray dosimetry, particularly H'(0.07) assessment.

An internal quality control on the system uncertainty using unknown doses (1 mSv to 500 mSv Co60 gamma-rays) under laboratory conditions, revealed the reported-to-nominal dose ratio, H_{rep} / H_{nom}, to be around unity with a coefficient of variation of 5%. The lower detection threshold was around 0.07 mSv. Participation in the annual PTB quality control for personal dosemeters with unknown dose equivalents between 0.1 mSv and 1 Sv (10 keV X-rays to Co60 gamma-rays) revealed $H_{rep}/H_{nom} = 1.3$, with a c.v. = 18%. A six-months field test with monthly evaluation including 40 customers (radiology, industry and nuclear plants), more than 240 persons and 1210 dosemeters showed the following results: Collective dose equivalent 128 mSv; mean dose equivalent per caput 0.72 mSv; 85% of the reported dose equivalents < 1 mSv; 13% between 1 and 10 mSv; 1.3% between 10 mSv and 50 mSv; 0.6% above 100 mSv.

German, Swiss and US (17) patents are pending for the ring dosemeter; an industrial prototype is under construction. Also the TL readout device was transferred to industry for prototype production.

5.2 Film dosemeter badge

The development and design of a new film dosemeter badge was completed, the absorption filter set optimized for the filteranalytic respectively linear combination method for dose evaluation of the detector films. The badge was accepted by the German authorities for official use. Because of the new German standardisation ordinance it was also necessary to apply

for pattern approval which was passed in 1989. Within the yearly PTB quality control for personal dosemeters the new badge type film dosemeter fulfilled all requirements for routine application; 30 reported dose equivalents showed a c.v. = 10%, the mean of the relative error was 1.015. The badge contains 4 filters (Cu: 0.05, 0.3 and 1.2 mm in thickness, Pb: 0.8 mm). There is an indicator for the radiation incidence direction out of "densimet" material. The badge design considers the automatic processing of the detector films. Integration of a TL assembly into the badge is continuing to provide a dual-system (TL for dosimetry, film for exposition analysis).

5.3 Albedo dosemeter

A thermoluminescent neutron albedo dosemeter was prepared for radiation workers in nuclear industry, based on a commercial TL-system with two pairs of lithium borate detectors enriched and depleted in the Li-6 and B-10 content and encapsulated in a boron cassette. Primary calibration refers to a Am-Be neutron source. In order to correct for the spectral response, on-site field calibration was performed at nuclear facilities using a 30 cm polyethylene sphere. Conversion coefficients were evaluated for different spectral conditions to convert apparent doses, in terms of Cs-137 gamma-rays, into dose equivalents.

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

- Japan Atomic Energy Research Institute, Tokai-mura, Japan

V. Publications:

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(22) Caldas, L.V.E.*, David, J.: Bremsstrahlung und beta radiation fields determination of an industrial radiactive source. Radiat.Prot.Dosim. 28, 47-51 (1989)
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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: B16-F-333-DK

Danish Cancer Society
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Institute of Cancer Epidemiology
P.O. Box 839
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Title of the research contract:

Late effects of Thorotrast among Danish patients 1932-1947

List of projects:

1. Late effects of Thorotrast among Danish patients 1932-1947.

Title of the project no.: BI6-F-333-DK

Late effects of Thorotrast among Danish patients 1932-47

Head(s) of project: Hans H Storm, M.D. and Ole Møller Jensen, M.D., Danish Cancer Society, Danish Cancer Registry, Institute of Cancer Epidemiology, and Helge Johansen, M.D., Oncological Dept. Rigshospitalet

Scientific staff:

Dr. Michael Andersson,M.D., Danish Cancer Registry. Ms Gerda Engholm, Statistician, Danish Cancer Registry.

I. Objectives of the project:

To study long term effects of continuous internal low-dose alpha radiation in a cohort of patients undergoing cerebral angiography with the contrast media Thorotrast (thoriumdioxide) during the years 1932-47 in Denmark. The cancer risk, morbidity and mortality of non cancer by radiation dose, age and sex, is studied using the risk in the general population as the baseline in a standard cohort analysis. The study of lung cancer will give dose-risk assessments for the impact of Thoron (^{220}Rn). The cancer risk in offspring of exposed females will be studied to evaluate the effects of intrauterine exposure to constant low-level alpha radiation.

II. Objectives for the reporting period:

Reestablishment of the cohort of patients undergoing examination with Thorotrast from an old card-file, computerising all information available in the file. Secure the identity of the patients by linkage to the Central Population Register, to the Mortality Register and by queries to Municipality Registers. Search for unidentified persons by scrutinizing their hospital records for errors in identification. Delete duplicate and unidentified persons in the file, and prepare the file for linkage with the Danish Cancer Registry, the Cause of Death Registry, and the Patient Discharge Registry.

III. Progress achieved.

Methodology:

The study will be based on the reestablished Danish Thorotrast cohort. The Cohort was first identified by Dr. Mogens Faber, the Finsen Institute, developed from the records of two neurosurgical hospitals (Faber,1978).

The study activity is divided into three phases. The first phase covering the first 14 months, is computerization of the manual Thorotrast files and identification of the cohort by linkage and search in the Central Population Register, the mortality files and municipality registers. The second phase is linkage to various morbidity and mortality registers for risk evaluation, and the third phase indepth studies on specific cancers (morphology) and offspring of exposed persons.

The study will be carried out as a population based cohort study, using well established routines in cancer epidemiology (Breslow and Day 1987) and proven valuable evaluating radiation effects (Storm 1988). It is possible to establish a fully identified cohort of patients undergoing examination with Thorotrast in Denmark, and follow the patients for the occurrence of cancer using record linkage techniques, linking with the population based National Danish Cancer Registry (in operation since 1943)(Storm 1987). Cancer rates in the general population will be applied to the personyears of observation, taking account of both sex, the ageing of the population, as well as calender year, using standard software under the hypothesis of a proportionate hazards model (Coleman et al. 1986). The ratio between observed and expected cancer will be determined for various subgroups of the cohort, stratified by variables such as sex, age at exposure, injected thorotrast dose, cumulative exposure, and reason for thorotrast examination. Likewise an analysis of mortality within the cohort will be carried out using linkage possibilities to the computerized National Danish Mortality Registry and mortality rates from the underlying danish population. The latter analysis will include

non-cancer mortality as well. Linkage with the Central Population Register in Denmark enables a study of morbidity among patients admitted to hospital since 1/1 1977 and thus recorded in the National Patient Discharge Registry.

Results:

Data from the original card-file was scrutinized and entered into a computerized data-base system, at the Cancer Registry computer, after approval was obtained from the Danish Data Inspection Agency. Altogether **1092** patients were recorded from the card file, after removal of a number of duplicates. Altogether **352** persons recorded alive in the card file were sought in the Central population Register and vital status obtained. 256 persons were still alive on 31/12 1987. By linkage on date of birth, date of death and first letter of names to the Danish Death Registry 1943-87, verification of vital status, date of death and identity was obtained for 626 persons. The identity on names is currently cross-matched with the names in the Thorotrast file and patients not identified by linkage procedures, are followed in municipality registers and hospital record files. Scrutiny of hospital records are carried out for all the patients.

The incomplete cohort was used for linkage with a cohort of epileptics, in order to remove patients undergoing examination with Thorotrast before analyzing the general cancer risk following anticonvulsive therapy (Olsen et al. 1989). The excluded patients were analyzed with respect to cancer risk and a significant 4,9 fold risk (95% confidence interval 3,8-6,3) was observed. The risk was highest for cancer of the liver 202 times that of the general population, but significantly elevated cancer risks was seen for gallbladder/biliary tract (RR=28), leukaemia (RR=17), brain (RR=23) and lung (RR=3,2). For lung cancer a 6 fold increased risk was observed after 20-29 years of follow-up, but the material was too small to evaluate any time trend in risk.

Discussion:

Even if the first phase of the study is not yet terminated, and the cohort not fully identified, it is obvious from the analysis of a fraction of the patients, that patients given Thorotrast represents an irreplaceable source of data on the potential long term effects of radiation exposure. The long term effects from exposure to longlived alpha-emitting nucleides, such as Plutonium is not well known, but knowledge is becoming important in light of the need to predict health hazards from accidental releases into the general environment. The Thorotrast patients will undoubtedly prove to be the most important source for evaluation of such health effects related to long lived alpha-emitting substances. The Danish study design enables evaluation both for cancer and for other health effects, and supplements the results obtained in the German Thorotrast Study (G. van Kaick et al. 1984) using a different methodology and by being population based.

The preliminary findings of an increased risk of non squamous cell lung cancer is provocative, considering the continuous exposure of the respiratory epithelium to evaporating Thoron, from the decay of Thorotrast. The findings may have implication for the radiation protection standards for exposure to indoor Radon. A detailed analysis calculating the dose to the lungs for the patients will be an important part of the ongoing project, as will an evaluation of health effects among offsprings of the female Thorotrast patients, being exposed in utero.

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The German Thorotrast Study, Prof. Dr. G.van Kaick, Institut für Nuklearmedizin, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg 1, FRG.

V. Publications:

Olsen J.H., Andersson M., and Boice J.D.Jr. Danish epileptics given Thorotrast in BIR report 21: Risks from Radium and Thorotrast. Eds. Taylor D.M., Mays C.W., Gerber G.B., Thomas R.G., British Institute of Radiology, London 1989, pp 116-118.

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RADIATION PROTECTION PROGRAMME
Final Report

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)]:

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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 Léroul

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 the main emphasis of the work has been put on the following:

The investigations reported in the previous reports of the simultaneous operation of electrofilters and ionizers have been repeated at intermediate aerosol levels and in rooms of different sizes. Further the effect of ion emitter geometry and ionizer location was investigated, and the variation of exposure and dose in different types of rooms was examined.

INTRODUCTION

Although the radiation dose from the radon gas itself is normally insignificant compared to the dose from its airborne shortlived daughter products, in many situations the most effective solution of radon–related problems is prevention of radon entry or removal of radon by ventilation.

Such methods, however, are often rather costly to establish, especially in existing houses, and for some methods, like the use of sealants, their long–time stability and reliability is questionable.

Methods for direct removal of airborne radon daughter products are, on the other hand, simple to apply, and even though their efficiencies are not as high as those applicable to the radon gas, methods like filtration and electrostatic plateout may be useful in many situations, either as the main solution, where only a fairly modest reduction is required, or as a supplement to other actions.

RADON DAUGHTERS, PROPERTIES AND EFFECTS

The effect on human beings of inhaled radioactive material must depend on the number of inhaled atoms of each radioactive nuclide, i.e. on the time the person has spent in the atmosphere considered, the breathing rate and the concentrations of the individual radon daughters.

The concentration of a given radon daughter in indoor air is determined by its rate of production and the rates with which it is being removed from the air. The only process with any significant rate of production is normally the radioactive decay of the airborne fraction of the immediate parent product. The contribution to indoor air radon daughter concentrations by outdoor air activity, brought in by ventilation, can normally be neglected in terms of radiological significance.

As far as the removal processes are concerned, the situation, however, is much more complex (1). Radon daughters may be removed from the air not only by the inevitable radioactive decay to the next daughter in the series but also by ventilation, plateout on room surfaces (possibly enhanced by electric fields) and filtration. In addition the rates of the various removal processes may be affected by the interaction of the radon daughters with the ambient aerosol.

Let us as an example consider the decay of a ^{222}Rn–atom. The recoil resulting from the emission of an α–particle causes the immediate decay product, ^{218}Po, to be a doubly charged positive ion.

This ion rapidly enters into interactions with trace gases, polar molecules, like water, or other ions in the air and in a time probably of the order of milliseconds gives rise to the formation of a small molecular cluster with a diameter of maybe a few nm. This cluster of molecules, which may be positively charged or neutral, is generally referred to as **unattached** or **free** ^{218}Po.

The corresponding **attached** form arises when unattached ^{218}Po attaches to ambient aerosol particles.

A series of mathematical models describing the interplay between the various processes affecting radon and its daughters have been developed over the last two decades. These models (2,3,4) are very useful in estimating the effect of a given type of air treatment on the concentrations of the radon daughters and their distribution between the attached and unattached state.

The potential alpha energy concentration (PAEC) or exposure rate of a given atmosphere can be determined directly from the concentration of the individual daughter concentrations, independently of their state of attachment.

The radiological dose, on the other hand, caused by the exposure to a given concentration of a given nuclide can only be estimated from dose models based on assumptions often lacking experimental verification, but which in all cases attribute a higher dose value per unit of PAEC in the unattached than in the attached state.

Although most methods of air treatment affect attached as well as unattached radon daughters, at the same time the aerosol conditions may also be changed thus affect–

ing the degree of attachment, resulting in a different relationship between measured PAEC and estimated dose before and after the air treatment has been employed.

The paper describes an investigation of the effect on the concentrations and properties of airborne radon daughters by electrofiltration and/or exposing the air to a combination of ionization and electric fields.

FILTRATION

When the air enters an electrofilter it passes through a region of high monopolar ionization by a corona discharge, usually from a thin wire kept at a high potential (5 – 20 kV). The ions move, by the field between the corona electrode and grounded surroundings transversely to the air flow. On their way the (small) air ions will attach to airborne particles, both inactive and radioactive, and these charged particles (or large ions) are brought by the air flow into a region, where an electric field will eventually deposit the particles on collection electrodes, with an efficiency that depends on the type of filter and the size distribution of the particles.
Most filters remove unattached and attached radon daughters and neutral aerosol particles from the air passing the filters with about the same efficiency, maybe 80 – 90 %, but the effect of the filtration on the steady state concentration of the species in question depends strongly upon the interplay between the various removal and attachment processes.
A given rate of filtration should, according to the models, lead to a certain decrease in the total (unattached and attached) concentration of a given radon daughter. If, however, the aerosol concentration is also substantially decreased by the filtration the attachment rate will be smaller and the fraction of the radon daughters remaining in the unattached state may increase.

ELECTROSTATIC PLATEOUT

As mentioned earlier the unattached radon daughters may be neutral or positively charged. The attached radon daughters can be assumed to be charged to the same extent as the type of aerosol particles to which they attach. In the typical 0.1 – 0.2 μm range most particles are charged with approximately the same number carrying positive and negative charges.
If a suitable electric field is established in a room, the charged radon daughters, and radon daughters attached to polarizable particles, will move towards and tend to plate out on the electrodes one of which is (normally) the room surfaces, which are essentially at ground potential (5,6).
The field may be established by placing a non–ionizing electrode at a high potential (with respect to ground) for instance near the ceiling.
If the dimensions of the electrode, however, are not impractically large the voltage will drop off rapidly over the first centimeter or so from the electrode, and the field strength will be very low in the rest of the room and especially at the surfaces, where the plateout takes place, and since the plateout rate is proportional to the field strength at the plateout surface, the effect of the field on removing the airborne radon daughters will be small.
If on the other hand the electrode is made to ionize the air in its immediate vicinity ions (with the same polarity as the electrode) will move away from the electrode and set up a space charge in the room and thus modify the field distribution in such a way that the field strength is lowered in the vicinity of the electrode and increased in the rest of the room, and therefore also at the boundaries, and as a result the electrical plateout rate may increase substantially.

EXPERIMENTAL RESULTS

FILTRATION

The initial measurements were performed in a room with a volume of 320 m^3 and a surface area of 360 m^2. The electrofilter used was an industrial unit, where the flow-rate could be varied from 0 to about 850 m^3/h or 2 – 3 h^{-1}. Under normal circumstances (no treatment) the aerosol concentration would stay very low, especially when filtration took place, about 1000 cm^{-3}. Since this is much lower than the normal level in ordinary houses, it was necessary also to conduct measurements at higher aerosol concentrations.

This was accomplished by the use of ordinary gas burners giving concentrations up to $2 \cdot 10^5$ cm^{-3}.

The effect of the aerosol concentration is demonstrated in *Table 1* (7)

	Aerosol concentration, cm^{-3}			
	< 1000	*50.000 –100.000*	*150.000 –200.000*	*"theoretical value"*
	Unattached fractions			
^{218}Po	*0.60*	*0.29*	*0.06*	
^{214}Pb	*0.08*	*0.08*	*0*	
^{214}Bi	*0.05*	*0.04*	*0*	
	Relative concentrations			
^{218}Po	*0.65*	*0.69*	*0.81*	*0.93*
^{214}Pb	*0.26*	*0.29*	*0.42*	*0.57*
^{214}Bi	*0.17*	*0.15*	*0.27*	*0.39*
	Equilibrium factor, e_p			
	0.25	*0.29*	*0.42*	*0.54*
	Unfiltered equilibrium factor, e_{po}			
	0.67	*0.69*	*0.90*	*1.00*
	Remaining Exposure			
	0.37	*0.42*	*0.47*	*0.54*

Table 1. Radon Daughter Parameters at Various Aerosol Conditions at a Filtration Rate of 1 h^{-1}

The figures marked "theoretical value" are calculated under the assumption that the radon daughters are being removed only by filtration and radioactive decay.

The deviation of the experimentally determined unfiltered equilibrium factor from the theoretical value of unity shows that plateout and the natural air exchange at low and intermediate aerosol concentrations remove about 30 % of the PAEC from the airborne state.

The effect of the filtration on the exposure can be expressed as the **REMAINING EXPOSURE**, defined as the ratio between the equilibrium factor at the particular rate and at zero ventilation rate.

For the experiments shown in Table 1 the filtration has reduced the exposure to between 37 and 47 % of the unfiltered value.

The major part of the filtration experiments were performed in two laboratory rooms with a volume of approximately 120 m^3 and an air exchange rate of about 0.2 h^{-1}. A number of commercially available filters were examined at varying aerosol conditions, and for each of the filters the remaining exposure was calculated as described above.

The effect on the radiological dose was determined in a similar manner. The dose rate D was estimated from the formula

$$D = (c_u \cdot f_u + c_a \cdot (1 - f_u)) \cdot E_p$$

where E_p is the PAEC, f_u is the unattached fraction of PAEC and c_u and c_a are the

dose factors for unattached and attached radon daughters.
The dose rates considered relate to the dose to the bronchial ephitheleum assuming 1) a mean aerosol diameter of 0.1 μm and 2) a doubling of the size of the unattached as well as the unattached radon daughters during breathing and before depositing. The dose rates are calculated using the parameter values $c_u = 169 \cdot 10^{-5}$ (Gy/year)/(Bq/m^3 EER) and $c_a = 10.15 \cdot 10^{-5}$ (Gy/year)/(Bq/m^3 EER), where the Bq/m^3 EER are the equivalent equilibrium activity concentrations corresponding to the PAEC of unattached and attached radon daughters respectively (8).
The **REMAINING DOSE** is then defined as the ratio between the dose rate when filtration is applied and the dose rate in the unfiltered state.

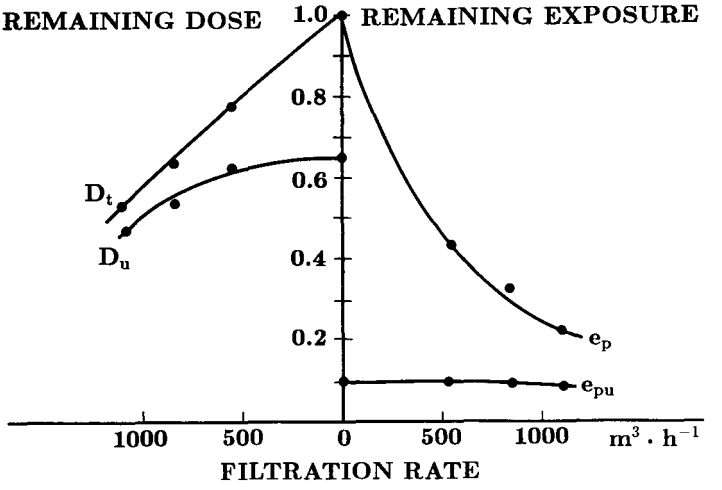

Figure 1. Remaining Exposure and Remaining Dose as a Function of the Filtration Rate.

In Figure 1 are shown some results of the measurements with one of the filters (ELIXAIR 1100) at intermediate aerosol concentrations. In order to get a better impression of what the filtration in detail is doing to the radon daughters the effects on the unattached and on the attached radon daughters are calculated separately.
On the right side of the figure is plotted the (total) equilibrium factor e_p as well as the equilibrium factor $e_{p,u}$ for the unattached daughters.
It appears that the filtration primarily affects the attached radon daughters rendering only a small decrease in the unattached PAEC as reflected by the unattached equilibrium factor $e_{p,u}$.
On the left side of the figure are shown the remaining doses of the unattached as well as of the total (unattached plus attached) daughter population.
At a filtration rate of zero it appears that even though the unattached PAEC is only about 10 % of the total PAEC, the unattached dose rate is estimated to be about 75 % of the total dose rate At a filtration rate of 1100 m^3/h the unattached dose rate D_{pu} is estimated to be about 90 % of D_{pt}.
These results show the main problem in using filtration for removal of radon daughters. It is possible effectively to remove the attached daughters and thereby reduce the exposure considerably. The activity concentration of the unattached daughters, however, will decrease only slightly, and because of the much higher dose value ascribed to the unattached daughters, the estimated dose will show a much smaller decrease than does the exposure.

In order to check whether the results obtained in laboratory experiments reflect the situation in real houses a series of measurements in houses in Sweden was performed in the period May – November, 1986 (9).
The main conclusion of the field experiments was that under comparable conditions the results from real houses are very similar to those obtained in laboratory experiments.

ELECTROSTATIC PLATEOUT

It appears from the results reported above, that although filtration is a fairly efficient method for lowering the exposure from radon daughters (as represented by the equilibrium factor) the effect on the estimated dose is rather modest.
It was therefore decided to investigate the possibilities of using electric fields supplemented with unipolar ionization for the removal of radon daughters.
The first step of the investigation consisted in the development of a method to study the charged fractions and mobilities of airborne radon daughters. The main results were (10):

a) no negatively charged unattached radon daughters could be detected, b) the fraction of positively charged radon daughters varied from 10 to 20 % for ^{218}Po and probably somewhat more for ^{214}Pb, while the activity concentration of ^{214}Bi in all cases was too low to allow a determination of the charged fraction with any reasonable degree of accuracy, c) the mobility of charged unattached radon daughters were determined to be higher than about $5 \cdot 10^{-4}$ $m^2V^{-1}s^{-1}$, d) about 70 % of the attached radon daughters seemed to be charged, more or less evenly distributed between positive and negative polarity, and e) the mobilities of charged attached radon daughters were in the range $10^{-7} - 10^{-8}$ $m^2V^{-1}s^{-1}$.

As the next step a theory for the variation of the electric field around an ionizing electrode was developed, and it was demonstrated that the variation of field strength with distance from the electrode is largely determined by the space charge created by the ions.
The actual electrostatic plateout was studied using an experimental set–up shown in Figure 2.

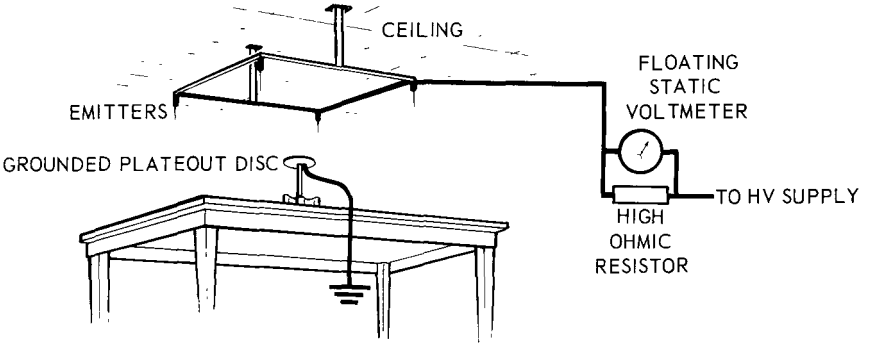

Figure 2. Experimental Set–up for Measuring Field–enhanced Plateout of Radon Daughters

About 0.5 m below the ceiling in a laboratory room (volume 120 m^3) four steel needles (emitters) were mounted, spaced approximately 1.5 m apart. The emitters were connected to a high voltage supply (range 0 – 30 kV). When the voltage exceeds a value of 2 – 7 kV (depending on the proximity of grounded objects) ionization takes place in a small region in front of the emitters and ions of the same polarity as the emitters will move away and modify the field in the room and, most importantly, increase the field at room surfaces to values higher than would be the case without the ionization.
In Figure 3 are shown some of the results of the study of the field–enhanced plate-out, represented in the same way as in Figure 1. It appears that with this purely experimental set–up it is possible by raising the emitter voltage to 30 kV to reduce the exposure to about one sixth and the estimated dose to about half of the values without ionization.

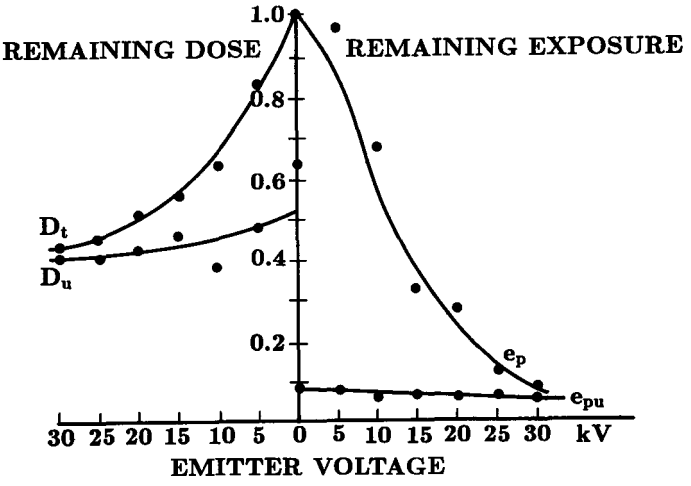

Figure 3. Remaining Exposure and Remaining Dose as a Function of the Emitter Voltage

It should be noted that all experiments show that a positive emitter voltage, and consequently positive ionization, gives about twice the reduction that does a negative emitter voltage.

A series of different ionizers, some of them commercially available and others home-made, have been checked for their effect on the radon daughters, either alone or in combination with electrofilters.
Most of these ionizers consist of a, rather simple, high voltage supply (from 5 to 18 kV, plus or minus) coupled to a corona electrode, either in the form of a steel needle or a carbon fiber brush.
The measurements seem to indicate, that the emitter voltage has to be about 10 kV or higher in order to create any significant effect.
In Table 2 are shown some results of the effect of an 18 kV positive ionizer (ION A (Laboratory of Applied Physics))) alone and in combination with various electrofilters (11).

Unit	*Filtration alone*		*Filtration + Ionization*	
	Remaining		*Remaining*	
	Exposure	*Dose*	*Exposure*	*Dose*
ELFI 62, LOW	*0.57*	*0.87*	*0.14*	*0.34*
ELFI 62, MED	*0.55*	*0.59*	*0.16*	*0.48*
ELFI 62,HIGH	*0.37*	*0.44*	*0.09*	*0.32*
ELIXAIR 400, LOW	*0.65*	*0.81*	*0.15*	*0.36*
ELIXAIR 400, HIGH	*0.63*	*0.77*	*0.22*	*0.40*
ELIXAIR 700, MED	*0.44*	*0.67*	*0.19*	*0.28*
ELIXAIR 700, HIGH	*0.41*	*0.50*	*0.14*	*0.37*
ION A			*0.27–0.35*	*0.33–0.46*

Table 2. Effect of Filtration and/or Ionization on Exposure and Dose at Intermediate Aerosol Concentrations

The results shown in Table 2 seem to indicate that the use of suitable ionizers will reduce the radon daughter levels as much and maybe even more than most conventional filters.
Other combinations of ionizers and filters have been checked in rooms of different sizes (different surface to volume ratios).
As an example the results of measurements in a 40 m^3 room the results for the combination of Elixair 400, High alone and in combination with the ionizer were

	Remaining		*Remaining*	
	Exposure	*Dose*	*Exposure*	*Dose*
ELIXAIR 400, HIGH	*0.33*	*0.74*	*0.04*	*0.34*

These results seem to indicate that the reduction, especially of exposure, is considerably greater in smaller rooms.

A possible disadvantage with using ionizers with an external field in a living area might be an electrostatic charging of persons in the room. Measurements of such chargings have shown lower values than what is often encountered by walking on ordinary floor coverings.
A more detailed charting of the plateout patterns, with the intention of directing the plateout, has been started, using nuclear track films.

GENERAL COMMENTS AND CONCLUSION

When deciding how to handle the radon problem in a given house, it is important to realize that there is no simple and only solution.
In most houses where radon in the soil gas is the main source, remedial measures, such as the use of barriers, aimed at preventing radon entry are to be preferred.
It should, however, be cautioned, that the long term reliability of many radon barrier techniques now being used have yet to be confirmed and verified experimentally.
Even where an effective barrier technique is properly installed the settling of a house over a period of a few years may cause new entry points to be formed. It is important to appreciate, that where conditions are favorable for radon entry (i.e. high soil permeability, an underpressure across the house foundation slab etc.) only a small entry point being available will allow significant radon flux into a house.
In discussing radon remedial methods it is often claimed that simple ventilation, or just opening the windows, is a method superior to all other methods because of its effect on the relative long–lived radon gas.

It can be assumed that the application of ventilation will reduce the PAEC and the dose to more or less the same extent, in practice down to maybe 20 % of the unventilated value.
Some caution should, however, be noted in respect to using ventilation as a radon control technique.
On a human level it may conflict with household comfort and energy budgeting and at a physical level it may, if incorrectly applied, cause an increase in indoor radon due to a depressurisation induced influx of radon–rich soil gas. In terms of overcoming these problems successful heat recovery systems are available.
In a well–designed system of this type highly efficient heat exchangers are used and the problem with depressurisation may be minimized by using a balanced indoor–outdoor airflow system.
A major disadvantage with the use of ventilation is that it often requires a rather complicated and costly installation, and especially in existing houses this may be the most important deterrent for its use.
As far as the removal methods, filtration and field–enhanced plateout, are concerned, they are in many respects equivalent to ventilation.
It should, however, be kept in mind, that neither filtration nor field–enhanced plateout, on the one hand, either increases or decreases the radon concentration, but, on the other hand, do have the advantage of not involving the energy–consuming side effect of introducing cold outdoor air.
The maximum effect of either process, used alone, seems under practical conditions, conservatively evaluated, to be a reduction in the exposure to about 30 – 40 % of the value in untreated air and a reduction in the estimated dose to maybe 40 – 60 %, depending on the device and the size of the room.
When used jointly the reductions are considerably greater.
Intercomparing the two methods, electrostatic plateout obviously has the drawback that the platedout material, although being removed from the air, is not being removed from the room, but is deposited on room surfaces.
As far as the radioactive plateout is concerned, this is, because of the fairly short half lives of the airborne radon daughters, hardly a health problem, but it should be kept in mind, that also any other airborne, charged or polarizable, material, like smoke particles and other type of particulates, will plateout on the room surfaces causing an unattractive smudging of the room walls.
This fact speaks strongly in favor of a combination of filtration and electrostatic plateout, where the filter essentially handle the normal "air pollution" and the combination reduces the radon daughter level.
The advantage of filtration and electrostatic plateout over ventilation is primarily a question of cost. For both methods installation is extremely simple, and, especially the ionization/field devices are also inexpensive in comparison with conventional ventilation appliances.
It should be mentioned that most of the tests of the use of filtration and field–enhanced plateout have been laboratory experiments, but they have, on the other hand, (also) been performed under conditions closely resembling normal living and working environments.
Unfortunately most commercially available ionizers have been developed for other purposes than radon daughter removal and are not optimally efficient for this purpose.
The experiences of this investigation, however, indicate that it is technically not only possible but rather simple to construct an efficient radon daughter removing ionizing unit.

Summarizing it seems that under certain circumstances realistic considerations argue strongly in favor of having indoor air treatment systems installed, either alone or as a necessary back–up to the primary radon barrier techniques.
Also considerations of economic cost, technical feasibility and maybe even human acceptability may in some cases suggest that a direct treatment of the indoor air is preferable.
This may especially be the case where the main or essentially only contributing source of indoor radon is the building materials of the house. In such situations direct removal of radon daughters from the air by filtration and electrically enhanced plateout may have advantages over traditional ventilation.
Some methods of direct treatment of the indoor air, may appear less effective in reducing the radiological doses, calculated on basis of the current lung dose models, than might be suggested by their high efficiency in reducing the overall exposure.
It should, however, be kept in mind that the lung doses are theoretical predictions based on models still under development, and it is thus prudent and common sense to assume that a reduction in the exposure will always be of beneficial nature.

ACKNOWLEDGMENT
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11. Jonassen, N., and Jensen, B., Removal of Radon Daughters by Filtration and Electrostatic Plateout, *Proc. EPA, The 1988 Symp. on Radon and Radon Red. Techn.*, Denver, October 1988.

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

Dr. J. P. McLaughlin, University College, Dublin, Ireland

V. Publications:

1. Jonassen, N., and Jensen, B., Are Exposure and Dose Determined by the Radon Concentration, American Assoc. Aeros. Res., 1989 Annual Meeting, October 1989, Reno.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Kernforschungszentrum Karlsruhe
GmbH, KfK
Postfach 3640
D-7500 Karlsruhe 1

Contract no.: BI6-F-128-D

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

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Inst.f. Neutr.phys.u. Reaktortechn.
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Telephone number: (7247) 82.24.40

Title of the research contract:

Methodology for evaluating the radiological consequences of radioactive effluents released in accidents.

List of projects:

1. Atmospheric dispersion and deposition in off-site accident consequence modelling.
2. The assessment of exposure due to deposited material.
3. Countermeasures to reduce the impact of accidental releases of radioactive materials.
4. Uncertainty analysis.

Title of the project no.: 1

Atmospheric dispersion and deposition in off-site consequence modelling

Head(s) of project:

J. Ehrhardt

Scientific staff:

H.-J. Panitz

I. Objectives of the project:

The overall objective of the project is to develop improved amd 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 MARIA2 programme (Methodes for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

In the final year of the project the models and their documentations have been finalized for the COSYMA code package. Especially, the models MUSEMET, RIMPUFF, and MESOS have been modified for the application in various accident consequence assessments. Some work has been invested in the development of a sampling scheme to define starting times of different weather sequences and their probabilities of occurrence.

III. Progress achieved:

Probabilistic comparative calculations

It was the essential aim of the study to identify improved atmospheric dispersion models which can be applied in ACA codes as a substitute of the conventional straight-line Gaussian plume model. Additionally, it was tried to give quantitative indications of the implications which different types of atmospheric dispersion models might have on the results of ACAs. Furthermore, attention was directed on the availability of meteorological input data and the computer time spent by the models. It is necessary in ACAs that the atmospheric dispersion model can be applied with reasonable computer time. Additionally, it has to be devised with the requirement that it uses real meteorological data extracted from routine observations which are recorded and reported continuously from meteorological stations. The only data needed by Gaussian-like models are measurements of wind speed, wind direction, and precipitation intensity, and, in addition, the stability class which can be derived from synoptic observations.

For these purposes probabilistic comparative calculations have been performed with different kinds of atmospheric dispersion models on the basis of artifical source terms [1]. The model types were:

- a Gaussian model (MUSEMET, [2]) which can be applied to three different modes:
  - the trajectory mode (referred to as mode 1) taking into account hourly changes of spatially homogeneous meteorological conditions;
  - the straight-line mode (mode 2) ignoring any information on the wind direction;
  - the straight-line model taking into account the wind direction preveailing at the beginning of hourly release phases (referred to as the Gaussian mode 3 model);
- a Gaussian-like trajectory puff model which is able to consider meteorological data which change temporally and spatially (RIMPUFF, [3]);
- a Eulerian grid-point model which solves the Diffusion-Advection Equation numerically and which is potentially able to consider explicitly the three-dimensional inhomogeneous and instationary wind- and turbulence fields in the atmosphere (TRANSLOC, [4]).

The main result of the study is that there are Gaussian-like trajectory models available to substitute the straight-line Gaussian model in ACAs. Under the assumptions of the source terms considered in this study and dependent on their characteristics, quantitative differences which were more or less pronounced arose in the accident consequences due to the application of different atmospheric dispersion models. The study showed that using a trajectory model leads to a spatial distribution of activity over larger areas (Figure 1) and an increase of the plume's residence time in the area over which it is dispersing combined with enhanced depletion and dilution by dry and wet deposition processes and tubulent diffusion. Therefore, the activity transported to farther distances may be significantly smaller (Figure 2)

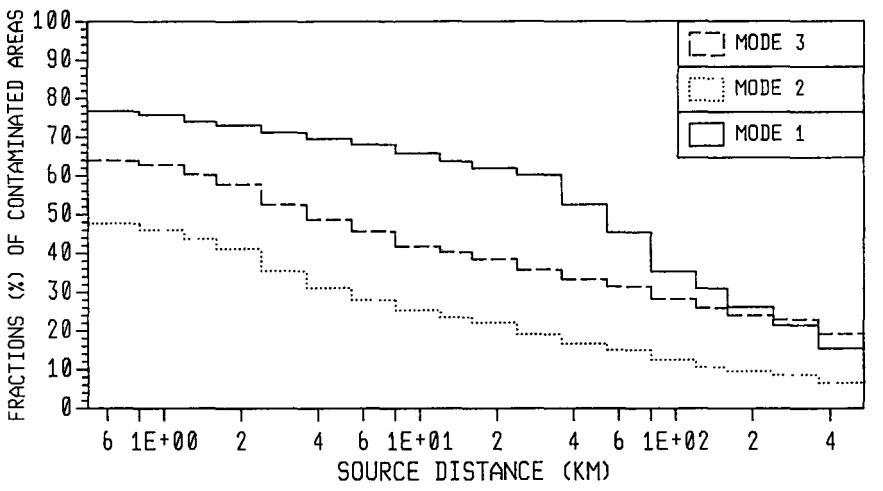

Figure 1. Mean relative size (%) of areas affected by different Gaussian model modes: nuclide considered: CS-137; unit release

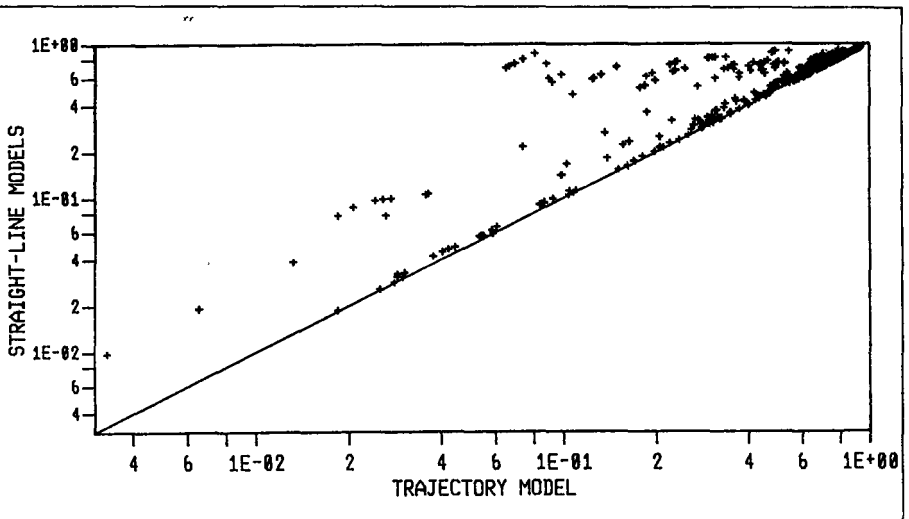

Figure 2. Scatter diagram of remaining normalized activity (maximum distance: 450km): Figures on axes are fractions of an unit release of CS-137

The application of a trajectory model might lead to contamination patterns completely different from those obtained by a straight-line transport. the study indicated that the radiological consequences in the early phase after the accident might be reduced. Thus, the straight-line Gaussian model may give conservative results in the assessment of early effects. But long-term consequences and countermeasures are underestimated if the simpler model is used and the conservatism argument does not hold any longer. As an example of the influence the choice of the atmospheric dispersion model might have on protective actions, Figure 3 shows the Complementary Cumulative Frequency Distribution (CCFD) functions of the areas with food-bans of other products than milk in the first year after the accident. It can be seen that under the conditions of the chosen source term the size of areas where the consumption and distribution of food should be interdicted increases especially if the trajectory model is applied.

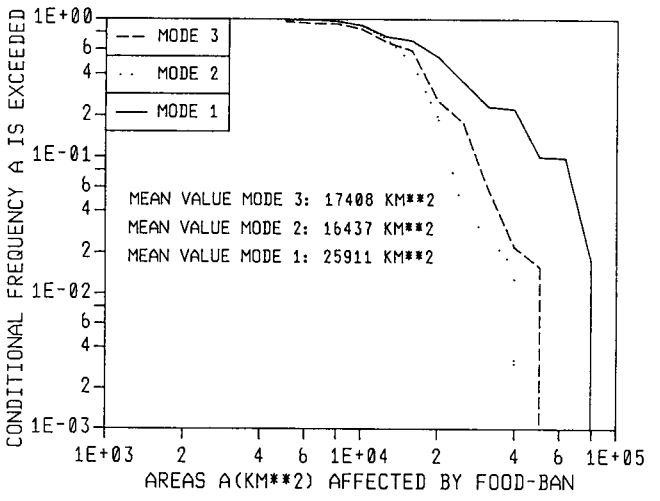

Figure 3. Conditional CCFDs of areas with food-bans execpt milk in the first year: (Release assumed)

The demands for availability of meteorological data and reasonable computer time are fulfilled by the segmented plume model as well as by the puff model, but with the restriction that for running the puff model a vector processor should be used. The principal reason why the numerical modelling concept has to be excluded from the application in ACAs is the CPU-time which is very much to high on a scalar computer and it can be assumed that also the use of a vector processor would not reduce this time to acceptable absolute values. With respect to the probabilistic results of an ACA the Eulerian model could also be applied successfully. But the study also demonstrated, that a reliable use of a numerical model in general depends on the quantity and quality of the

required meteorological data which are mainly three-dimensional temperature, wind- and turbulence fields. Because such data are generally not available from routine measurements in a sufficient quantity, they have to be derived from boundary-layer models using observations - an additional effort which is too high with respect to computing time in the context of ACAs.

Generally, it can be stated that the application of trajectory models provides more realistic results of ACAs than the straight-line models because the consideration of changes of wind direction gives a more realistic picture of the atmospheric conditions. There are no stringent arguments to apply only a straight-line Gaussian model in ACAs. This comparative study showed clearly that Gaussian-like trajectory model are as easy to apply as the straight-line plume model. Regardless whether the differences between the trajectory and the straight-line model are larger or smaller than a factor of two for a certain source term, the acceptance of the results of an ACA will increase if the improved model is used. Furthermore, the applicability of ACA codes will increase from the comprehensive and realistic assessments of consequences towards a powerful tool in a decision-making framework (e.g. emergency planning, siting, research priority setting).

UFOMOD	
Near range model ($\leq$ 50km)	Far range model ($\geq$ 50km)
Atmospheric dispersion	
MUSEMET (KFA) RIMPUFF (RISO)	MESOS (ICST) Wind fields in the region 10°W - 50°E, 36°N - 62°N
synoptic data recorded and reported continuously at 1h intervals	synoptic data of 1982 and 1983 reported from more than 800 measuring stations at 3h intervals

Figure 4. UFOMOD: Modelling of atmospheric dispersion: (adapted from [5])

Therefore, it was a logical consequence from this study to apply trajectory models in the new program system UFOMOD [5] for assessing the consequences of nuclear accidents. This led to a completely novel concept of atmospheric dispersion modelling in the new UFOMOD (Figure 4). Due to the facts, that

- strictly, Gaussian plume and puff models are only applicable for travel distances up to a few tens of kilometres and that they become increasingly inaccurate or unreliable at longer distances,
- site-specific characteristics are only relevant in the near range and vanish at farther 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,
- many applications of ACA refer to only one of both distance ranges

different ranges of validity are distinguished and assigned to respective trajectory models:

- the near range ($\leq$ 50 km), where modified versions of the atmospheric dispersion models MUSEMET and RIMPUFF are used;
- the far range ($\geq$ 50 km), where the computer code MESOS is applied [6]. It is a long-range dispersion model simulating the transport of radioactive material over large areas up to thousands of kilometres. It combines the requirement of short computing time with the ability to disperse radioactive material along precalculated wind fields derived from synoptic meteorological data measured within whole Europe.

UFOMOD and COSYMA: Atmospheric dispersion and deposition

The application of different trajectory models with ranges of validity near to the site and at far distances, respectively, is a significant step foreward to an appropriate treatment of site-specific problems and questions arising in connection with the transportation of radioactive material over large land areas up to thousands of kilometres. The new structure of UFOMOD clearly reflects this problem-oriented modelling by the division into three subsystems each built to assess accident consequences resulting from acute or chronic exposure [5].

The UFOMOD system contains four different models for atmospheric dispersion which are appropriate for different applications [7].

In the two near range versions UFOMOD/NE and UFOMOD/NL the segmented plume model MUSEMET [2]and the puff model RIMPUFF [3]can be applied. Both are Gaussian-like trajectory models. MUSEMET, as the standard atmospheric dispersion model of the UFOMOD system, is implemented as a subroutine in the UFOMOD code. RIMPUFF is a stand-alone code with an appropriate interface to UFOMOD. MUSEMET assumes that hourly meteorological data of one single meteorological station are known and that these data are constant in consecutive hourly time intervals. Further, it is assumed that all material released in a particular phase is transported along one trajectory which is divided into several straight-line segments, but material released in different phases can follow different trajectories. In the multiple puff model RIMPUFF all puffs which are emitted at intervals of a few seconds to minutes experience a change of meteorological conditions instantaneously. Each puff will follow its own trajectory according to the weather data. By simple interpolation methods spatial varying wind fields and precipi-

tation patterns are determined from meteorological data measured at a series of stations in the area affected by the dispersing plume.

For the application in the far range (> 50km, version UFOMOD/FL), where the site-specific characteristics become less important, the MESOS model [6] is available. It is a trajectory-puff model which simulates the transport, dispersion, and deposition of airborne material up to distances of thousands of kilometres, thereby taking into account the changing local meteorological conditions obtained from a data base of standard meteorological observations from synoptic stations and ships every 3 hours across Europe. In MESOS a 3 hour release is simulated by explicitly tracing the histories and developments of puffs released at the beginning and at the end of the 3 hourly period. Further it is assumed that a continuous release over 3 hours is treated by a continuous sequence of intermediate puffs following intermediate trajectories. Their position, dispersion, and depletion will be calculated by interpolation between the tracked puffs leading to a contamination of the whole area along and between the calculated trajectories. MESOS is provided in the UFOMOD system as a stand-alone program. It is appropriate only in the FL sub-system of UFOMOD.

In addition, the special straight-line Gaussian model ISOLA [8]has been implemented to estimate the spatial concentration distribution for low-level long-duration (weeks or months) releases of radioactive material.

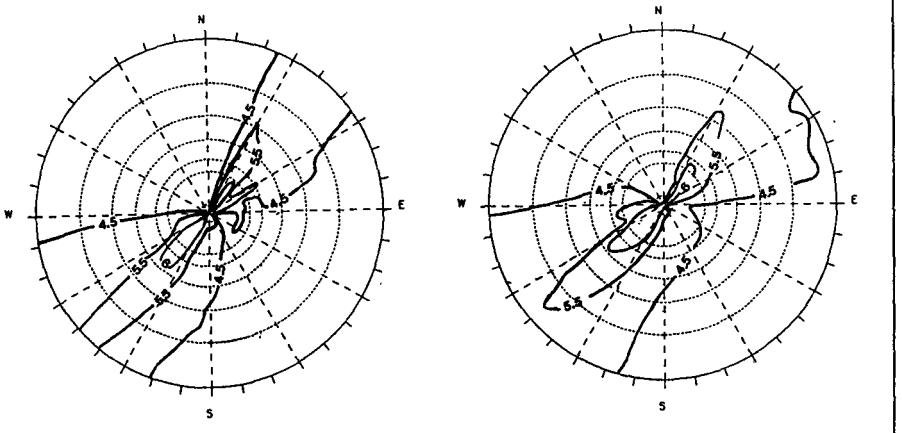

Figure 5. Ground concentrations after a long duration release (144h): isolines represent the decadic logarithms of concentrations
left: MUSEMET right: ISOLA outer radius: r=87.5km

It is a stand-alone program, appropriate for use with the NL and FL versions of UFOMOD. The meteorological conditions during the long-duration releases are evaluated sta-

tistically. Changes of atmospheric conditions are not considered during the travel of material. The concentration is averaged across the sectors considered, assuming that the wind direction is evenly spread throughout the sectors and allowing for material crossing the boundaries of the sectors. To test this concept a comparison between the trajectory model MUSEMET and ISOLA has been performed. As an example, Figure 5 shows the ground concentrations after an 144 hours release. It can be seen that the results of both models are in good agreement. Thus, the statistical evaluation of rather long atmospheric sequences leads to similar results as the explicit consideration of dispersion situations by a trajectory model.

The atmospheric dispersion models mentioned above predict the spatial and temporal distributions of activity in the air near to the ground and on the ground surface. The models MUSEMET, RIMPUFF, and ISOLA use horizontal and vertical dispersion parameters which are functions of atmospheric stability and release height, with two sets of parameters for smooth and rough underlying surface. The dispersion coefficients were derived experimentally, with the smooth surface values based on work at the Belgian Nuclear Energy Centre, Mol, [11], and the rough values from the Karlsruhe - Jülich experiments. In MESOS the lateral expansion of the plume is dominated by the synoptic spread of successive trajectories, while the small scale lateral turbulent diffusion of an individual puff only leads to a broadening of the plume beyond the trajectories forming the boundaries of a plume. This small-scale horizontal diffusion is assumed to be proportional to travel time [9]. The vertical dispersion parameters are derived from Smith's scheme for estimating the vertical dispersion of a plume from a source near ground level [12]. Mechanisms for removal of activity from the cloud are considered by each of the models, these being mainly dry and wet deposition processes, with parameter values for noble gases, aerosols, and iodine in elemental and organic forms, or bound to aerosols. The washout coefficients are also functions of rainfall rate. The source depletion model is applied to account for the reduction of activity in the plume due to the removal processes. Radioactive decay during the dispersion and the built-up of radionuclides from radioactive decay chains are not considered in the atmospheric dispersion models themselves but in a subsequent module of the UFOMOD system. Dependent on the release characteristics special features are modelled. Plume rise due to the buoyancy of the released activity is included in MUSEMET and RIMPUFF using the Briggs model [10], which also allows for the possibility of material released into a building wake not lifting-off. The down-wash and mixing in the wake of a building are included in MUSEMET, RIMPUFF, and ISOLA using a virtual image model. The upper boundary of the mixing layer varies with stability. Generally, it is assumed that this boundary is formed by an inversion which cannot be penetrated by the plume. An exception is the MESOS model where parts of the radioactive material remain above the inversion if the mixing layer shrinks. This material is prevented from diffusing to the ground until the mixing layer depth subsequently increases to include it. The models MUSEMET, RIMPUFF, and ISOLA do not explicitly model the daily variations of the depth of the mixing layer. Preselected values depending on the stability class are used. The height of the mixing layer increases with decreasing stability, but it is not allowed to decrease if the stratification becomes more stable.

To consider external irradiation from the radioactive cloud in the near range ($\leq$ 20 km) cloud correction factors are evaluated in MUSEMET and RIMPUFF, which correct the dose conversion factors with respect to the finite extent of the cloud and the non-uniform activity distribution in the cloud.

To define starting times of weather sequences and the probabilities of occurrence of these sequences, it is convenient to apply stratified sampling. Therefore, a preprocessing program package called METSAM has been developed to perform the sampling. METSAM is designed in such a way that all possible weather situations are classified according to the initial wind direction, the time a plume need to leave a predefined area around the source, and the total amount of precipitation fallen during the dispersion. Afterwards, at least one weather sequence is sampled randomly from each class. This procedure ensures that the whole spectrum of weather conditions is taken into account. Especially infrequent sequences which might lead to severe consequences near to the plant will not be overlooked. In order to present the consequences probabilistically, the METSAM procedure is able to determine the probability of each chosen weather sequences.

The weather sampling procedure METSAM is more aiming at generic ACAs. Therefore, the correlation with population distributions is not taken into account. But for site-specific ACAs it is inevitable to apply meteorological sampling schemes to select weather sequences in correlation with the population distribution around the site. Therefore, as a further improvement of the program package COSYMA [13], a sampling scheme will be provided which has been developed at the National Radiological Protection Board (NRPB, Chilton/ UK) [14].

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V. Publications:

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RISO, Report Riso-M-2840 / KfK-4685, (to be published in 1990)

Title of the project no.:

PROJECT 2

THE ASSESSMENT OF EXPOSURE DUE TO DEPOSITED MATERIAL

Head(s) of project: J. Ehrhardt

Scientific staff: I. Hasemann, W. Raskob, C. Steinhauer

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 to atmosphere. The exposure pathways considered include: external irradiation from material deposited on the ground and on buildings, transfer of radionuclides through terrestrial foodchains, radionuclide transfer in freshwater bodies and in the marine environment and inhalation of resuspended material. The work forms part of the MARIA-2 Programme (Methods for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

Preparation of the COSYMA program package for distribution in Europe, especially the implementation of codes and data for foodbans based on activity concentrations, for doses and health effects assessments from the ingestion pathways with the agricultural production method, and for tritium behaviour in the environment.

III. Progress achieved:

1. Introduction

In 1983, the European project MARIA (Methods for Assessing the Radiological Impact of Accidents) was initiated as a part of the CEC Radiation Protection Research Programme with the aim to review and build on the nuclear accident consequence assessment (ACA) methods in use within the European Community. The main contractors are the Kernforschungszentrum Karlsruhe GmbH (KfK, FRG) and the National Radiological Protection Board (NRPB, UK).

One of the objectives of the second period of the project (MARIA-2, 1985-1989) was to develop a joint accident consequence assessment computer program package, COSYMA (Code System from MARIA) for use in the CEC, which should represent a fusion of ideas and models from the KfK program UFOMOD, the NRPB program MARC, of new developments within both institutions and of contributions from other MARIA contractors. COSYMA will be made available after the CEC seminar on "Methods and Codes for Assessing the Off-site Consequences of Nuclear Accidents" in Athens, May 7 - 11, 1990.

Between 1985 and 1988, KfK worked mainly on a complete revision of the probabilistic accident consequence assessment methodology UFOMOD, which lead to the release of the new UFOMOD program system in 1988 [1]. This version of UFOMOD, including all preprocessor and evaluation programs, together with some additional features from the corresponding NRPB-model MARC [4] not included in UFOMOD, formed the initial outfit of the European code system. For potential users of UFOMOD, but also for those interested in COSYMA, a "Seminar on UFOMOD and the development of an European accident consequence code" was organized by KfK in October 1988. In the time period after this seminar, the work at KfK was centered on the preparation of COSYMA for distribution in Europe by implementing additional models and data sets into the code.

The work was performed both within the frame of MARIA-2 and the GERMAN RISK STUDY - PHASE B. Contributions from other contractors actively involved in the two programmes were extensively utilized and included in both codes.

In this report, the KfK-contributions with respect to the the following features of COSYMA are briefly described:

- the general structure,
- the modelling of health effects,
- the modelling of the ingestion pathways,
- the model for radiation exposure from HT and HTO.

2. General structure of COSYMA

The general structure of the COSYMA program package was taken over from UFOMOD. As can be seen in Fig. 1, it consists of three independent ACA programs for the assessment of the consequences of acute exposure in the near

range (subsystem NE) and of long-term exposure in the near range and in the far range, respectively (subsystems NL and FL).

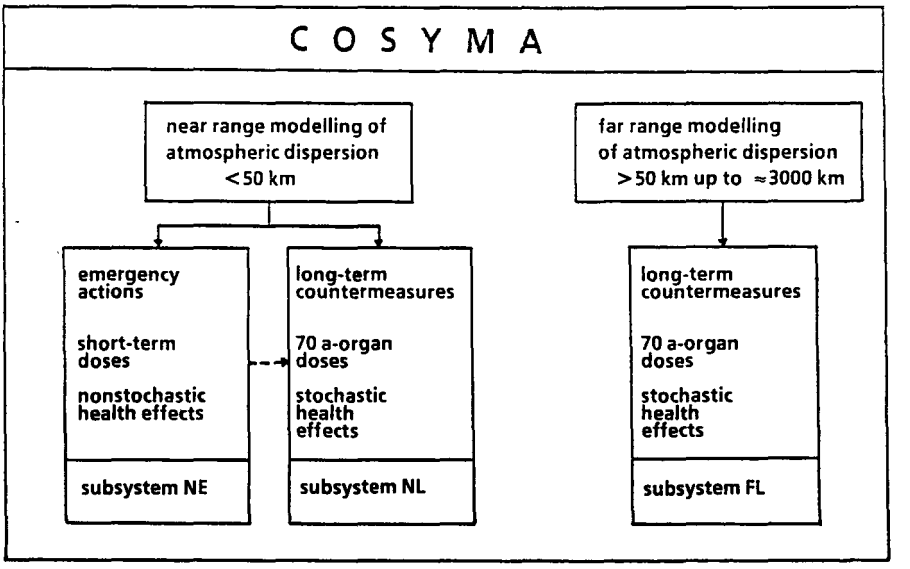

Fig.1: General structure of the COSYMA program system

This division was motivated as well from investigations concerning the range of applicability of atmospheric dispersion and deposition models in probabilistic ACAs described in the progress report for Project 1, as from the fact that the calculations of emergency actions and of the consequences from acute and longterm exposure require different models and different degrees of spatial resolution. In addition, many applications of ACAs refer only to one of both distance ranges and accident consequences.

Output from each subsystem are - among others - individual organ doses and health effect 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 representations of the results, for instance distributions of statistical quantities (e.g. expectation values, percentiles) as functions of distance, and pie diagrams of the contributions of radionuclides, exposure pathways and foodstuffs. Furthermore, three special evaluation programs were developed, which allow to correlate nuclide specific activity concentrations with the areas affected, individual organ doses with the number of individuals affected, and collective doses with individual doses. Presented graphically, such a form of results may be of considerable help in the analysis and interpretation of accident consequences especially at farther distances or for small source terms.

3. Health effects models in COSYMA

During the reporting period, a new "Health Effects Model for Nuclear Power Plant Accident Consequence Analysis" was developed for the USNRC [2], and a new model for stochastic somatic health effects by the GSF in the FRG [3], [5]. It was felt that these models set a standard which could not be ignored in the development of a new health effects model for UFOMOD, and later COSYMA, and that a certain harmonization would be of general benefit with respect to probably upcoming acceptance discussions. Therefore, two workshops with participants from the three countries were organized by KfK in October 1985 on "Radiological Health Effects Models for Nuclear Consequence Assessments" and in January 1986 on "Harmonization of Modelling in Nuclear Accident Consequence Codes". Another meeting on "Quantitative Evaluation of Health Effects of Ionizing Radiation" was organized by the CEC in Brussels. Based on the results of these three meetings the health effects model of UFOMOD was fixed. In a slightly expanded form, this became the model in COSYMA, which is outlined below.

3.1 Early effects

The models for the nonstochastic health effects are implemented in the modules for assessing individual risks of the subsystem NE. The health effects considered include all those causes for deaths for which a dose-response relationship has been specified either by NRPB or by USNRC. However, it does not include all the non-fatal effects of irradiation which have been considered. The subset selected for inclusion in COSYMA consists of those effects which lead to a severe disability of the affected individuals for the rest of their lifes or which require continuous medical treatment and/or social care. Those non-fatal effects which are transitory and leave no permanent health detriment are not quantified.

The assessments of the health risks is based on the hazard function approach of the USNRC [2]. The parameters defining the dose-response curves, i.e. the median doses, the slope parameters, the effective threshholds, and, eventually, time intervals for dose protraction, are set up by user input.

3.2 Stochastic somatic effects

Both fatal and non-fatal effects are considered. Age-, sex- and time-dependent dose-risk coefficients of 10 fatal cancer types have been provided by the GSF. The coefficients for leukemia and bone cancer are based on the assumption of an absolute risk model, for all other coefficients, relative risk models have been used. The number of incidences is calculated using a linear dose-response relationship. For cancer morbidity, GSF recommended the use of correction factors from the BEIR-III study.

For the new program system UFOMOD, the concept of activity-risk-coefficients (ARCs) has been developed to calculate the number of the fatal late health effects; this formalism is also used in COSYMA. These precalculated coefficients are normalized to the initial unit activity concentrations in

the air or on ground, and contain all information of the age and lifetime distributions in the population, the time and age dependence of the intake of activity for internal exposure pathways, the time and age dependence of dose accumulations for all exposure pathways, and the time and age dependence of individual risk. During an ACA run, individual risks for fatal cancers are simply calculated by multiplication of these coefficients with the initial activity concentrations; an intermediate dose assessment step is not necessary.

With the activity risk coefficients, it is for the first time possible to estimate within an ACA the individual risks of fatal late health effects as a function of time after the accident. An an example, the expectation values of the total number of fatal leukemia cases and lung cancers as a function of time are shown in Fig. 2. 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. - The results were obtained with UFOMOD using the PWR release category FK2 of the German Risk Study - Phase A.

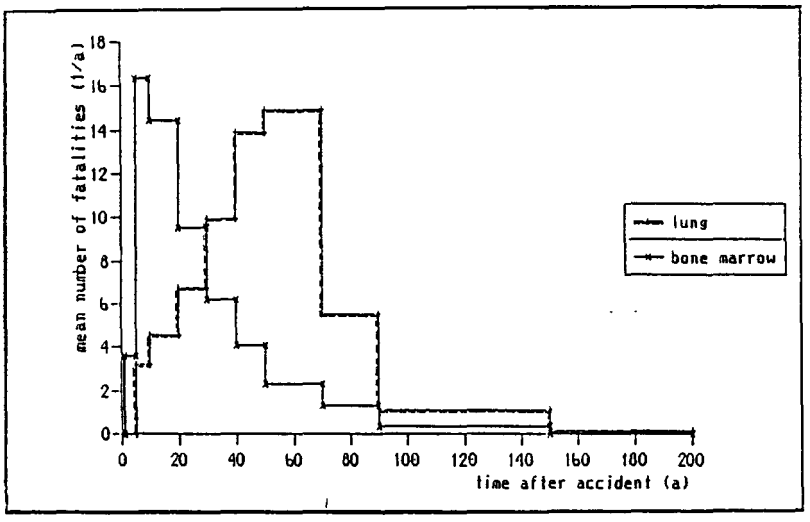

Fig.2: Example for the time-dependence of fatal leukemia and lung cancer

For the ingestion pathways, the ARC-method implies the assumption of local production and consumption of the foodstuffs. An alternative possibililty to estimate the number of late health effects from these pathways in COSYMA makes use of the fact that the assumption of a linear dose-response-relationship means that the total number of incidences in an exposed population is related to the collective dose. The total activity intake by the population from ingestion can be estimated from the quantity of activity appearing in agricultural produce predicted with a foodchain transport model, which is then assumed to be ingested by the population. Multiplication of the total activity intake with an appropriate dose conversion factor gives the collective dose, and multiplication of the collective dose with an

average dose-risk factor yields the total number of the corresponding health effects.

The second method will give a more accurate estimate of the overall number of late health effects than the ARC method for most accident scenarios considered. The disadvantage is, that no information is obtained on the ranges of individual doses and risks which make up the collective doses and risks.

4. Foodchain modelling in COSYMA

In the models calculating foodchain-related accident consequences in the subsystems NL and FL, nine foodstuffs are currently taken into account: milk (including milk products), beef (including veal), pork, mutton, grain products, potatoes and three types of vegetables (leafy-, nonleafy-, root). The time dependent foodchain concentrations are implemented in COSYMA as precalculated data obtained with a general terrestrial foodchain transport model for use in the CEC recommended by NRPB and GSF as a result of a comparison study between the foodchain models FARMLAND (NRPB) and ECOSYS (GSF). The data will become available in 1990 and contain activity concentrations and time integrals normalized to unit mass of foodstuff and initial deposit for 12 different times after the assumed date of the accident.

To account for seasonal effects, two such data sets are implemented in UFOMOD and COSYMA, one for a release on January 1st to represent an accident in winter ("November-March") and one for a release on July 1st to represent an accident in summer ("April-October"). In the calculations, each weather sequence is, depending on it's real date, attributed either to the winter or to the summer period and the corresponding foodchain data set is selected by the program. When using weather sequences covering at least one complete year, the results obtained in this way approximately represent averages over the different seasons. In addition, it is also possible to generate results for one of the above time periods alone.

Foodbans may be imposed on the basis of either the activity levels in foodstuffs or on dose criteria. More details about the corresponding UFOMOD/COSYMA models are given in the progress report for Project 3.

5. Tritium model

Since 1988, a model is being developed at KfK to assess within the frame of an ACA the atmospheric transport/deposition and the radiation exposure of tritiated water (HTO), gaseous tritium (HT), and fission products from postulated airborne releases from hypothetical fusion reactors. The fission products have the same phyco-chemical and radiological properties as nuclides from fission reactors and can be treated in UFOMOD/COSYMA by simply adding the corresponding data to the data bases already installed. Tritium, however, is chemically identical to hydrogen and thus interacts directly with water and organic substances, making processes like conversion of HT to HTO, reemission after deposition, and the conversion of HTO into organically bound tritium (OBT) relevant, all of which may modify the total balance of the

available HT or HTO inventories. The exposure pathways of significance for tritium are inhalation, ingestion and the radiation exposure from skin absorption, the latter playing no role for "conventional" radionuclides.

The consequences of a tritium release are commonly estimated in two separate ways. On the one hand, Gaussian (trajectory) dispersion models will describe all atmospheric transport and deposition processes which result in radiation exposure from inhalation and skin absorption. On the other hand, and separated from the dispersion process, compartment models will describe the transfer of tritium through the foodchains and the resulting radiation exposure from ingestion.

KfK took a somewhat more realistic approach by coupling directly an atmospheric dispersion model describing the atmospheric transport and deposition processes under consideration of all relevant transfer processes in the environment (soil, plant and animal) for approximately 100 hours after the release event, when the atmospheric transport plays the dominant role, with a first order compartment model [6] describing the transfer of tritium through the foodchains. The resulting computer code, UFOTRI (Unfallfolgenmodell für Tritiumfreisetzungen) will in future be used as a submodule in COSYMA. The computing times are in the range of several minutes for a one-hour release followed by a 100-hour re-emission period, which is reasonably short in order to be applicable in probabilistic accident consequence assessments.

The present version of UFOTRI is based on the Gaussian trajectory model MUSEMET which is in use in the program system COSYMA. MUSEMET was slightly modified for describing the behaviour of tritium in the environment in both chemical forms, i.e. gaseous tritium and tritiated water vapour.

The importance of the re-emission process necessitates dual modelling of the atmospheric dispersion. Primarily, MUSEMET calculates the dispersion after a single release event and the subsequent deposition onto soil and plants. Source terms of more than one hour are divided into one-hour intervals and calculated individually. In a second step, the re-emission of tritium from soil (evaporation) and plants (transpiration) is taken into account by an area source model which was specially developed for and combined with MUSEMET. In addition, allowance is made for the conversion of HT into HTO in the soil, the transport of tritium into deeper soil layers, and all other transfer processes with respect to plants and animals which are considered in the later ingestion part.

Some days after the release, when all the transport processes from grid point to grid point no longer significantly change the concentration distribution in the environment, the extensive dispersion module will be stopped and the calculated actual and integral concentrations of all compartments are then input for the ingestion submodel.

In this submodel, a first order compartment model is used, which describes the transport processes between the foodchain compartments in the form of

exchange rates. The compartments are autonomous parts of the overall system; they may exchange substances with other compartments, take up substances from outside, or give off substances to outside. The differences between the ingestion submodel and the ingestion model in the atmospheric dispersion and deposition submodel are the following:

- The transport between two different grid points is suppressed.
- The transfer rates will not be re-calculated each hour dependent on the environmental conditions, but are mean values representing the vegetation period.

Apart from this, the transfer ways and transfer rates considered in both submodels are identical.

Output of UFOTRI are the doses due to inhalation and skin absorption as well as the doses due to the ingestion pathways in a variable polar coordinate system.

Previous applications of UFOTRI and benchmark calculations have demonstrated the importance of the re-emission process, especially for HT releases, and that the doses from the ingestion pathways are about equal or higher than the doses from inhalation and skin absorption, as can be seen from Table 1 below:

release height	early dose (inhalation and skin absorption) from plume passage (Sv)	early dose (inhalation and skin absorption) from pl. pas. + re-emission (Sv)	early dose from ingestion (Sv)
10 m	3.1 E-7	1.8 E-4	3.6 E-4
20 m	2.1 E-7	8.9 E-5	2.2 E-4
60 m	5.9 E-8	9.3 E-6	3.7 E-5
20 m with building wake effects	2.3 E-7	1.2 E-4	2.4 E-4

Table 1. Plume centerline dose in 1000 m distance for a HT release of 100g and dispersion category F

Some modelling approaches in UFOTRI, e.g. the plant and soil models, are still rather simple, and further investigations are necessary to minimize the uncertanties due to the model assumptions.

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Title of the project no.: 3

Countermeasures to reduce the impact of accidental releases of radioactive materials

Head(s) of project:

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Scientific staff:

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I. Objectives of the project:

The aim of this project is to quantify the benefit of countermeasures in case of accidental releases to atmosphere in order

- to improve modelling of protective actions in ACA codes and
- to provide a greater input into emergency response planning.

Sheltering, prophylactic evacuation, and evacuation based on measurement of dose distributions in the environment are alternative countermeasures suitable to mitigate or to avoid death due to acute radiation syndrome. In case of alternatives an improved knowledge of risks and benefits tends to facilitate both decision making of the emergency management in a real case and the anticipation of these decisions in risk studies and ACA codes.

II. Objectives for the reporting period:

The main objectives of the work in the reporting period was to complete the countermeasures models for COSYMA, to increase their flexibility for allowing due consideration of strategies, plans and criteria existing in various countries, and to develop an economic module for implementation in COSYMA.

III. Progress achieved:

1. Countermeasure models and criteria

The modelling of countermeasures in COSYMA has been extended in comparison to UFOMOD and MARC to allow the user considerable freedom in specifying a wide range of emergency actions and criteria at which these actions will be imposed and withdrawn, so that most of the recommendations and criteria adopted in different EC countries and some of those which may be suggested in future can be modelled. Some of the countermeasures can be initiated automatically in an area which can be defined by the user. In general, countermeasures are defined in the program on the basis of dose criteria. The corresponding doses are summed over specified exposure pathways, and the user has the option of selecting which of these routes should be considered.

1.1 Sheltering

Sheltering can be initiated as a single countermeasure within a predefined circle or an area determined by an isodose line. The dose can result from any combination of the inhalation, deposited gamma and cloud gamma doses, with the periods over which the doses should be integrated specified by the user. The duration of sheltering is also a free parameter.

1.2 Evacuation

Evacuation is considered in COSYMA to be an action aimed at reducing short-term exposure. It can be initiated automatically in a geometrically defined area or on the basis of a dose criterion, and can be preceded by a period of sheltering.

The dose considered in the criterion is any combination of the inhalation, deposited gamma and cloud gamma doses, with the periods over which the doses should be integrated specified by the user. The doses considered are those to people out of doors at the time of plume passage. COSYMA allows the user to specifie criteria for several organs, with different dose levels and integration periods for each. Evacuation is assumed if the doses in any of the organs exceeds the level specified.

The modelling adopted in COSYMA to allow for the effects of sheltering and evacuation when calculating the doses received is complex and allows the user a great deal of flexibility in modelling the likely actions. The area of automatic evacuation is denoted "area A", while that where evacuation is triggered by a dose criterion is denoted "area B". The sequence of events assumed is illustrated in Figure 1. The actions are assumed to take place first in area A. After a delay for the authorities to initiate the actions, people are assumed to take shelter, while the plume remains overhead. Evacuation is assumed to begin at a specified period after the end of the release. The dose received during the period while people are evacuating is determined from the time taken to drive out of area A and the dose rate at the initial point. This driving time is determined from information specified by the user. Evacuation of area B is assumed to begin after the evacuation in area A has been completed.

The method adopted for calculating doses during the sheltering period includes the effects of different shielding factors in different types of house. Three groups of the sheltering population can be considered with different shielding factors. The program also allows for some

people in area A and B to "self-evacuate" rather than to shelter, and for some people remaining out of doors because they were unaware of the warning having been given. However all people in area A and B are assumed to be evacuated.

1.3 Relocation

Relocation is considered in COSYMA to be an action aimed at reducing longterm exposure. It is initiated only on a criterion based on the effective dose.

The doses considered in the criterion are the external gamma dose or the dose from inhalation of resuspended material or the sum of the two. The user can specify the periods over which the deposited gamma dose and the intakes from resuspension are integrated. This period can be any time up to 1 year, and could be sufficiently short that the criterion becomes effectively one of dose rate.

COSYMA will give the user the option of increasing the dose considered in the criterion at two specified down-wind distances. This enables the user to model a desire not to relocate people from large towns at some distance from the site.

There are two possible ways of specifying the time at which people are relocated. The first is simply to specify the time directly. The second is to use a time derived from the area to be located and a user-specified relocation rate expressed in area relocated per day, when it is assumed that all people are relocated at this same average time.

1.4 Return from evacuation or relocation and decontamination

The same criterion is used for returning from evacuation and relocation. Returning and decontamination are considered together in COSYMA.

Return will be assumed once the effective dose in the next year, from deposited gamma and/or resuspension, falls below a level specified by the user. Return is only considered at a discrete series of times. Decontamination is assumed in any time period where people could return at the end of that period if decontamination has been carried out, but not if it has not. Thus not all areas from which people have been evacuated or relocated will be decontaminated. The user can specify a decontamination factor for each time period considered.

1.5 Stable iodine tablets

The effect of taking stable iodine tablets on the thyroid dose from radioactive iodine depends on the time delay between inhaling the radioactive iodine and taking the stable iodine tablets. However the form of this relationship is not well known. Therefore a simple approach is adopted in COSYMA, and the user is required to input a value for the dose-reduction factor which is used for all locations, irrespective of the assumed times of plume arrival and of taking the tablets.

Iodine tablets are assumed to be distributed automatically within a circle whose radius is specified by the user. This simple model is adopted because it is felt that the dose estimates required for a more sophisticated system would not be available following an accident.

1.6 Foodbans

Foodbans are considered in the NL and FL sub-systems of COSYMA. Bans can be imposed or withdrawn on the basis of intervention levels optionally for activity levels in food or for individual doses. In either case the user has considerable freedom to specify his requirements.

If based on activity levels in food, the user can input the levels for a range of food categories and nuclide groups. The program will sum the concentrations of the nuclides from each group for comparison with the corresponding intervention level. It is assumed that the foodbans are imposed and withdrawn at the same concentrations.

If based on doses, the user can select which organs and foodstuffs shall be taken into account for banning. Individual foodstuffs can be combined to foodgroups in the sense that the sum of the doses from all members of each group will be compared with an intervention level holding for the whole group rather than for the individual members. A ban is assumed to be imposed for all foodgroup members, if the intervention level for any one of the organs to be considered with the group is exceeded; the intervention levels used for imposing and withdrawing the bans need not be the same.

The calculation of the doses is done with age-dependent consumption rates given by the user and corresponding age-dependent dose conversion factors. If the intervention levels are based on the average rather than the critical group, delays between harvest and consumption can be specified for each foodstuff. Losses of activity during normal food processing are always accounted for.

After the calculations of the foodbans with one or the other of the methods above, all foodstuffs can be combined to secondary foodgroups in the sense that the most restrictive ban time found for any one member of a foodgroup is also assigned to all other group members; this grouping is again steered by user input.

2. Development of an ECONOMICS Module

A joint model has been developped by NRPB and KfK within the MARIA programme to assess the economic cosequences of accidental releases of radioactivity. The aim of this model is to assess in detail the economic costs of early and late health effects, as well as of different countermeasures that are considered in order to reduce the number of health effects in the affected population. These countermeasures include sheltering, evacuation, relocation, food bans and decontamination.

2.1 General Structure of the System

The respective modules - ECONOM for the calculation of costs and EVAECO for the evaluation of costs - will be included in the overall COSYMA code system, therefore the same general structure as in COSYMA has to be applied. The COSYMA code system is split into the following three subsystems:

- subsystem **NE**, covering the **N**ear range and **E**arly measures and effects,
- subsystem **NL**, covering the **N**ear range and **L**ate measures and effects,
- subsystem **FL**, covering the **F**ar range and **L**ate measures and effects.

Subsystems NE and FL can be operated independently from the others, whereas subsystem NL needs information from subsystem NE. Within the different subsystems of COSYMA, the same loop structure, in which calculations are performed, is applied in each module. The outermost loop refers to different **nuclear sites (NS)** and the following loop to different **weather sequences (L)**. Within the loop of weather sequences calculations are based on a polar coordinate grid sytem (I,J; I = radius, J = azimuthal sector) with the center point at the location of the nuclear site. In the sequence of loops, **distance (I)** and **sector (J)** are the next loops used for calculations.

It is evident that the modules ECONOM and EVAECO have to make use of the same sequence of loops (NS,L,I,J) in order to be consistent with all other modules because of the transfer of stored data. This information stored on data files may either be of an integral, grid-independent type, or refers to each grid element. This means that cost calculations have either to be performed on an average, grid-independent level, or on the basis of grid element by grid element.

The following health effects and countermeasures can be treated in each of the subsystems NE, NL, FL of COSYMA, and the results calculated in previous modules may be used as a basis for cost calculations in ECONOM:

- subsystem **NE**
  - evacuation,
  - early health effects.

- subsystem **NL**
  - sheltering (only if not followed by evacuation, relocation and decontamination,
  - evacuation,
  - relocation,
  - decontamination,
  - food ban,
  - early health effects (taken over from the NE version),
  - late health effects.

- subsystem **FL**
  - relocation,
  - decontamination,
  - food ban,
  - late health effects.

With respect to the input information required for cost calculations in the module ECONOM, three different kind of data have to be handled:

Input data that are directly provided by the user; these kind of data are treated in COSYMA on the basis of default values and read in within a special input module. "Default values" means a procedure, in which all data of this kind are stored as reference values in the code, whereas the the user has the chance to change most of them, if he wants to do so.

Data sets in which the user will use information on a grid-by-grid basis.This kind of information is needed, if e.g. cost calculations will be performed on the basis of economic regions, or if land-use grids will be used. Data sets of this kind have to be provided separately for use in the module ECONOM.

Calculated results from preceding COSYMA modules that have been stored on data files for reuse.

2.2 Principal Procedure of Cost Calculations

In the following, the modelling concept for the calculation of economic costs will be described for each of the categories of countermeasures and health effects mentioned above.

Sheltering

Sheltering of people may lead to an interruption of the total economic production in the sheltering area during the time of sheltering, the costs of which are calculated in the same way as in case of evacuation.

Evacuation

The course of an evacuation process leads to three principally different cost categories:

Transport costs. This cost category includes all expenditures that arise in connection with the movement of people away from and back to the evacuation area. These costs are independent from the duration of evacuation.

Accomodation costs. This cost category comprises the additional expenditures for housing and food of the evacuated people away from the evacuation area. These costs are dependent of the duration of evacuation.

Costs due to losses in economic production. The movement of the total population out of the evacuation area leads to an interruption of the total economic production in this area during the time of absence; this comes out to be a loss to the overall national economy, because people are not able to earn money. These costs are, of course, also dependent of the duration of evacuation.

The primary input information that is submitted by preceding COSYMA modules is the number of people (and the respective area) evacuated, i.e., cost calculations are performed on a per-person basis.

Relocation

The course of a relocation process leads to four principally different cost categories, whereas three of them - transport costs, accomodation costs and costs due to losses in economic production - are the same as in case of evacuation and are also treated in the same manner. The fourth category is:

Costs due to the non-use of capital. The non-use of capital value and investments for economic production (housing, all tangible assets, land) as well as the non-use of private and general public property (e.g. consumer durables) during the time of absence of people leads to losses in value that may be calculated in a similar way as the "normal" depreciation of capital.

The primary input information that is submitted by preceding COSYMA modules is the number of people and the respective area relocated, i.e., cost calculations are performed on a per-person basis, except for the capital category "land", which is treated on a per-area basis.

Decontamination

In case of decontamination there is only one cost category to be calculated, i.e. the expenditures that arise in connection with decontamination procedures. The primary input information that is submitted by preceding COSYMA modules is the area of decontamination (and the number of people associated with this area). A first approximation is to perform the calculations on a per-area basis. This method does not take into consideration a distinction between decontamination in rural and urban areas which may be rather different, both in methods and costs. Therefore, calculation on a per-person basis will also be included, representing decontamination in urban areas.

Food ban

Three different cost categories will be handled in connection with food ban:

Costs of lost produce. The calculation of this cost category is similar to that of costs due to losses in economic production in case of evacuation or relocation, because of the agricultural sector being one sector in total national economy. There is one peculiarity in case of agricultural production: it cannot be interrupted immediately after the beginn of the ban; so in the first year, it is the the total value of products that will be taken as economic losses. COSYMA allows for a break-down of agricultural production into different products (foodstuffs).

Costs of lost agricultural capital. Again, the calculation of this cost category will be treated in a similar way as that of costs due to the non-use of capital in case of relocation.

Costs of disposal of food. Agricultural products, that have been produced in the first year of a food ban and will not be used, have to be disposed, which leads to direct expenditures of the disposal process.

The primary input information that is submitted by preceding COSYMA modules is either the area that is banned for each agricultural product, or the amount of product of the total area.

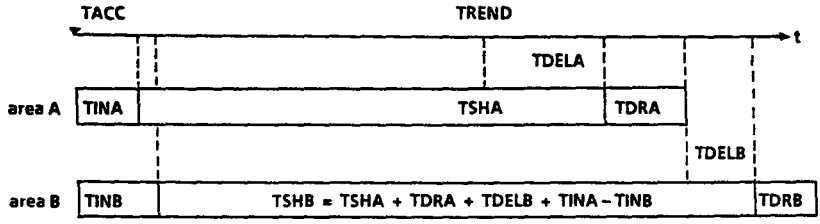
Health Effects

In COSYMA, a distinction is made between early and late health effects. With respect to cost calculations, both categories are treated in the same way; the only difference is that early effects are supposed to occur immediately after the radioactive release, whereas in case of late effects a time-dependency of occurrence has to be taken into account. Fatal and non-fatal effects are considered in both cases. There are two different cost categories that will be treated:

Medical treatment costs. These costs comprise all direct expenditures in connection with the treatment of a health effect.

Costs arising from the lost contribution of an individual to society suffering from a health effect.

The primary input information that is submitted by preceding COSYMA modules is the number of health effects cases, which can be used immediately for cost calculations.



TACC	=	time of accident (end of chain reaction)
TREND	=	time of end of release (≈ 95 %)
TINA, TINB	=	initial delay, area A, B
TSHA, TSHB	=	duration of sheltering period, area A, B
TDELA	=	delay time between end of release and end of sheltering period, area A
TDELB	=	delay time between end of evacuation in area A and begin of evacuation in area B
TDRA, TDRB	=	driving time to leave area A, B

Figure 1: COSYMA: Timing of emergency actions

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K. BURKART, J. EHRHARDT, I. HASEMANN
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The program system UFOMOD for assessing the consequences of nuclear accidents
Karlsruhe, Report KfK-4330 (October 1988)

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 were to understand, apply and refine uncertainty and sensitivity analysis methods with respect to the program UFOMOD subsystem NE

- to get a deeper insight into the propagation of parameter uncertainties through its different modules (the atmospheric dispersion module, the module describing early protective actions, the module calculating short-term organ doses, the healths effects module),
- to quantify their contribution to the confidence bands of the intermediate and final results of an accident consequence assessment (ACA) and,
- to combine the most important parameters from all submodule uncertainty analyses to a final overall uncertainty analysis.

III. Progress achieved:

Introduction:

UFOMOD version NE consists of many submodels with varying degrees of complexity, which have a large number of parameters associated with significant uncertainties. It is of considerable importance to understand the nature and magnitude of these uncertainties and their influence on the accuracy of the assessed consequences. This is a pre-requisite in decision making, where knowledge of the inherent uncertainties in the information being evaluated is essential if balanced and well considered judgements are to be made. It is equally important for the identification of modelling weakpoints and thus areas for further improvements and supporting research and development activities.

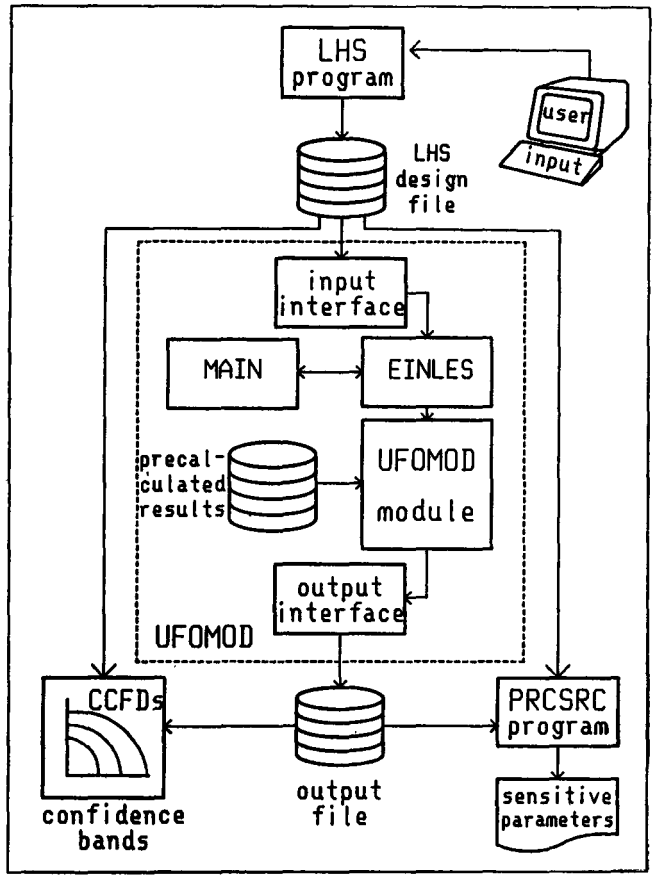

Figure 1. Scheme of UFOMOD - uncertainty and sensitivity analyses

Appropriate techniques are available for propagating parameter uncertainties through complex models like the program system UFOMOD. Their main task is the generation of a set of parameter vectors for which the ACA codes are run repeatedly. The parameter values of each vector are sampled from the probability distributions describing their variability. A variety of sampling techniques are in use for uncertainty analyses. For the investigations with UFOMOD, the Latin Hypercube Sampling (LHS) program [15] developed at Sandia National Laboratories has been used together with the corresponding sensitivity evaluation program (PRCSRC) [16] calculating correlation and regression coefficients. A comprehensive description of the procedures adopted is given in [9]. Intermediate and final results of uncertainty analyses of UFOMOD have been presented in several conference reports (see for instance: [2], [4], [5], [6], [7], [8] and [17]).

subject of uncertainty analysis	no. of paramters	no. of runs	CCFDs with (5%, 95%) confidence bounds			
			activity concentrations	radiation doses	individual risks	no. of early effects
atmospheric dispersion ATM	20	≤100	12	6	2	2
early protective actions CTM	20	60	-	6	2	2
dose model DCF	20	60	-	6	2	2
health effects HEM	8	40	-	-	6	5
overall model OAL	24	100	12	6	2	2

Figure 2. Overview of uncertainty analyses with UFOMOD / NE

Uncertainty/sensitivity analysis procedures:

Before starting the uncertainty and sensitivity analyses, a detailed discussion of the parameter uncertainty has to take place. The estimation of the physically possible range of variations and their probability distribution, and the possible correlation between parameters is an important but often tedious task, when the lack of experimental data requires expert opinion with subjective judgements. A detailed justification of the parameter uncertainties is given in [10], [11], [12], [13] and [14].

Having defined ranges and distributions for model parameters it is necessary to select specific values for each of the uncertain model parameters to be used in each run of UFOMOD, i.e. to have a suitable sampling scheme. For a sampling scheme to be effective the generated model parameter values should adequately span the model parameter space. The Latin hypercube sampling (LHS) procedure in contrast to the well-known random sampling design (RSD) forces the entire range of each model parameter to be sampled.

Each UFOMOD run produces *one* complementary cumulative frequency distribution (CCFD). Confidence bands have to be estimated for each set of CCFDs. The width of the bands is an indicator of the sensitivity of model predictions with respect to variations in parameters, which are imprecisely known.

To quantify the relative importance of the uncertain model parameters to the output of the accident consequence model some sensitivity measures are needed to 'rank' the parameters with respect to their influence on the consequences.

The partial (rank) correlation coefficient PCC or PRCC, respectively, are measures that explain the relation between a consequence variable and one or more model parameters. When a nonlinear relationship is involved it is often more revealing to calculate PCCs between variable *ranks* than between the *actual* values for the variables. The numerical value of the PRCCs can be used for hypothesis testing to quantify the confidence in the correlation itself, i.e. by statistical reasons one can determine which PRCC values indicate really an importance (significance) of a parameter or which PRCC values are simply due to 'white noise' (for details see [8], [9], [11], [12], [13] and [14]). Moreover, it is possible to calculate the percentage contribution of each uncertain model parameter to uncertainty in consequences by use of so-called *coefficients of determination* (R^2).

Figure 1 indicates in a schematic way the steps of uncertainty and sensitivity analyses done at KfK.

The last step in performing uncertainty analyses is to present and interprete the results of the analyses.

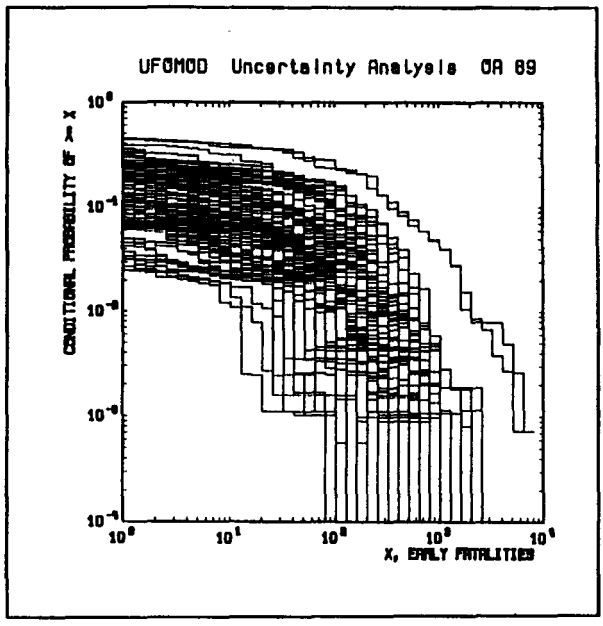

Figure 3. Complementary cumulative frequency distributions (CCFDs): of number of early death from pulmonary syndrome (assuming release has occured)

To summarize some general findings at first with respect to sampling:

- uncertainty (UA) and sensitivity analyses (SA) with random sampling (RS) and LHS do not lead to significantly different results,
- UA: sample sizes of 1.5 x # (model parameters) are sufficient,
- SA: to get statistically stable results, larger sample sizes are needed,
- distribution effects: for some distribution types there are differences between raw and rank values in the correlation matrices of the LHS - code.

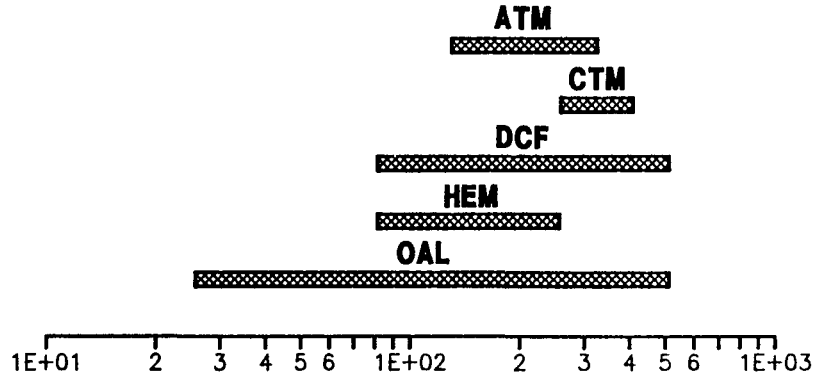

Figure 4. (5%,95%) confidence bands of 99% fractiles:
of number of early death from pulmonary syndrome (assuming release has occured)

(This may be important in the appreciation of induced raw or rank correlation structures between parameters. For details see [8], [13] and [14].)

To summarize some general findings with respect to the evaluation of sensitive parameters:

- Not every PRCC value makes sense:
  Therefore significance tests have to be used;
- a large absolute PRCC value is not in every case an indication for a considerable amount of responsibility for uncertainty in consequences:
  Therefore use PRCC values *and* coefficients of determination R^2,
- in most cases the number of PRCCs, which are above the 'white noise level', increases with the sample size
- for the atmospheric dispersion submodule of UFOMOD it was tested:
  - the most important parameters are stable in their rankings regardless of the distributions (predefined by experts or all uniform, respectively),
  - for the less important parameters:
    the PRCCs vary, but even then in most cases the ranking is the same.

Uncertainty/sensitivity analysis applications:

In a series of investigations with the mentioned submodules, a great deal of experience was gained with methods and evaluation techniques for uncertainty and sensitivity analyses. Especially the influence on results of different sampling techniques (random, Latin hypercube) and sample sizes, parameter distributions and correlations could be quantified and the usefulness of sensitivity measures like partial rank correlation coefficients (PRCC) and R^2-values (coefficients of determination) for the interpretation of results could be demonstrated.

Figure 2 gives an overview of the number of uncertain parameters identified for each submodel, the corresponding number of UFOMOD runs performed and the results evaluated with respect to confidence bands and most important input parameters.

The complementary cumulative frequency distributions (CCFDs) of the following quantities were evaluated:

	ATM	CTM	DCF	HEM	OAL
$\sigma_z(E,F)$	87 % (−)				11 % (−)
VD(IOD)	14 % (−)				
PAUFA1		17 % (−)			
PAUFA5		5 % (+)			
TINA		71 % (+)			2 % (+)
DCFIO			38 % (+)		32 % (+)
ARATIH			53 % (+)		27 % (+)
LGFD50				99 % (−)	29 % (−)

Figure 5. Percentage contributions of model parameters to the uncertainties in the number of early deaths (pulmonary syndrome)

- air and ground activity concentrations of the two nuclides I-131 and Cs-137 at three distances (0.875 km, 4.9 km and 8.75 km),
- individual bone marrow and lung doses at the same distances,
- acute risks of hematopoietic and pulmonary syndrome at the same distances,
- number of early health effects from hemetopoietic and pulmonary syndrome.

Each submodel leads for each of these endpoints to a list of parameters contributing significantly to the (5%-95%) - confidence bounds of mean values and 99% - fractiles, which have been chosen for evaluation. The most important parameters were selected and combined for the final overall analysis (OAL) as indicated in Figure 5. As source term the release RL 2 (RL 2 was derived from the release category FK 2 of the German Risk Study - Phase A by multiplying the amount of iodine and aerosols with the arbitrary factor 0.2, leaving the noble gases unchanged and disregarding the energy content of the release.) was used in all calculations For details see [1] and [3]. As a typical example of the results Figure 3 shows the set of CCFDs obtained from the parameter variations for the number of deaths from pulmonary syndrome: at the 99th percentile the (5%-95%) - confidence bound extends from 25 to 512.

From the preceding analyses, the contributions of the single submodels and their parameters to the uncertainties are known. The main results are summarized in Figure 4. It compares the (5%-95%) - confidence bounds of the 99% - fractiles of the number of deaths from pulmonary syndrome caused by each submodel with the overall analysis. It is obvious, that the parameters of the dose model dominate the uncertainties, followed by the uncertainties of parameters in the atmospheric disperion model and the health effects model. In Figure 5 details to Figure 4 are given: it shows the explained percentage uncertainties relevant in the submodel and in the overall analyses. The dose conversion factor of I-131 (DCFIO), the breathing rate (ARATIH) and the LD_{50} of the dose - risk relationship for the pulmonary syndrome (LGFD50) contribute each to about one third to the overall uncertainty estimates. The vertical diffusion parameter σ_z mainly of category F causes about

10%. All other parameters, like the dry deposition velocity of iodine (VD(IOD)), the percentage of people staying outdoors during sheltering (PAUFA5) or evacuating spontaneously (PAUFA1), and the initial delay time of emergency actions (TINA) are unimportant with respect to the uncertainties of the consequences 'death from pulmonary syndrome'.

Other parameter combinations and contributions are obtained for other consequence endpoints, like activity concentrations, organ doses or or individual risks at the various distances. It is one of the main conclusions of the investigations, that not only the strength of the source term but also the consequence types considered may lead to completely different conclusions about confidence bands and model parameters responsible for them.

IV. 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

V. Publications:

[1] J. Ehrhardt, K. Burkart, I. Hasemann, C. Matzerath, H.-J. Panitz, C. Steinhauer
The program system UFOMOD for assessing the consequences of nuclear accidents
Kernforschungszentrum Karlsruhe GmbH, Report KfK 4330,
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[2] J. Ehrhardt
Uncertainty and sensitivity analyses of UFOMOD - Applications -
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Role and importance of uncertainty analysis
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p. 769 - 786, ISBN 92-825-5991-2

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August 30 - September 4, 1987, Swiss Federal Institute of Technology (ETH), Zürich, Switzerland
in:
Probabilistic Safety Assessment and Risk Management, PSA '87, Vol. III, 939 - 944
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January 25 - 29, 1988, Rome (Italy)
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Uncertainty and sensitivity analyses of UFOMOD - Methods -
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[9] F. Fischer
Procedures for uncertainty analyses of UFOMOD - A user guide -
Kernforschungszentrum Karlsruhe GmbH, Report KfK 4626,
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[10] F. Fischer, J. Ehrhardt
Uncertainty Analysis with a View Towards Applications in Accident Consequence Assessments
Kernforschungszentrum Karlsruhe GmbH, Report KfK-3906,
September 1985

[11] F. Fischer, J. Ehrhardt, K. Burkart
Uncertainty analyses of the countermeasures module of the program system UFOMOD
Kernforschungszentrum Karlsruhe GmbH, Report KfK 4472,
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[12] F. Fischer, F. Ehrhardt, I. Hasemann
Uncertainty and sensitivity analyses of the complete program system UFOMOD and of selected submodels
Kernforschungszentrum Karlsruhe GmbH, (Report KfK 4627 to appear 1990)

[13] F. Fischer, J. Ehrhardt, J. Raicevic
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Uncertainty analyses for the the atmospheric dispersion submodule of UFOMOD with emphasis on parameter correlations
Kernforschungszentrum Karlsruhe GmbH, Report KfK 4447,
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A FORTRAN 77 program and user's guide for the generation of Latin hypercube and random samples for use with computer models
Sandia National Laboratories, Albuquerque NM (USA)
March 1984
SAND 83 - 2365
NUREG CR - 3624

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Sandia National Laboratories, Albuquerque NM (USA)
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Commission of the European Communities, Report EUR - 10397 EN (1986),
p. 831 - 850, ISBN 92-825-5991-2

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-F-114-GR

Greek Atomic Energy Commission
GAEC
NRC "Democritos"
153 10 Aghia Paraskevi
GR- Attiki

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

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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 centers from the operation of nuclear facilities for population distribution criteria.

Head(s) of project:

Dr. J.G.Kollas, Dr. I.A.Papazoglou

Scientific staff:

Dr. N.Catsaros, Mr. M.D.Christou, Ms. E.Daoukou

I. Objectives of the project:

The main objective of the project is the estimation of the radiation risk resulting from the operation of nuclear power plants, in the framework of the extensive non-uniformity of population and environment in Greece, putting emphasis at the same time on adopting appropriate population distribution criteria. This risk will be also set in perspective by comparing it to the corresponding risks of alternate energy sources and other technological activities in Greece.

II. Objectives for the reporting period:

Same as above.

III. Progress achieved:

The activities of the first project throughout the period 1985-1989 fall into three main categories, namely : siting criteria development in the presence of a large population center, optimization of emergency response in the event of a nuclear accident, and assessment of the radiation risk from severe nuclear accidents.

1. Methodology

The methodology developed concerning nuclear power plant (NPP) siting criteria in the presence of a large population center is based on assessing the average over a large spectrum of meteorological conditions whole body collective dose resulting from a postulated reference severe accident. The assessment of this dose is performed by code CRAC.GAEC, the NRCPS "Demokritos" version of the CRAC2 code. A representative collective dose is chosen as a measure of the social radiation risk, and is compared to the dose corresponding to a level of social risk encountered historically in energy production as a whole, by relating indirectly the risk of energy production to the risk of natural background radiation. The collective dose of the inhabitants of the population center for the first year after the postulated reference accident, which exceeds the corresponding doses of all other years following the accident, is chosen as the representative dose. The outcome of the comparison can be the determination of one or more sectors of acceptable sites for a set of specific conditions considered, such as the reactor characteristics.

A methodology for the optimization of the short-term emergency response in the event of an accident in a nuclear power plant has been also developed. Emergency response planning (ERP) refers to the protective actions that should be taken for the population around a nuclear plant in the event of an accident. Since absolute zero risk is not possible to achieve, the methodology addresses the question of how two different emergency plans can be compared in the view of the fact that the possible consequences to the public are measured in several dimensions and in addition these consequences are uncertain.

The reduction of the possible effects of the accident, which is the objective of the protective actions taken by ERP, can be achieved by reducing the population exposure to radioactive releases both in the early phases of the accident and in later periods. The developed methodology, however, is concentrated only in the early phases of the accident and, consequently, protective actions consist of evacuation of certain areas, sheltering people in other areas and finally relocating population in some other areas. An ERP would, therefore, determine which areas must be evacuated and when, as well as where people should be sheltered and for how long

in the event of an accident.

The concept of optimization of emergency response planning incorporates two main issues: 'adequate protection' and 'uncertainty'. First, the need for determining a level of adequate protection is obvious, since otherwise one would try to provide absolute or extremely high levels of protection which are either impossible or would be provided at extremely high socioeconomic cost. In trying to provide adequate protection an emergency plan tries to minimize the potential adverse health effects of an accident and at the same time minimize the associated socioeconomic impacts of the plan. These objectives are in general conflicting. Additional conflicting objectives appear whenever a policy tends to decrease a particular health effect (e.g. acute deaths) while at the same time it increases another (e.g. latent cancers). Second, all the checks and balances in the search for the best solution are done under conditions of uncertainty. This uncertainty characterizes both the details of the accident (amount of radioactivity release, energy, timing, inventory, etc.) and the prevailing weather conditions at the time of the accident.

The general methodological approach followed here is that of multiobjective optimization. Three criteria have been used as objective functions of the multiobjective programming problem: acute fatalities, latent fatalities and the socioeconomic cost of the emergency response policy. The optimization procedure defines the 'efficient frontier', which is the set of emergency response policies that are not dominated by any other response policy in all three criteria mentioned above. The reason for establishing the efficient frontier is to avoid the controversial problem of assessing preferences among different levels of the consequences of each policy. In addition, this set of non-dominated policies provides useful insights on the range of available policies and the corresponding consequences and many times the 'optimum' solution can be derived from it without formal preference assessment. Furthermore, a preference assessment, if required, can be better focused in the reduced area of consequences that correspond to the 'efficient' set.

The assessment of reactor accident consequences in all activities of the project, e.g. optimization of emergency response, Greek research reactor (GRR) accident analysis, etc., was performed by employing code CRAC.GAEC.

Shielding factors of typical Greek houses were estimated by the SHIELD-F code, which is a modified Fortran-77 version of the Danish computer code DEPSHIELD.

2. Results

From the methodology developed concerning NPP siting criteria, techniques were formed for estimating the minimum

siting distance from a large population center, for identifying acceptable sites around a large population center, for estimating the maximum allowable size of a NPP in different sectors with respect to a large population center, etc. In all applications the level of risk resulting from a postulated reference severe accident of a typical NPP to the inhabitants of the large population center, is less than the level of risk associated with the same population and corresponding to energy production as a whole.

The methodology of multiobjective optimization mentioned previously was implemented in computer code GOERP (Greek Optimization of Emergency Response Planning). First, the area around the nuclear plant has been divided into a number of cells at various distances and angles from the plant. The following six possible protective actions have been considered for the population within each cell:

- evacuation,
- sheltering in houses for one day,
- sheltering in houses for seven days,
- sheltering in large buildings for one day,
- sheltering in large buildings for seven days, and
- continuation of normal activity.

The distinction between sheltering in large buildings and sheltering in houses lies in different shielding factors and ventilation characteristics due to their structure. An emergency response plan is determined by a combination of the above protective actions for all the area cells around the plant (e.g. evacuation for cells 1 and 2, sheltering in large buildings in cell 3, home sheltering in cells 4,5 and 6, and continuation of normal activity for all the other cells). Therefore, all the possible combinations of protective actions determine the alternative ERPs, which constitute the alternative policies for the problem.

The effects (consequences) of these policies are measured in three dimensions:

- acute fatalities,
- latent health effects, and
- socioeconomic cost of the ERP.

The above three types of consequences, which can be assessed via any consequence assessment code, constitute the objective functions of the optimization problem. The number of possible combinations of ERPs increases geometrically with the number of spatial cells that are considered (actually, for **n** spatial cells, 6^n combinations of emergency policies must be considered). The problem was approached via a 'dynamic programming' procedure. In other words the optimization problem was solved in steps. In each step, corresponding to a spatial interval, the corresponding cumulative consequences until this interval are calculated and the set of the non-dominated policies is determined. In this framework, a policy is rejected if there exists at least one other policy which 'dominates' the first, i.e. it is better than

the former in all aspects of the consequences (less early and latent health effects and less socioeconomic cost). This way, after the last step has been processed, the set of all 'efficient' or 'non-dominated' policies is assessed.

The uncertainties of the problem have been also taken into consideration in the optimization procedure by using the expected values of the consequences. This presupposes full probabilistic analysis of the effects of the accident and use of probabilistic models for their assessment.

As a demonstration, the methodology was applied in a hypothetical site of a nuclear power plant with uniform population distribution around the plant. The plant has been assumed to be a PWR, similar to an existing one for which a full Probabilistic Risk Analysis has been performed. The emergency response planning for a distance of 16 km around the plant has been 'optimized'. The 16 km distance was divided into 5 intervals (at 2.4, 4.8, 8, 11.3, and 16 km), which correspond to the steps of the dynamic programming approach. For this application the 'socioeconomic' cost has been assumed proportional to the weighted sum of the total number of people affected by each policy. The weights reflect the differences among the various policies (e.g. person evacuated versus person sheltered in large building). The results of the application were interesting and meaningful, assessing the set of non-dominated ERPs, which consists of 32 policies out of $6^5 = 7776$ possible alternatives. The consequences of these 32 policies, measured in the three-dimensional space of acute fatalities, latent cancers and socioeconomic cost (all in LOG scale), are shown in Fig. 1. In addition, Fig. 2 depicts the relation between acute fatalities and policy's cost for the points of the efficient frontier. In Fig. 2 it is clearly shown that the efficient

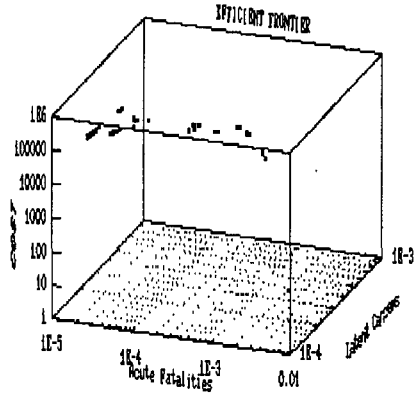

Fig. 1. Expected Consequences of Non-dominated Policies.

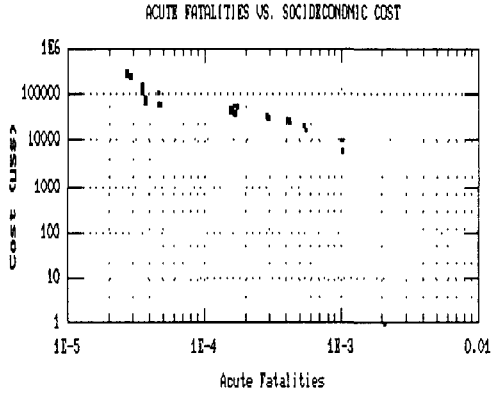

Fig. 2. Acute Fatalities vs. Socioeconomic Cost

frontier includes both policies providing high protection level at high cost and policies with low protection level at low cost. The policy of continuation of normal activity everywhere (with no cost at all) is also included in the efficient set. Furthermore, a preference assessment can evaluate the points of this set and assess the optimum emergency policy.

Concerning the assessment of the potential consequences of certain accidents postulated for GRR, two scenarios were employed, namely the severe design basis accident (DBA) of the reactor, a 20% core melt loss-of-coolant-accident, and a realistic coolant flow blockage accident. Both the presently highly enriched uranium core and a future low enriched uranium core of GRR were analyzed. The results of the analysis indicate : (a) that for the DBA no essential difference of consequences should be expected with the use of low enriched uranium in GRR, and (b) that in the event of a realistic accident release the consequences are not significant. Limiting effects are the thyroid dose and the thyroid latent health effects.

The sensitivity of the radiological consequences of the design basis accident of GRR to the operating schedule of the reactor was additionally assessed. The results of the analysis indicate that there is a direct relation between consequences and duration of operation. In all cases the thyroid dose and the thyroid latent health effects were the limiting consequences. It was also shown that the influence of the meteorological record on predicted consequences is not very significant, and the utilization of a substitute meteorological record from a nearby meteorological station instead of the record of the reactor site, could be acceptable for performing consequence analyses.

The consequence analysis of accidents of nuclear powered ships to the population residing in large ports indicated that the consequences of a severe accident could be substantial.

The analysis of large scale nuclear accidents with transboundary consequences leads to the conclusion, that the magnitude of the potential effects from the severe releases that were examined suggests, that multinational emergency planning for nuclear installations may be required even when nuclear power stations do not lie in proximity to national borders.

The mechanisms of deposition of radioactive materials on various surfaces has been analyzed, as well as the theoretical background for the calculation of shielding factors for buildings and houses. The Fortran-77 computer code SHIELD-F has been developed for the calculation of shielding factors for multi-storey buildings and single-family houses. An estimation of the average shielding factor for the Attica basin was performed.

The risk induced on humans and the environment by coal fired power plants and the radioactivity levels of Greek lignites have been also reviewed. The latter have been found to be significantly above the levels encountered usually elsewhere.

The analysis of the radiological impact of the Chernobyl accident in Greece made apparent that this impact can be considered as a minor one, with estimated average individual doses of 0.5 mSv and 1.6 mSv for the first year after the accident and all lifetime respectively.

Finally a critical review of emergency plans established in European Community countries has been performed, and the major basic recommendations and practices have been underlined with aim to be employed in future Greek emergency plans. The critical examination of the different approaches performed led to some recommendations for effectiveness improvement and optimization.

3. Discussion

The methodology developed on siting criteria is aimed to deal with the problems stemming from the demographic idiomorphy of Greece, where one third of the population of the country is concentrated in Athens city and suburbs, with the rest of the country exhibiting small population densities. Using this methodology, it becomes clear that by comparing the risk of a postulated severe nuclear accident to the risk of energy production, a decision aid tool for siting nuclear power plants near large population centers is formulated in respect to the social radiation risk. This approach has three

advantages : (a) it can be used in conjunction with other approaches strengthening thus the final decision, (b) it takes into account the social risk and it can be considered thus as a very desirable supplement to the more common site selection approaches used up to now, which satisfy mainly individual risk criteria, and finally (c) it is very simple and can ease public fears to some extent by being conservative in certain aspects, e.g. the postulated occurrence of a severe reactor accident, even the employment of a worst case wind-rose in the calculations if desired, etc.

The present methodology can be utilized as a siting decision aid tool in the presence of a large population center, when deciding on the acceptability of sites, or when two or more sites, which are equivalent otherwise, are compared. It can be also used to determine the maximum allowable magnitude of a NPP at different sites, that will limit the risk from the reference severe accident to levels below those of energy production.

The developed methodology for ERP optimization satisfies the need for provision of 'adequate protection' by setting conflicting objectives and trying to optimize them all. In the application described above, it is noteworthy that evacuation stands as an adequate protective action only within the first 2.4 km from the plant, while for further distances other policies 'dominate' it. Of course the policy of deciding evacuation for areas within, say, 16 km provides high level of protection. However, the cost for this is very high and, as it has been indicated by the analysis, there is another policy providing such high protection level at lower cost.

The problem of uncertainty has several times been proposed to be addressed by the so-called 'worst-case scenario'. This approach assumes that if an ERP has been planned to confront the worst possible conditions, it can also provide adequate protection in the case of less severe accidents, which in addition are more frequent. In most cases, planning against the worst accident will possibly result in deciding evacuation of large areas. However, evacuation is not always the best policy, since, for example, if the wind speed is such that the cloud is going to catch the evacuees, sheltering and leaving the cloud pass will certainly be a better policy. As this example indicates, the uncertainty in the parameters of the accident strongly affects the ERP and it should be taken into account when planning against emergencies.

It has also been proposed to use a single criterion, such as radiation dose, as a measure of effectiveness of an ERP. However, particular problems have been created from such use of radiation dose, problems deriving from the fact that the health effects are not linear functions of the dose. For example, we are not interested in the exact level of dose, if it has exceeded a certain limit, since above this limit any

level of dose produces the same health effects. Therefore, it seems that explicit use of societal health effects as measures of effectiveness of a particular ERP provides a much more clear understanding of the consequences and hence a framework for evaluating alternative ERPs that facilitates communication of the results to both decision makers and interested parties.

The assessment of the consequences of GRR under various operating conditions indicated that serious consequences are to be expected in the event of the DBA for the continuous operation schedule, and rather not significant consequences, with the exception of the thyroid dose and the non-fatal thyroid latent health effects, for the current operating schedule. In the event of the most severe credible accident the major part of the non-significant consequences would be due to the early exposure.

The policy of avoiding port entry of a nuclear powered ship in a large port, would not be different in essence from the siting policy followed in land based nuclear power stations, where proximity to large population centers is avoided.

When examining the different emergency plans, some points appear to need further improvement. Such points are : the standardization of nomenclature regarding the seriousness of emergencies together with comprehensive notification procedures which will be of a great help in establishing cooperation between neighboring countries; the generalized use of dedicated communication lines between authorities and services involved in an emergency planning procedure; the knowledge necessary to establish efficient decision making capabilities as far as sheltering and/or evacuation of a given population group is concerned - the "natural" shielding factor presented in buildings and houses around a given nuclear power plant is an important element to be taken into account in decision making procedures; the mechanisms for controlling the contamination of foodstuffs taking into account that the recent Chernobyl accident brought out the lack of adequate preparedness.

Concerning the shielding activities, the ALGOL-60 code DEPSHIELD of the Danish Riso National Laboratory for the calculation of shielding factors of buildings and houses has been significantly modified and rewritten in Fortran-77. The modified version, named SHIELD-F, has been used to estimate an average shielding factor for the population of the Attica basin.

In conclusion the activities concerning all main objectives of the project have been successfully carried out.

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

V. Publications:

A. Scientific Journals, Conference Proceedings

1. J.G.Kollas and N.Catsaros, **A Social Radiation Risk Criterion in Nuclear Power Plant Siting**, Intl. J. Energy Systems, v.6(3), p.81-85, 1986.

2. N.Catsaros and A.Vassiliou, **An Assessment of the Average Shielding Factors for the Population of the Attica Basin Using the SHIELD-F Code**, Rad. Prot. Dosimetry,v.21(1-3), p.97-102, 1987.

3. A.Vassiliou and N.Catsaros, **Indoor Dose-Rate Point-Kernel Approximation Using a Modified NAG Double Integration Routine**, NAG Newsletter 2/88, p.17-22, 1988.

4. J.G.Kollas and E.Daoukou, **The Risk of Nuclear Powered Ships at Large Ports**, Intl. J. Modeling and Simulation, v.9(3), p.90-94, 1989.

5. J.G.Kollas, **A Comparison of the Radiological Consequences of a HEU and LEU Fueled Research Reactor**, Proc. Intl. Mtg. on Reduced Enrichment for Research and Test Reactors, Petten, The Netherlands, Oct. 14-16, 1985, p.113-22, D.Reidel Publ. Co., 1986.

6. J.G.Kollas and J.Anoussis, **An Assessment of the Consequences of a Research Reactor's Credible Accident Release**, Proc.IVth European Congress, XIIIth Regional Congress of IRPA, Saltzburg, Austria, Sept. 15-19, 1986, p. 162-66, 1988.

7. J.G.Kollas, **The Radiological Impact of the Chernobyl Accident in Greece**, Proc. CEC Workshop on the Radiological Consequences of Chernobyl, Brussels, Belgium, Feb. 3-5, 1987.

8. J.G.Kollas and M.Tombrou, **Modeling Nuclear Reactor Acci-**

dents - Sensitivity of the Consequences to the Meteorological Record, Proc. IASTED Intl. Symp. on Modeling, Identification and Control (MIC'87), Grindelwald, Switzerland, Feb. 17-20, 1987, p.438-41.

9. J.G.Kollas, **An Estimation of the Chernobyl Accident Consequences in Greece - A Theoretical Approach**, Proc. Symp. on the Consequences of the Chernobyl Accident in Greece, Athens, Greece, Nov.19-20, 1987, p. 145-50 (in Greek).

10. J.G.Kollas, **Accidental Dose and Risk Assessment in the Immediate Vicinity of a Research Reactor**, Proc. 14th Regional Congress of IRPA, Kupari, Yugoslavia, Sept. 29-Oct. 2, 1987, p.445-48.

11. I.Papazoglou, **Optimization of Emergency Protective Planning for Major Industrial Accidents**, 1st Conf. of the European Section of the Society for Risk Analysis, IIASA, Luxenburg, Austria, Nov.10-11, 1988.

12. J.G.Kollas, **Radiation Risks of Large Scale Nuclear Accidents - A Case Study**, Proc. 7th Intl. Congress of IRPA, Sydney, Australia, April 10-17, 1988, v.2, p.995-98.

13. J.G.Kollas, **Accident Consequence Assessment and Siting Criteria Development**, Proc. Joint CEC/NEA(OECD) Workshop on Recent Advances in Reactor Accident Consequence Assessment, Rome, Italy, Jan. 25-29, 1988, EUR 11408, p.395-404.

14. J.G.Kollas, **Risk Comparisons as Decision Aid for Siting Nuclear Power Plants near Large Population Centers**, Proc. IAEA Intl. Conf. on Radiation Protection in Nuclear Energy, Sydney, Australia, April 18-22, 1988, IAEA-STI/PUB 783, v.1, p.413-21.

B. Short Communications, Internal Reports

15. J.G.Kollas and N.Catsaros, **Transfrontier Consequences to the Population of Greece of Large Scale Nuclear Accidents - A Preliminary Assessment**, DEMO-NTD-851, and DEMO 85/7, 1985.

16. J.G.Kollas, **An Assessment of the Radiological Consequences of the Greek Research Reactor's Design Basis Accident with the Use of Low Enriched Uranium**, DEMO 85/6, 1985.

17. J.G.Kollas, **The Risk of the Low Enriched Uranium Fueled Greek Research Reactor on the Personnel of the Greek A.E.C.**, DEMO-NTD-852, 1985.

18. J.G.Kollas and J.Anoussis, **A Comparison of the Consequences of the Design Basis Accident of the Greek Research Reactor with those of a Serious Realistic Accident**, DEMO 85/12, 1985.

19. N.Catsaros, **Electricity Generation from Coal: A Review of Impacts on Human Health and the Environment**, DEMO 85/5, 1985.

20. J.G.Kollas, **The Influence of the Operating Schedule of the Greek Research Reactor on the Radiological Consequences of the Reactor**, DEMO 86/2, 1986.

21. ... , J.G.Kollas, et. al., **The Consequences of the Chernobyl Nuclear Accident in Greece**, DEMO 86/4, 1986.

22. ... , J.G.Kollas, et. al., **The Consequences of the Chernobyl Nuclear Accident in Greece - Report No. 2**, DEMO 86/9, 1986.

23. N.Catsaros, **Critical Examinations of Emergency Plans for Nuclear Accidents** (in French), DEMO 86/5, 1986.

24. N.Catsaros and A.Vassiliou,**Radioactive Deposition on Surfaces and Shielding Factors for Buildings - A Preliminary Assessment for the Greek Territory** (in Greek), DEMO 87/1G, 1987.

Title of the project no.: 2

Wind Flow and Dispersion Aspects of Radiation Risk Assesment

Head(s) of project: J. G. Bartzis

Scientific staff: Antoniadis J., Assimakopoulos D., Catsaros N., Karras G., Konte K., Megaritou A., Notaridou V., Pissimanis D., Varvayanni M.

I. Objectives of the project:

Transient three-dimensional atmospheric dispersion capability under any atmospheric stability taking into consideration realistic topography including, among others, mountains, hills, surface water and islands incorporated into the computer code ADREA-I. The code is intended to be on one hand a "production" code for Environment Institutions and on the other hand a "module" useful to accident consequence analysis. The study of the flow field of the lower atmosphere in the Greek territory in the framework of specifying boundary conditions and input data for the atmospheric modeling and the accident consequence analysis.

II. Objectives for the reporting period:

Same as above.

III. Progress achieved:

METHODOLOGY

A. During the present work the computer program ADREA-I, has been developed particularly suitable for atmospheric transport and dispersion calculations over realistic topographies, of any degree of complexity. ADREA-I is intended to be a useful tool in analysing the radiation risk in complex environments, where simple codes reach their limits

The characteristics of the code can be classified as follows:

On Modeling

1. A coherent geometry and physical modeling is offered for treatment of time dependent transport processes in a complex three dimensional domain.

2. The code is based on the finite volume concept in Cartesian coordinate system. Treatment of complex domains is achieved by allowing a boundary surface of arbitrary irregularity to cross a calculation cell. An increase in the terrain complexity is not translated in increase of problem complexity.

3. The fluid under consideration consists of a mixture of dry air and water vapour. A certain number of passive fluids or/and substances (eg. released contaminants) can also be considered.

4. In order to maintain the computation time and storage at realistic levels, turbulence closure modeling is limited to eddy viscosity/diffusivity concept. Anisotropic effects are also included.

5. The fluid dynamics and thermodynamics are described by the mixture continuity, momentum and energy equations whereas mass conservation of the passive fluid component is fulfilled through a separate concentration equation.

6. The conservation equations include the compressibility effect and the hydrostatic and Boussinesq approximations are not used although the latter exists as option.

7. An option of the ADREA-I code allows the energy exchange between the atmosphere and the adjacent soil layer. In this option, the conductive, the convective and radiative contributions of heat to the ground surface are balanced. The layer heat transfer mechanism is limited to one dimensional heat conduction perpendicular to the underlying surface. The prediction of ground surface temperature by this technique permits feedbacks between the surface temperature and the prevailing mesoscale system.

On Numerics

1. For the numerical solution, the SIMPLER/ADREA algorithm is used, based on the SIMPLER algorithm given in Patankar(1980). The mixture mass conservation equation is turned to a full pressure (Poisson) equation including the transient term. Pressure correction is avoided. Underrelaxation factors are also avoided.

2. The solution per variable is obtained either by the Gauss - Seidel point iteration method or by a direct solution utilizing the NAG library sparce matrix techniques

3. The time step is automatically selected based on "convergence error bands".

On Code Structure

1. The ADREA-I code is a user oriented code and it has been designed with the purpose to be transferred easily from one computer to another. Therefore it has a relatively simple structure and it is written in standard FORTRAN 77.

2. It can run with computers like CRAY, AMDAHL, IBM, CDC, VAX, PRIME and Personal Computers, with minor modifications.

3. The code is intended to be a "production" code utilized by persons even with a relatively low expertise. It is designed in a way to make the user decision process as simple as possible offering however at the same time to the specialists a large degree of flexibility in decision making. Special attention has been given to the code structure so that the input data volume and the computer time and storage are commensurate with the degree of complexity of the particular application.

On Applications

The code is capable to predict many aspects of the windfield distribution and the atmospheric dispersion.

It offers the possibility to simulate phenomena during the contaminants dispersion process in cases where:

- the terrain is characterized by a high degree of complexity including mountains of any height and/or water surfaces.
- the ambient conditions are complex (e.g., stable/ unstable conditions, stagnant conditions, etc).

B. For the flow field study in the 850mbs, the Greek territory was divided to four parts (NW, NE, SW, SE) based on the local climatological behaviour. The analysis which covers the 1976-1985 period is based on the synoptic maps and the sounding data of the stations, Bari (NW), Instabul-Plovdiv (NE) and Athens-Heraklion (SE). For the flow field on the surface, the meteorological data obtained from the major stations in the Greek territory have been utilized.

RESULTS

A. During the present contractual period, besides code development (1,9), a great effort was made to demonstrate and verify the capability of ADREA-I to treat the problems that is designed for.

The work performed for code development, demonstration capability and verification studies can be classified as follows:

1. Reviews

a. In view of the ADREA-I application to terrain consisting of a land/water interface, a review on the sea breeze modeling was carried out. The techniques mostly used by the sea breeze investigators reported in existing literature are extensively discussed (10).

b. In view of air/ground interaction modeling, to be incorporated in the ADREA-I code, a review on techniques existing in this field was carried out. The methodologies used in mesoscale models to represent air/ground interaction are discussed. Additionally, a first "heat budget" model adopted in ADREA-I is recommended (11).

2. Applications

The applications performed to demonstrate and verify the code capability are the following:

a. The building block problem was selected as a demonstration to three dimensional analysis. A building block with an inner yard was considered and the protection of this yard against the nearby road (ground pollutant source) was studied. The performed calculations provided predictions of wind velocity and ground concentration distribution (2,3).

b. The triangle obstacle problem was carried out in order to check the code performance in nonrectangular terrain. The predictions were compared with the wind

experimental results, for a two dimensional triangular ridge having a slope 2 to 1 obtained in the EPA experimental tunnel (3,4,12).

c. The Nebraska planetary boundary layer problem was selected to confirm the capability of the code in diurnal effect studies. A verification study based on the Nebraska Great Planes Experiment provided results for the planetary boundary layer diurnal evolution (3,13) and the vertical distribution of potential temperatures.

d. The Alaskan Beaufort Sea breeze problem was chosen as the first verification study of the ADREA-I code on terrain induced circulations. It was based on data referring to the Alaskan Beaufort Sea Coast. This case was selected, as corresponding to a well documented sea breeze analytical study (Kozo 1982), suitable for comparison with the model adopted. The results are shown in terms of sea breeze circulation development, boundary layer growth and temperature field evolution over the coastal area under consideration (5,14).

e. The studies of dispersion over mountainous coastlines were performed in order to examine the code ability to treat more complicated situations, where terrain irregularities (i.e. raised ground) and surface inhomogeneities (i.e. land/water interface) coexist. Three different cases were investigated. In the first case, a two dimensional, 1000m mountain range, parallel to the N-S axis, surrounded by sea and representing an idealized west-east cross section of the Athens basin was considered. A point source located at the eastern side of the mountain, 36km offshore was assumed. The flow and contamination patterns were predicted for 12 hours, starting from 0600 LST with stable conditions and a 5m/s synoptic wind blowing from west. The temperature field and the boundary layer growth were also predicted for the studied period (6). In the second case the same calculations were made but for a prevailing synoptic calm, in order to examine the code capability in

stagnant conditions (7). The predicted contamination pattern for 1100 LST is shown in Fig. 1. In the third case the mountain assumed was adjacent to a sea surface only at the eastern side and the background situation was again assumed stable and calm (8). The analysis provided results for the flow field and the concentration distribution, in low wind conditions.

f. The Wangara data problem was analysed within the framework of verification studies of ADREA-I. This approach started a series of calculations and tests against experimental data, focusing on the Australian Wangara experiment (July and August 1967) (15). In this first approximation, the mean wind, the temperature and specific humidity profiles as well as the boundary layer growth were predicted for the 0900 to 1800 CST period.

The experimental Wangara data were also used for the first verification study of the surface heat budget model adopted in the code. In this first application, the model was tested on the horizontal ground, the Wangara data are referred to. Twelve hours calculations were made, starting from 0900 CST (16). The vertical temperature profiles, the boundary layer growth and the surface heat flux variation were predicted for the period studied.

B. A seasonal analysis was 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 (17).

DISCUSSION

A. The model verification studies performed up to now showed satisfactory agreement with experimental results increasing the confidence as an appropriate transport model in complex terrain environment. However, further verifica-

tion studies of the code, based on experimental data, are needed. The main conclusions drawn from the applications performed during this period are summarized in the following paragraphs.

1. The first verification and demonstration studies (ie. triangular ridge problem, building block problem and Nebraska boundary layer) showed the capability of the code to treat the complex domain satisfactorily. It should be underlined the "comfort" of the ADREA-I code to handle non-rectangular obstacles.
2. The sea breeze verification study, based on the Alaskan Beaufort Sea Coast data, showed that the results obtained were in relatively good agreement with those obtained by Kozo (1982). This verifies the capability of the code to apply successfully to problems including land/water interfaces.
3. The studies of the effects of a large natural barrier on sea breeze and contamination patterns gave reasonable results for the diurnal evolution of the phenomena. All these applications demonstrate the code ability to treat complicated dispersion problems, where terrain disturbances and inhomogeneities and complex atmospheric conditions, such as stagnant prevailing atmosphere, are combined. In all the above cases, the main conclusion was that the mountain presence seems to exert a drastic control on the pollutants distribution, keeping the lower altitudes of the opposite mountain side relatively clear.
4. The first test of the ADREA-I code, against real field data, (Wangara experiment), provided quite satisfactory results for the mean boundary layer structure.
5. The air/ground interaction modeling review shows that the majority of the mesoscale theoretical studies either utilize a periodic heating function to prescribe the ground surface temperature variation, or use the surface heat energy budget equation. The

latter is a more advanced approach being the most appropriate in complex terrain simulations and permitting feedbacks between the ground temperature and the atmosphere. In ADREA-I code, both cases are included.

6. The first test of the surface heat budget model, reproduced the experimental Wangara data satisfactorily.

B. The flow field data in the 850mbs over the Greek territory show that the prevailing wind directions are either of the W, SW sector (December, March) or N 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 interchanges).

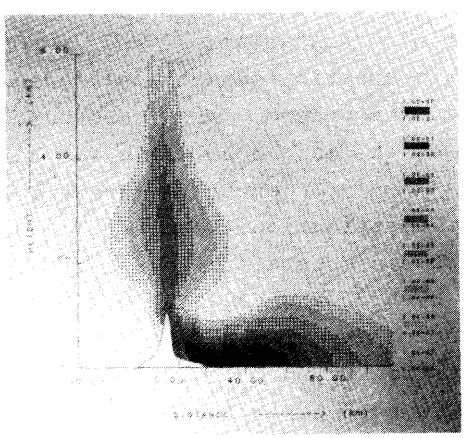

Fig. 1: Concentration pattern at 1100 LST

IV. 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

V. Publications:

V1. Publications in scientific journals, monographs, etc.

1. Bartzis J.G, Venetsanos A.G., Varvayanni M., Catsaros N., Megaritou A. : ADREA-I, a transient three dimensional transport code for complex terrain and other applications. Submitted for publication to Nucl. Techn.
2. Bartzis J.G. : ADREA-I, a transient three dimensional transport code for atmospheric and other applications. ENC4, EURATOM IX, V. 3, P. 603-608.
3. Bartzis J.G. : Turbulent diffusion modeling for windflow and dispersion analysis. Workshop on recent Advances in Reactor Consequence Assessment, Rome, Jan. 27-29 1988.
4. Bartzis J.G. : Flow modeling in complex terrain for atmospheric applications. Intern. Symp. in Env. Meteor. Wurzburg F.R.G. Sept. 29 - Oct. 1, 1987.
5. Varvayanni M., Bartzis J.G. : Sea breeze wind field predictions in atmospheric dispersion modeling. 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., Catsaros N. : A theoretical investigation of natural barrier effects on sea breeze and contamination patterns. Workshop on recent advances in reactor accident consequence assessment, Rome, Jan. 27-29, 1988.

7. Bartzis J.G., Varvayanni M., Cornelios N. : An analytical study of natural barrier effects on sea breeze and dispersion. Meteorology and Atmospheric Dispersion in Coastal Area, Riso Nat. Lab., Denmark Oct. 25-27, 1988.
8. Varvayanni M., Bartzis J.G. : Numerical analysis of dispersion over a near mountain coast. Eurasap Meeting on Atmospheric Dispersion in low windspeeds and foggy conditions, Sept. 5-7, Torino, Italy 1989.

V2. Internal reports.

9. Bartzis J.G. : ADREA-I, a transient three dimensional transport code for atmospheric and other applications. Some preliminary results. DEMO report 85/3 NCSR "Demokritos", Aghia Paraskevi, Greece 1985.
10. Varvayanni M., Bartzis J.G. : Sea breeze modeling review. DEMO report 87/9, NCSR "Demokritos", 1987.
11. Varvayanni M., Bartzis J.G. : Air/Ground interaction modeling review. To be published.
12. Bartzis J.G. : Flow modeling in complex terrain. DEMO report, NCSR "Demokritos", 1986.
13. Bartzis J.G. : ADREA-I code development: Atmospheric stability modeling and verification studies. DEMO report 86/7, NCSR "Demokritos", 1986.
14. Varvayanni M., Bartzis J.G. : ADREA-I code development: Sea breeze modeling and calculations. DEMO report 87/8, NCSR "Demokritos", 1987.
15. Varvayanni M., Bartzis J.G., Catsaros N. : ADREA-I verification studies based on Wangara experiment. To be published.
16. Varvayanni M., Bartzis J.G. : The surface heat budget model in ADREA-I code and its application to Wangara experiment. To be published.
17. Pissimanis D., Karras G., Notaridou V., Bartzis J.G. : Preliminary study on the flow field over Greece. DEMO report 89/1, NCSR "Demokritos", 1987.

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
Dr. V. Kamenopoulou
Mrs. Ch. Chaloulou
Mr. M. Probonas

I. Objectives of the project:

A. Main objectives:
1. Investigation of areas with elevated concentrations of natural radioactivity (Greek radon spas, well water supplies, building materials, indoor radon). Estimation of the related occupational and population doses.
2. Investigation of the behaviour of long-lived radionuclides into coastal marine ecosystems (uptake and retention by marine organisms, transfer through various trophic levels).
B. Objectives related to the Chernobyl accident:
1. Investigations on the delayed and late impact of the Chernobyl accident on the Greek environment.
2. Studies on the "hot particle" form of the Chernobyl pollutants.

II. Objectives for the reporting period:

As above.

III. Progress achieved:

1. Methodology.

The main efforts in the field of methodology have been related to the development and/or improvement of methods for determination of radon and its short lived decay products in various environmental media. This includes:

a) The development of an alpha-spectrometry laboratory method for separate determination of the potential alpha-energy concentrations (PAEC) of Rn222 and Rn220 decay products in air. This is a grab sampling filter method with sampling duration of 1200 s and filter counting during the time intervals 120-3915 s and 10200-18480 s after the end of sampling. The method is characterized by low detection limit of 10 and 40 micro-WL for the decay products of Rn222 and Rn220 respectiv.

b) The development of an express variant of the 3-interval total alpha-counting method, suitable for work in areas of elevated radon concentrations in air. The sampling interval is 1 min and the measuring - (1-5),(6-10) and (11-15) min after the end of sampling. The LLD is 25 Bq/m3 equilibrium equivalent concentration of Rn222. A computer code has been developed later for rapid calculation of the relations between the air concentrations of Po218, Pb214 and Bi214 and the total alpha-counts after any set of sampling/measuring intervals. The relations are calculated in both directions, which allows the use of the code not only in any case of "non-standard" measurement, but also for quick optimization of any variant with fixed total duration.

c) The clasical circulation extraction method for determination of Rn222 in liquid samples has been improved by introduction of a single sampling/extraction unit, vacuum sampling, temperature and volume corrections. As a result a considerable reduction of certain systematic errors has been achieved.

d) A system for determination of the exhalation rate of Rn222 from various materials (of volume up to 2000 cm3) has been developed. The LLD is $2 \cdot 10^{-5}$ Bq/s of Rn222. The system has been used also for determination of the diffusion of radon through various materials and the selection of the most suitable for construction of pots free of radon losses to be used for gamma-spectrometry of Ra226. The system is used also as a small calibration chamber for track-etch detectors.

e) A day-by-day monitoring of the concentrations of Rn222 and Rn220 decay products in the outdoor air ("Democritos" region) became possible after the introduction of 2 new measurements of the total-beta activity of the filters used for the regular environmental control. This provides approximate values, which nevertheless can be used to observe seasonal effects and correlations with various meteorological and air pollution parameters.

f) The track-etch detector technology for determination of the integrated exposure to radon has been introduced during 1989, as a result of collaboration with the Radiation Dosimetry

Institute of the Chechoslovakian Academy of Sciences. This includes the whole circle of the method and will allow fully independent indoor radon surveys to be organized in the near future. It was possible to develop a user-friendly method of manual track counting, based on the projection of the etched surface on a semi-transparent screen after suitable color filtering.

A survey of the natural radioactivity of the Greek soils is in progress during the last two years. The survey will provide useful information about the major source of indoor radon. The results are of use also for the estimation of the external exposure of the Greek population to the gamma-rays from the terrestrial radionuclides. In order to confirm the reliability of the relations between the concentrations of U238, Th232 and K40 in soil and the resulting external dose rate on its surface, a number of paired measurements has been performed in areas of various absolute and relative (one to another) content of the above basic natural radionuclides. The results indicate that the external dose rate can be determined with an error not exceeding typically 20%, while no correction for the cosmic ray component is necessary. This enabled us (in collaboration with the Athens Technical University) to provide the NRPB group for the "Natural Radioactivity Atlas of Europe" data covering most of the Greek territory.

Certain methodological work has been done in the field of the quantitative evaluation of gamma-spectra for natural radionuclides. A computer code has been developed and used after the stage of peak analysis, in order to optimize the determination of specific nuclides of the U238 and Th232 series.

The detection of "hot particles" in the Chernobyl fallout has been followed by the development of methodology for determination of their surface density in order to estimate their integrated air concentrations and the probability for inhalation in the region of Athens (11).

2. Results.

2.1. The major Greek radon spas and springs have been investigated. This includes 23 sources of thermal water with concentrations of Rn222 in the range of 50 kBq/m3 to 6 MBq/m3. The sources are located in the regions of Ikaria island, Kamena Vourla, Loutraki and Edypsos (Evia island). The concentrations of Ra226 in water and Rn222 and its daughters in the air of the balneological premises have been determined as well. The results have been reported in (1,2,4,13,17) and are summarized in Fig.1. The additional annual doses to the patients and the personnel due to inhalation of radon decay products have been estimated. The maximum values refer to a modern unit in Kamena Vourla and are of the order of 3 mSv/a for a typical patient and 100 mSv/a for the most exposed members of the personnel.

2.2. The concentrations of Rn222 in well waters of the Attiki region have been determined. The values lay in the range 0.15 - 13 kBq/m3 and the consumer-weighted average is 4 kBq/m3.

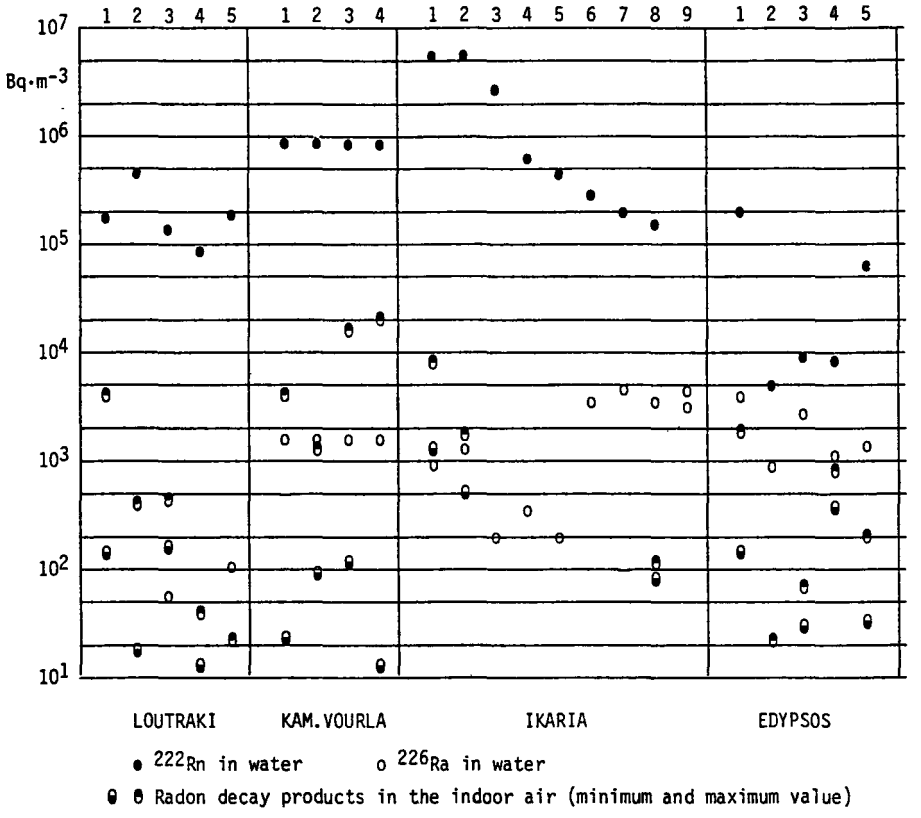

Fig.1. Concentrations of natural radionuclides observed in 23 Greek radon spas and springs. Note that the units in Kamena Vourla use a common source of thermal water.

The additional dose due to the systematic domestic use of well water does not exceed 50 μSv/a.

2.3. The concentrations of Rn222 decay products in air have been determined in over 200 Athens and Thessaloniki residences, by use of a sensitive total alpha-count filter method. The results have been reported in (4) and (20). The values of the equilibrium equivalent concentration vary typically in the range of 3 - 20 Bq/m3 and are lower, in average, for the Athens region.

A special case of houses, where lignite ashes has been used as a foundation-forming material, has been investigated in Milaki, Evia Island (8). The enhanced content of Ra226 in the ashes (500-1000 Bq/kg) results in elevated values of the radon concentration indoors, reaching, under conditions of "winter ventilation" values of 50 Bq/m3 EEC.

The outdoor concentrations of Rn222 and Rn220 decay products in the region of "Democritos" Center have been monitored continuously. The EEC of Rn222 daughters vary in the renge of 0.3 - 3.5 Bq/m3 (generally) and 0.8 - 2 Bq/m3 typically and no significat seasonal variations can be observed. The EEC of Rn220 daughters vary in the range of 0.007 - 0.12 Bq/m3 (generally) and 0.01 - 0.08 Bq/m3 typically and a definite trend of increase during the summer and decrease during the winter is observed, in a ratio up to 4:1.

2.4. The concentrations of natural radionuclides in the constituent materials and the final products of a major Greek cement producer have been investigated, mainly in order to evaluate the radiological impact of the use of fly ashes constituent of enhanced Ra226 content (240-530 Bq/kg). The results indicate an insignificant increase of the Ra226 content in the final product, resulting in values in the range of 30-45 Bq/kg, while the exhalation of Rn222 from the ashes material is very low (the last seems to be a property of the ashes itself rather than of the final product).

2.5. The radiological impact from the operation of an experimental geothermal power plant in Milos island has been studied, in collaboration with the Public Electricity Company. The results do not indicate any measurable effect on the environment. In contrary, during the measurements in selected "reference" regions of the island it was found that most of the ores excavated in tens of open mines are characterized by enhanced levels os natural radioactivity (up to 100 Bq/kg Ra226, 120 Bq/kg Th232 and 1500 Bq/kg K40). It has been a perfect example of unjustified public concern about a new activity of assumed radiological importance in a region of traditional works under conditions of enhanced natural radioactivity.

2.6. More than 450 samples of Greek soils have been analyzed by use of high-resolution gamma spectrometry. The results provide a good picture of the variations of the natural radioactivity in the country and enable the estimation of the external doses from the terrestrial gamma-rays. The results are summarized in the table following and refer to dried and sifted material collected from the upper 20 cm soil layer.

Nuclide	min.	max.	aver.	st.d. (Bq/kg)
Ra225	7	252	46.2	25
Th232	2	123	41.3	19
K40	26	1475	599	250

2.7. The concentrations of long-lived natural and artificial radionuclides in samples from the Greek marine environment have been determined during the whole duration of the project. A sampling network has been established, including a number of reference stations as well as stations located in regions of enhanced natural radioactivity of the coastal and sea bed materials (Fig.2).

The influence of the enhanced coastal radioactivity on various marine organisms has been studied (stations of Chalkidiki, Lavrion, Milos island, Evia gulf) and the results have

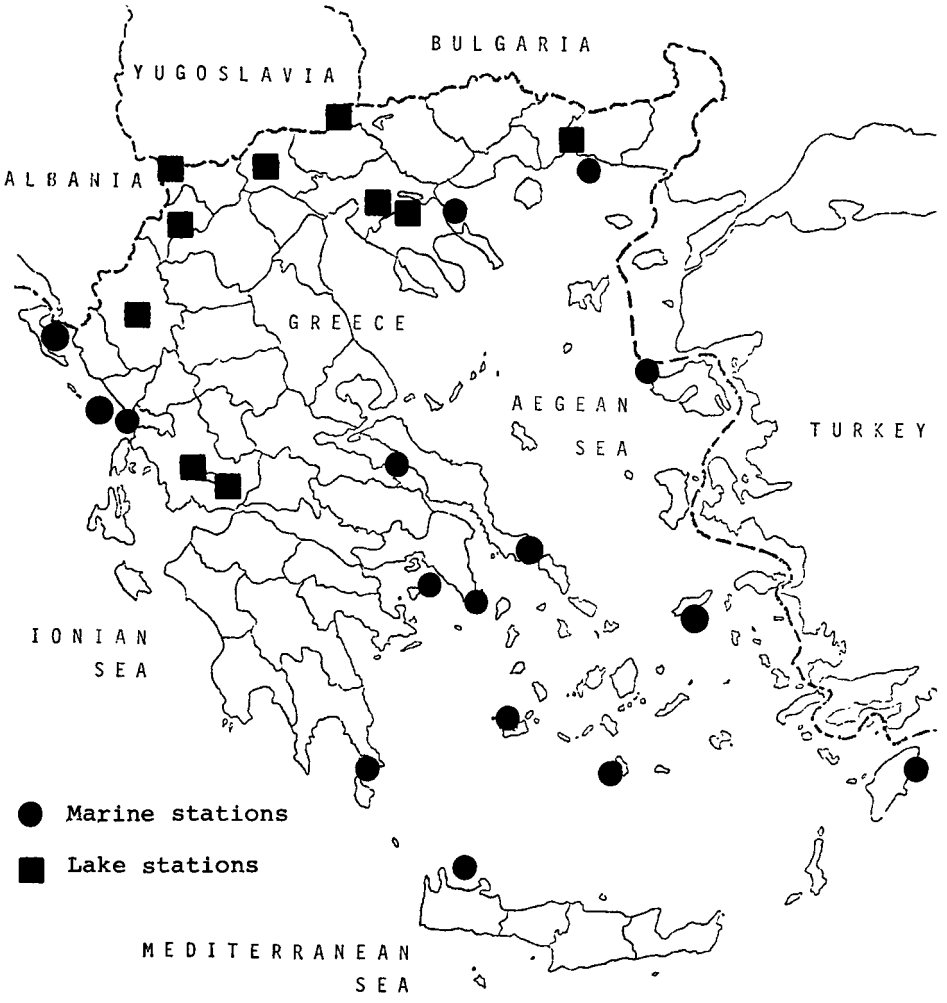

Fig.2. Studies of marine radioactivity: map of the sampling network. The lake sampling stations are shown as well.

been reported in (3,5,7,14). Concentrations of Ra226 up to 1 order of magnitude higher than the typical have been measured in certain algae samples, while a significant bio-accumulation of thorium by algae has been demonstrated by typical values for Th234 of 20-40 Bq/kg dry and maximum values of 150 Bq/kg dr. A special case of terrestrial influence has been studied in the island of Ikaria, where radon spa waters are continuously released to the sea. Maximum concentrations of 700 Bq/kg Ra226 and 5500 Bq/kg of Th234 have been measured in algae species collected close to the points of water release. The results have not been published yet.

2.8. The significant impact of the Chernobyl accident on the Greek environment created certain new priorities and resulted in new, non-planned studies. Especially:

a) More than 20000 samples (air, water, soil, deposition, foods) were analyzed by use of high-resolution gamma-spectrometry during the first year after the nuclear accident. The results served as the main basis for the evaluation of the radiological impact of the accident in Greece, as presented in the national reports (26) and (27).

b) The impact of the accident on the marine and lake environment has been studied separately and the results have been reported in (12,18,21,23). The contamination of the marine environment appeared to be of low radiological significance and the related doses to the population - small and transient. In contrary, total caesium concentrations up to 200 Bq/kg have been observed in certain lake fish samples during 1988 and 1989, indicating the possibility of prolonged influence of this pathway to the late population doses due to the accident.

c) The contamination of the Greek soils with radioactive caesium has been investigated. About 500 samples have been analyzed in order to obtain a relaible picture of the caesium deposition pattern in the country. The country average is approx. 9 kBq/m2, while the regional averages vary between less than 2 and 45 kBq/m2. Hot spots (areas of the order of 1 km2) with deposition up to 100 kBq/m2 have been observed. The results have been reported in (9,22). A large number of paired measurements - soils/plants - has been performed as well, in order to determine the extent of root uptake of caesium by plants of major dietary importance.The results indicate the low significance of this pathway for the long-term population dose (10,15,24).

d) We have provided one of the first reports on the observed unconventional form of "hot particles"(HP) in the Chernobyl pollutants (6). A more detailed investigation (11) included estimations of the probability for inhalation of HPs in the Athens region, based on the observed surface dencity of these particles in carefully selected un-disturbed areas. The average adult in Athens could have inhaled more than 5 HPs of initial activity in the range of 1-10 Bq, while certain individuals could suffer an internal exposure to HPs of more than 10 kBq of Ru103/Ru106.

3. Discussion.

3.1. Greece appears to be one of the European countries with the highest concentrations of Rn222 in thermal waters used in balneotherapy. The violation of the justification and ALARA principles during this "use" of ionizing radiations has been repeatedly pointed out in our publications, although the benefits from this practice (if related to radioactivity at all) seem to be confirmed by centuries of application.

3.2. The relatively low content of Ra226 in soil and the warm climat (high average ventilation rates) of the 3.5 millions Athens region are the reasons of the observed low concentra-

tions of Rn222 decay products in the indoor air. Higher values have been observed in Thessaloniki, while there are similar indications from other regions of Northern Greece. The use of track-etched detectors for a wider radon survey is planned to begin during 1990, but it seems that the Greek population will appear to be exposed to indoor radon in a less extent than the population of most of the E.C. countries.

3.3. The Greek building materials investigated so far do not create any concern from the radiological point of view. This is confirmed not only by the observed indoor radon levels, but also by the values of the exposition rate measured in parallel. There are nevertheless certain cases of mine areas where further investigation is necessary.

3.4. The results of the survey of natural radioactivity in Greek soils indicate that the country is characterized, generally, by "conventional" levels, as compared to the E.C. area.

3.5. The studies of natural and artificial radioactivity in the Greek marine environment have created a good reference base. It was used after May 1986 to evaluate the impact of the Chernobyl accident and can be used for any similar comparisons in the future. In addition, they enabled the selection of certain marine organisms (espacially algae) as suitable bioindicators for certain natural and artificial radionuclides.

3.6. The basic comments on the findings related to the impact of the Chernobyl accident are as follows:

a) The properties of the Greek soils favour the fixation of caesium and reduce its availability to the growing plants. The 1987 dose to the average Greek adult due to the root uptake pathway (including projections for meat and milk through the animal feedingstuffs) does not exceed 10 μSv.

b) The most important short-term pathways include the consumption of animal products, grain and pastry during the winter of 1986-87, i.e. reflect the contanination of the 1986 harvest.

c) The major late pathway is the external irradiation due to the radioactive caesium deposited in rural areas. The 50-years critical group dose from external irradiation approach 3000 μSv, while for the average Greek its value does not exceed 200 μSv.

d) The special concern about the "hot particles" is related to the fact that they lead to a type of internal exposure not comparable with any natural one (in fact this could be a reasonable radiological definition of the HPs). The collective dose due to inhalation of HPs seems to be relatively low (even if a "quality factor" of 10 is used with respect to the conventional form of radioactive materials), but the peak values of HP activities observed call for further research and special epidemiological attention.

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

1. The Marine Radioecology Laboratory, National Center of Marine Research, Hellinikon, Athens (Mrs. H. Florou).

2. The Soil Science Laboratory, Inst. of Biology, NCRPS "Democritos", Aghia Paraskevi, Athens (Dr. E. Papanicolaou).

3. The Radiation Dosimetry Institute, Chechoslovakian Academy of Sciences, Prague (Dr. F. Harvat).

V. Publications:

1. P. Kritidis and P. Angelou, "Recherche des Concentrations du Rn222 et de Ses Produits de Filiation dans les Sources Thermales de Loutraki et de Kamena Vourla", Proc. FITEC Congress 1985, Isthmia/Kam. Vourla (Greece), 23-30 Oct. 1985, p. 136.

2. P. Kritidis and P. Angelou, "Concentratios of Rn222 and its Short-lived Decay Products in a Number of Greek Radon Spas", Proc. III Intern. Conf. "Low Radioactivities 85", Bratislava (Chechoslovakia), 21-25 Oct. 1985, Nucl. Instr. and Methods in Phys. Res. B17, p.p. 537-539 (1986).

3. H. Florou and P. Kritidis, "Gamma Spectrometry in Marine Sediments and Organisms from a Mining Waste Deposition Area", Thalassographica 9, Fasc.2., p.p.23-28 (1986).

4. P. Kritidis, P. Angelou, S. Pournis and A. Tsiapali, "Investigation of Radiation Exposure in Greek radon Therapy Centers and in Athens Residences", Proc. IV Europ. Congress of IRPA, Salzburg (Austria), September 15-19, 1986, p.209-211.

5. H. Florou and P. Kritidis, "Natural Radioactivity in the Marine Environment of a Mining Waste Disposal Area", Rapp. Comm. int. Mer Medit. 30, 2 (1986).

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7. H. Florou, P. Panaiotidis et al., "Monitoring of Background Levels of Radioactivity in Selected Areas Around the Greek Peninsula", XXX CIESM Congress, Mallorka (Spain), October 20-25, 1986 (Special Chernobyl Session).

8. P. Kritidis and P. Angelou, "A Region of Lignite Ashes Depositions: Investigation of the Radiological Impact", Proc. XIV Regional IRPA Congr., Kupari (Yugoslavia), Sept.29-Oct.2, 1987, p.p.101-104.

9. 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 press).

10. 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. 2-4, 1987 (in Greek).

11. P. Kritidis, N, Katsaros and M. Probonas, "Hot Particles in Greece after the Chernobyl Accident. Estimations of the Inhalation Probability", Proc. Intern. Workshop "Hot Particles in the Chernobyl Fallout", Theuern (FRG), Oct.28-29, 1987, p.p.115-118.

12. H. Florou, P. Kritidis, S. Synetos and Ch. Chaloulou, "Aspects radioecologiques de l'influence par les polluants radioactives au milieu marin" (Grece) apres un accident nucleaire", SFRP-AIRP Congr., Rome (Italy), Oct.12-13, 1987 (in press).

13. P. Kritidis and M. Probonas, "The Greek Radon Spas: Hot Spots of Natural Radioactivity in the Mediterranean Area", Proc. Intern. Conf. "Environmental Radioactivity in the Mediterranean Area", Barcelona (Spain), May 10-13, 1988, p.p.53-59.

14. H. Florou and P. Kritidis, "Enhanced Natural Radioactivity in some Areas of Aegean Archipelago", Proc. Intern. Conf. "Environmental Radioactivity in the Mediterranean Area", Barcelona (Spain), May 10-13, 1988, p.p.93-99.

15. E. Papanicolaou and P. Kritidis, "Contamination of the Agricultural Land of Greece with Radioactive Caesium and its Effect on the Growing Crops", Intern. Conf. "Environmental Radioactivity in the Mediterranean Area", Barcelona (Spain) May 10-13, 1988, p.p.457-466.

16. P. Kritidis, "The Lessons of the Chernobyl Accident in Regard to the Environmental Radioactivity Control", Intern. Scient. Conf. of the Balkan Academies on Environmental Protection, Varna (Bulgaria), 19-23 Sept. 1988 (in press).

17. P. Kritidis, "Radioactive Balneological Spas and Radiation Protection Principles", II Nation. Conf. for the Thermometallic Waters, Thessaloniki (Greece), 7-9 Oct., 1988.

18. P. Kritidis, H. Florou and S. Synetos, "The Contribution of Fish Consumption to the Dose Received by the Greek Population due to the Chernobyl Accident", XXXI CIESM Congr., Athens (Greece), 17-22 Oct., 1988. To be published in "Thalassographica", Suppl. 1989.

19. P. Kritidis, "Radiological Characteristics of the Drinking Water", Proc. Nation. Conf. "The Drinking Water", Athens (Greece), 7-9 Febr., 1989 (in press).

20. P. Kritidis and H. Florou, "Natural Radioactivity and Radioactive Pollution of the Environment", Proc. Nation. Congress of Environmental Science and Technology, Mytilini (Greece), 4-7 Sept. 1989, Vol.B, p.p.24-34 (in Greek).

21. H. Florou, P. Kritidis and S. Synetos, "Comparative Study of Radiocaesium in Marine and Lake Fish", Proc. Nation. Congress of Environmental Science and Technology, Mytilini (Greece), 4-7 Sept. 1989, Vol.B. p.p.19-23 (in Greek).

22. P. Kritidis and E. Papanicolaou, "Delayed and Late Impact of the Chernobyl Accident on the Greek Environment", Proc. Intern. Radiation Protection Symposium, Dubrovnik (Yugoslavia), 2-6 Oct. 1989, p.p. 370-375.

23. H. Florou, P. Kritidis and S. Synetos, "Comparative Study of Caesium in Marine and Freshwater Fish Two Years after the Chernobyl Accident", Proc. Intern. Radiation Protection Symposium, Dubrovnik (Yugoslavia), 2-6 Oct. 1989, p.p.376-380.

24. C. Apostolakis et al., "A Study of Radioactive Caesium in Relation to Soil Properties in Greece", Proc. Intern. Workshop "The Transfer of Radionuclides in Natural and Semi-natural Environments", Udine (Italy), Sept. 1989 (in press).

Reports:

25. P. Kritidis, "Natural Radioactivity of the Indoor Air and Energy Saving Measures", Proc. Nation. Coll. "Research and Development in Energy", Athens, 3-4 April 1985, p.p.99-103 (in Greek).

26. "The Consequences of the Chernobyl Nuclear Accident in Greece", Report DEMO 86/4, p.p.6-17 and 50-80, Greek Atomic Energy Commission, 1986.

27. "The Consequences of the Chernobyl Nuclear Accident in Greece", Report DEMO 86/9, p.p.2-4 and 15-21; Greek Atomic Energy Commission, 1986.

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-105-F

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

Dr. J. Lochard
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B.P. n° 48
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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.:
Occupational radiological protection optimization in the nuclear fuel cycle and in medical applications. (BI6-105-F, Part 1)

Head(s) of project:

J. Lochard - CEPN - BP 48 - 92263 Fontenay-aux-Roses, Cedex - France

Scientific staff:
M. Benedittini
C. Lefaure
J. Lochard
C. Maccia

I. Objectives of the project:

The overall objective of the project was to develop case studies of optimization for occupational radiological protection in the nuclear fuel cycle and in the domain of medical activities. Two aspects were considered with a particular attention : the role of work management actions within dose reduction policies and the integration of dose distribution in the optimization process.

II. Objectives for the reporting period:

Since this is the final contract report, these are identical to the objectives of the project itself, described above.

III. Progress achieved:

1. Background

The project has been implemented in close relation with the CEPN-NRPB joint project on "methods for the practical implementation of the ALARA principle" with the objective of identifying, at the level of ALARA applications, the areas where progress had to be made to facilitate the diffusion of the principle for both the design and the operation of installations.

At the beginning, the intention was to develop case studies in parallel in various installations of the nuclear fuel cycle and in the field of medical activities. In the course of the project because of difficulties to have access to the pertinent data, especially in the medical field, most of the efforts have been refocussed on occupational dose control at nuclear power stations and especially pressurised water reactors (PWRs).

Retrospectively, the question of having access to adequate data to work with, appears to be, apart from some methodological obstacles, the main issue in the implementation of the optimization principle. Having quite early recognized this point, a large part of the work has been devoted to the compilation of occupational radiation dose statistics from PWRs in France and in others countries. Beside an explicitation of the general trends about collective and individual exposures, the analysis has demonstrated that synthetic indicators are of poor use for identifying areas for potential ALARA investigations. As a complement, in depth analysis of particular maintenance works in some reactors have been performed to delineate the driving parameters of collective and individual occupational exposures at the level of specific operations, in an ALARA perspective. As a consequence, during the last phase of the project, attention has been given to the design of specific job dosimetry systems to collect the key informations to implement ALARA i.e. ambient dose rate and job time associated to all elementary tasks to be performed in the course of an operation to be evaluated, as well as data on the working conditions.

Parallel to this investigation on exposure data, in conformity with the objectives of the project, case studies have been designed to adress the potential of work management actions for dose reduction with a particular attention to the impact on individual dose distributions. As a matter of fact, the first generation of ALARA studies in the field of occupationnal exposures at nuclear power plants was mainly focussed on evaluating actions aiming at reducing or protecting sources, reflecting the emphasize put on this aspect of dose control by engineers. At the time the project started it was already obvious that large efforts had been done in the direction of source reduction and that further improvements were not really

cost-effective compared with potential dose saving related to working time reduction. However, it was also stated that quantifying work management actions was certainly beyond the capabilities of the ALARA procedure. In this context the question of the role of robotics and remote control to perform repetitive operations in nuclear installations became an important issue. Due to the large investments requested to develop and implement these techniques, many utilities were reluctant to start with large programmes unless it was possible to demonstrate quantitatively the potential for significant dose savings both in terms of collective and individual doses. The last phase of the project has been mainly devoted to the performance of case studies aiming an evaluating robotic actions in an ALARA perspective.

2. Results

2.1 Occupational exposure data collection

The compilation performed during the project includes collective and individual exposures at PWRs, for the period 1975-1988, in eight countries : Belgium, Federal Republic of Germany, Finland, France, Japan, Sweden, Switzerland, United-States. Only reactors in commercial operation since july 1974 are included in the analysis. At the end of 1989, all together the data base comprizes a total number of reactors of 157 representing 1352 reactor-years.

Globally the mean annual colective dose per reactor is decreasing over the years to reach 2.42 man-Sv in 1988. The most important reduction concerns the US reactors (46 % decrease since 1982) and those of the Federal Republic of Germany (49 % decrease since 1981). This general trend is closely related to the dose reduction programmes adopted in most countries, combined with the positive impact of the past experience. However, despite this tendancy, one may note a small increase of the collective occupational exposures for four out of the eight countries during the last perod. The gap between lower and higher values is also continuously reducing to reach respectively 0.88 man-Sv in Finland and 3.08 man-Sv in the US in 1988 (factor of 3.5). Excluding Finland, the difference is reduced to a factor 2. These results show a clear tendency towards an uniformization of practices and operations.

Annual collective doses presented as a function of the number of operating years confirm previous results : for all units, one may notes a first period with a collective dose increase, followed by a relative stability or even a reduction, and then for some of them by a second

increase. In 1988, the averge annual collective dose per reactor for all PWRs is about 1.27 man-Sv after one operating year, 2.58 man-Sv after three years, and respectively 4.16, 3.86 and 3.96 man-Sv after seven, ten and fourteen years.

The analysis of collective doses per reactor according the startup date and the electrical output confirms that for a same generation of reactors, the higher the electrical output is, the higher collective exposures are, and for a given electrical output, values are smaller for units put in commercial operation lately. For the three generations of reactors (74-80 ; 80-84 ; 84-88) the evolution of the collective dose per unit is very similar, but values are firmly decreasing for the younger generations.

As far as mean individual doses per worker are concerned, results have to be interpreted carefully since outside personnel data are given per nuclear power plant and never for the whole country, and significant differences exist between countries in the way of counting the number of workers. The average annual individual dose by plant is 3.3 mSv in 1988.

2.2 Development of a new dosimetry system

The classical dose monitoring systems in use in nuclear power stations allow to perform analysis as presented above but are inappropriate to serve the purposes of the ALARA approach as no links can be reasonably and reliably established between the collective or individual doses and the exposure conditions in which the different elementary tasks are performed during an operation. As a matter of fact, without a good knowledge of ambiant dose rates, job times and conditions of work, it is practically impossible to interpret the evolution of doses from one operation to the next one, and even more difficult to identify on which parameters to play in order to reduce effectively the doses if necessary.

Having recognized these limitations, it was decided to devote efforts to delineate the basic principles and features of a dosimetry system fitting with the ALARA needs. To differentiate such a system from those focussing only on the monitoring of individual doses, either on a monthly, daily or even shorter basis, the denomination of analytical dosimetry has been adopted, by analogy with the distinction made in the French accounting system between general (or financial) accounting and analytical (or management) accounting, where the first one aims at establishing final results and the second one allows to explain how these results are obtained.

Based on the first developments, Electricité de France and Framatome, have asked CEPN in 1987 to adapt this analytical dosimetry for reactors and to design a computed based support systems for the engineers, technicians and health physicists involved in the preparation and the accomplishment of maintenance or backfitting works, with the objective of maintaining occupational exposures ALARA. As a result, CEPN developed the DOSI-ANA microcomputer software which is now currently in use in French PWR's, for planning, follow up and evaluating operations in an occupational dose control perspective.

2.3 Work management

A large part of the project has been devoted to the analysis of past experience with occupational exposure related to maintenance operations in French reactors to identify the main work management parameters influencing collective and individual dose evolution and the protection actions to be developed to reduce further exposures. A study, developed in close cooperation with Electricité de France, on the dosimetric evolution of the shot-peening operations performed in 1985 and 1986 in many reactors to prevent steam generator tubes from deterioration, allowed to identify some key elements to controll doses such as :

- the integration of radiation protection considerations at the design and planning level of operations,
- the preparation of operation (tunning of equipements, training of operators, coordination between power station and contractor staffs),
- the follow-up of operation to implement immediate corrective actions,
- the analysis of past experience for feedback,
- the planning of corrective actions when adopted.

The analysis showed also that the reduction of collective doses with time, resulting from the impact of both corrective actions and the "learning effect" was accompanied by a concomitant decrease of the highest individual doses within the dose distributions.

2.4 Robotics and remote control

Within work management actions aiming at controlling exposures, the use of robotics and remote control appears "a priori" to be of particular interest to reduce working time in areas with high dose rates. However, the use of robotics is not straightforward. First investments are costly, and secondly the installation of robots or tools is also dose consuming. In such a context, decisions about the use of robotics in an ALARA perspective, imply to answer two

main questions : 1) under which conditions is it dosimetrically justified to replace workers by robots ? and 2) what is the optimal use of robot ? Past experience analysis, followed by prospective studies have been performed in collaboration with the French nuclear industry (EDF, COGEMA and FRAMATOME) to first develop a methodology and then to test it in view to answer to the above questions.

As a first result, it was demonstrated that the use of robotics generally reduce both collective and individual exposures. The analysis of the evolution of steam generator tube plugging techniques in France has shown that the mean collective dose per plug fixed, which was 0.82 mSv in 1981 when the operation was fully manual, decreased to 0.25 mSv in 1987 after having introduced automatic tools. During the same period individual doses were divided by 3 for the most exposed workers.

Furthermore, economical analysis of various operations has also demonstrated that the introduction of remote toolings and robotics may also result in operating cost savings because of the possible decrease of on-site-acess-costs, training costs, wages, protective suits costs (including conditionning and storing of contaminated suits) related to the reduction of the number of workers, as well as the decrease of other cosumables. In many cases these savings outweight the increase of costs associated to robot drivers and maintenance specialised workers.

With regard to the evaluation of robotic actions, a set of management concepts have been elaborated as, for example, the dosimetric break-even point and the operating cost break-even point which allow to identify the minimum conditions of use of robots or remote-tooling ensuring first a reduction of the collective exposure and secondly a reduction in operating costs. For example, to illustre these last points, it has been demonstrated that in the case of the tube plugging operations already mentionned, taking into account the current technique, the dosimetric break-even point is for about 30 plugs and the operating costs break-even point is for about 80 plugs.

3 - Conclusions

Stated in general terms, the main results of the project can be summurized as follows :

- Statistics on occupational exposures generally available in nuclear installations are not adapted to perform ALARA studies. A new generation of dosimetry systems allowing the

link between elementary tasks performed by workers and corresponding exposures needs to be developed.

- Beside actions to directly reduce sources or increasing protections to decrease ambiant dose rates, there is a large potential for dose savings through work management actions which can reduce significantly exposed working time in controlled areas. First attempts to quantify such actions in an ALARA perspective have been successfull and it is worthwile to continue in this field in the future to enlarge the integration of the optimisation principle within the operating and maintenance procedures for nuclear installations.

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

V. Publications:

/1/ LOCHARD J., BENEDITTINI M. - Expositions professionnelles dans les réacteurs à eau pressurisée : comparaison internationale de quelques indicateurs globaux entre 1975 et 1985. Rapport CEPN-R-103, Septembre 1987.

/2/ LOCHARD J., BENEDITTINI M. - Expositions professionnelles dans les réacteurs à eau pressurisée : comparaison internationale de quelques indicateurs globaux entre 1975 et 1986. Rapport CEPN-R-132, Mars 1988.

/3/ LOCHARD J., BENEDITTINI M. - Expositions professionnelles dans les réacteurs à eau pressurisée : comparaison internationale de quelques indicateurs globaux entre 1975 et 1987. Rapport CEPN-R-145, Mars 1989.

/4/ BENEDITTINI M., TABARE M. - Expositions professionnelles dans les réacteurs à eau pressurisée : comparaison internationale de quelques indicateurs globaux entre 1975 et 1988. Rapport CEPN-R-164, Décembre 1989.

/5/ LOCHARD J., LEFAURE C. - Caractéristiques et principes de fonctionnement d'un système informatisé de dosimétrie analytique pour les grands chantiers de maintenance. Rapport CEPN-R-119, Septembre 1987.

/6/ LOCHARD J., LEFAURE C. - DOSI-ANA : A microcomputer software for Occupational Dose Management at NPPs. In : New Developments in Occupational Dose Control and ALARA Implementation at Nuclear Power Plants and similar Facilities, Workshop, Brookhaven National Laboratory, Upton, New-York, September 18-21, 1989.

/7/ LOCHARD J. - L'évaluation technico-économique de la protection radiologique des travailleurs. Revue Générale Nucléaire, n°6, 1986, pp. 511-520.

/8/ LOCHARD J., PAGES P., FAGNANI F., HALLER P., BLAIN M., BREGEON B. - 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.

/9/ LOCHARD J. - Cost-effectiveness of Robotics and Remote Tooling for Occupational Risk Reduction at Nuclear Facilities. In : Proceedings of an International (AIEA/CCE/OCDE/AEN). Conference on Man-Machine Interface in the Nuclear Industry (Control and Instrumentation, Robotics and Artificial Intelligence). Tokyo, Japan, 15-19 February 1988, pp. 725-736.

/10/ LEFAURE C., LOCHARD J., BLAIN A. - Evaluating Remote Control and Robotics Actions in NPPs in an ALARA Perspective : Lessons from the Evolutions of Steam Generator Tube Plugging Technique in France. In : In : Radiation Protection Optimization 'Advances in Practical Implementation', Proceedings of the Third European Scientific Seminar held in Madrid 12-14 September 1988, Commission of the European Communities, 1989.

/11/ FEYLESSOUFI A., LOCHARD J., LEFAURE C. - A Model for Evaluating Robotics and Remote Tooling in Nuclear Installations. Radiation Protection - Theory and Practice. Session 5. 4th Int. Symp., Malvern, June 1989.

/12/ LEFAURE C., LOCHARD J., SEGUY J. - An ALARA approach for robotics and remote tooling evaluation in nuclear power plants. International Workshop on new developments in occupational Dose Control and ALARA Implementation at NPPs and Similar Facilities. Brookhaven National Laboratory NY. 11973, September 18, 21 1989.

/13/ LEFAURE C., LOCHARD J., BLAIN A. - Weighing up the costs and benefits of remote maintenance. Nuclear Engineering Internal, June 1989.

Title of the project no.:

Methods for the practical implementation of the ALARA principle (BI6-105-F, Part 2)

Head(s) of project:

J. Lombard - CEPN - BP 48 - 92263 Fontenay-aux-Roses, Cedex - France

Scientific staff:

P. Hubert
J. Lochard
J. Lombard
C. Maccia
A. Oudiz
P. Pagès

I. Objectives of the project:

These were formally stated as follows :

(1) To review the difficulties that have arisen in the practical implementation of ALARA;

(2) To suggest methods whereby these difficulties may be resolved;

(3) To develop a simple structural framework for future ALARA studies;

(4) To demonstrate the use of this framework by applying it in example studies.

II. Objectives for the reporting period:

Since this is the final contract report, these are identical to the objectives of the project itself, listed above.

III. Progress achieved:

1. Background

The second European Scientific Seminar on the Optimisation of Radiological Protection which was held in Luxembourg on 8th and 9th November 1983 explicitly identified a need for clearer guidance on the implementation of the ALARA principle in practical radiation protection programmes. As a consequence, the Centre d'étude sur l'Evaluation de la Protection dans le domaine Nucléaire (CEPN, France), and the National Radiological Protection Board (NRPB, UK) decided to joint their effort to initiate and develop a project with the formal objectives listed above. It was clear from the beginning of the project that, in order to meet for these objectives, it would be necessary to combine expertise on the theoretical aspects of optimisation techniques with the experience of practical radiation protection problems. Appropriate staffs were identified within CEPN and NRPB to cover both aspects of the project with NRPB dealing more specifically with the industrial and medical fields, whilst CEPN because of its institutional background (CEA/EDF), concentrated on applications in the nuclear fuel cycle. The whole range of activities of radiation protection was thus covered.

By means of a series of meetings and annual correspondence between technical staffs a general framework for performing optimisation studies was progressively developed. At the same time each organisation was identifing radiation protection problems that could be used to test the applicability of the general framework and use the experience gained to refine this framework. In addition, it was agreed to review other published ALARA studies to check that they, too, could have been approached from the perspective of applying the general framework.

By the end of the project, it was recognized that to facilitate the implementation of the ALARA concept into radiation protection programmes a document summurizing, in language appropriate to the practitioner, the ideas behind the ALARA principle and describing the practical measures for implementing it together with examples from real situations would be appreciated. It was then agreed to prepare such a document based on all facets of the work performed during the course of the project.

2. Results

Following a review of the concepts underlying ALARA and from detailed consideration of the role that ALARA studies should play in aiding decisions on radiation protection matters,

a general framework for applying the ALARA principle to practical radiation protection problems has been developed. This framework was given the name the "ALARA Procedure", and a schematic diagram (see Figure 1) illustrates its composition. It provides a simple structured approach that can be applied to any problem involving optimisation of radiation protection. CEPN staff has applied the ALARA procedure to several case studies addressing practical radiation protection problems, including all kinds of decisional problems (routine and accidental exposures to the public or occupational, long term considerations). The structured approach, in all cases, provided a way of thinking that assisted and clarified the decision-making process. From this work, many scientific papers were published. Some of the most important are listed at the end of this report. They provide detailed account of the work performed on the application of the ALARA procedure to optimisation problems.

The most visible result is the synthetic document prepared at the end of the project that explains the ideas behind the ALARA principle, the ALARA procedure, and measures for implementing them into radiation protection programmes. Entitled "ALARA - from theory to practice", the document is written in language that makes it accessible to radiation protection practitioners and is supplemented with practical examples to illustrate the points being made. Many of these examples are from the case studies performed in the course of the project. The table of content of this document is included hereafter in order to facilitate an appreciation of the topics covered. It is hoped that this will be a useful addition to the literature and, more importantly, be read extensively.

Apart from this final report, the two CEPN and NRPB staffs have organized in cooperation with the CEC Ispra center, two one week seminars on the implementation of ALARA (17-21 June 1985 and 22-26 September 1986), based on the material resulting from the project with lecturers originating from CEPN and NRPB mainly.

Furthermore, staff from NRPB and CEPN were also heavely involved in the organisation of the Third European Scientific Seminar on Radiation Protection Optimisation organised by CEC in Madrid from 12th to 14th September 1988 which provided a form for the presentation of the work carried out under the project. Some 30 % of the papers that presented were prepared by CEPN and NRPB. Subsequent discussions and comments on these papers were taken into account in producing the final version of the document on ALARA described above.

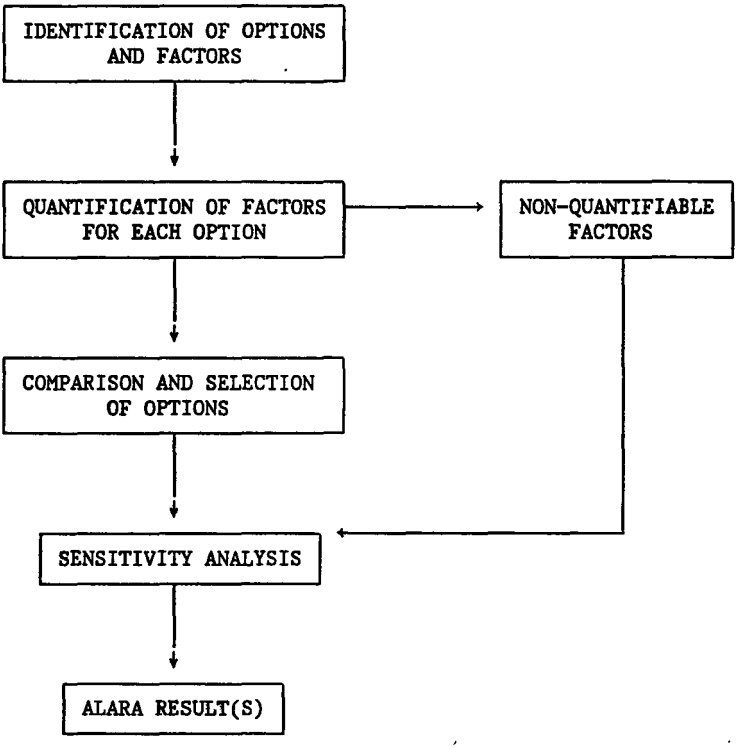

Figure 1 – The steps of the ALARA procedure

Finally, it should be noted that the developments made during the course of this project have fed into the work of the ICRP Task Group set up in 1984, which produced ICRP Publication 55 - *Optimisation and Decision-making in Radiological Protection.*

3. Conclusions

Several general comments can be made about the state of development of the work on optimisation of protection. It is now clear that the ALARA principle is a workable concept and that tools for structuring optimisation problems and aiding decisions, such as the ALARA Procedure, exist and have been shown to work in practice. It is also clear that a structured approach can be applied to all levels of radiation protection problems, but that the ressources allocated to each problem need to be commensurate with the level of decision involved. For example, in many cases of low-level decision, intuitive thinking by experts coupled with ALARA awareness may be all that is necessary. At high levels of investment, an extensive desk-top ALARA study involving several man-months of effort may be needed. It is also clear that methods for incorporating these ideas into radiation protection programmes (eg. ALARA audits and ALARA predictive plans) exist, and again have been demonstrated to work in practice.

What is required now is that these ideas, principles, tools, and methods be actually used widely in radiation protection programmes. The document produced on ALARA is an important step in this, but there is additionally a need to provide manuals of good practice, training on the implementation of these methods, and simple computer tools to aid decision-making as well as guidance on the application of these methods to more complex problems. It has also been recognised that there is a lack of relevant data on dose-saving techniques and associated costs for detailed application of quantitative methods to operations warranting them. Future work is needed to address these specific areas.

Contents of the final report "ALARA - From Theory to Practice"

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

National Radiological Protection Board (NRPB)

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United Kingdom

V. Publications:

/1/ LOMBARD J., OUDIZ A., ZETTWOOG P. - A contribution to optimizing radiological protection in a u-mine. Health Physics, Vol. 50, n° 4, 1986, pp. 473-483.

/2/ RINGOT C., TOMACHEVSKY E., PAGES P., HUBERT P. - Application au transport des matières radioactives de la méthode d'évaluation du risque pour l'aide à la décision. In : Optimization of Radiation Protection, Proceedings of a Symposium, Vienna, 1986, pp. 389-403.

/3/ LOMBARD J. - Comment tenir compte de la distribution temporelle du détriment radiologique ? In : Optimization of Radiation Protection, Proceedings of a Symposium, Vienna, 10-14 March 1986, Jointly organized by IAEA-NEA (OECD), IAEA Vienna 1986, pp. 529-540.

/4/ LOMBARD J. - Les méthodes d'évaluation de la valeur monétaire de l'homme-sievert. In : Optimization of radiation Protection, Proceedings of a Symposium, Vienna, 10-14 March 1986, Jointly organized by IAEA-NEA (OECD), IAEA Vienna 1986, pp. 499-518.

/5/ HUBERT P., PAGES P., RINGOT C., TOMACHEVSKY E., HAMARD J. - Assessing Costs and Effectiveness of Safety Measures for the Transit of Small Type A Packages through Road Tunnels. In : Packaging and Transportation of Radioactive Materials (PATRAM' 86), Proceedings of an International Symposium organized by the IAEA, Davos Switzerland 16-20 June 1986, IAEA Vienna 1987, pp. 513-521.

/6/ WEBB G.A.M., OUDIZ A., LOCHARD J., LOMBARD J., CROFT J.R., FLEISHMAN A.B. - Development of a General Framework for the Practical Implementation of ALARA. In : Optimization of Radiation Protection, Proceedings of a Symposium, Vienna, 10-14 March 1986, Jointly organized by IAEA-NEA (OECD), IAEA Vienna 1986, pp. 123-136.

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/8/ MACCIA C. - L'évaluation de l'exposition professionnelle dans le domaine du radiodiagnostic médical. Application du principe ALARA. In : Actes du XIVème Congrès ATSR (Association pour les Techniques et les Sciences de radioprotection), Paris, 26-28 novembre 1986, 18 p.

/9/ JAMMET H., LOMBARD J. - Towards a general Model of Health Detriment Cost Evaluation. Health Physics, Vol. 52, N° 1, 1987, pp. 91-101.

/10/ LOMBARD J., COULON R., DESPRES A. - An ALARA Approach of the Radiological Control of Foodstuffs following an Accident Release. Risk Analysis, Vol. 8, n° 2, 1988, pp. 283-290.

/11/ WEBB G.A.M., LOMBARD J. - Decision-Aiding Techniques for Radiological Protection. In : IAEA-CN-1, International Conference on radiation Protection in Nuclear Energy, Sydney, Australia, 18-22 April 1988, 9p.

/12/ CROFT J.R., LOCHARD J. - Status of achievements reached in applying optimisation of protection in design and normal operation of reactors. The Application of Optimisation of Protection in Regulation and Practice. Proceedings of an NEA meeting, 16-18 March 1988. Paris.

/13/ LOCHARD J., CROFT J.R. - Key Issues in the Implementation of ALARA in Operation. British Journal of Radiation Protection, Vol. 8, n° 2, 1988, pp. 87-96.

/14/ VANO E., GONZALEZ L., MACCIA C., WALL B.F. - ALARA and Radiodiagnostics. In : Radiation Protection Optimization 'Advances in Practical Implementation', Proceedings of the Third European Scientific Seminar held in Madrid 12-14 September 1988, Commission of the European Communities, 1989.

/15/ SAUNDERS P.J., CROFT J.R., WRIXON A.D., LOMBARD J., LOCHARD J. - Methods for the Practical Implementation of the ALARA Principle : The CEPN/NRPB Joint Project. In : Radiation Protection Optimization 'Advances in Practical Implementation', Proceedings of the Third European Scientific Seminar held in Madrid 12-14 September 1988, Commission of the European Communities, 1989.

/16/ LOMBARD J., HUBERT P., PAGES P. - ALARA and Waste Disposal. In : Radiation Protection Optimization 'Advances in Practical Implementation', Proceedings of the Third European Scientific Seminar held in Madrid 12-14 September 1988, Commission of the European Communities, 1989.

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-207-F

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

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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 industrial activities.

Head(s) of project:

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Scientific staff:

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F. Fagnani	C. Le Galès	P. Pagès
F. Farhi	J. Lombard	T. Schneider

I. Objectives of the project:

These were formally stated as follows :

(1) To put into perspective the methodologies aiming at the management of radiological risk and other industrial risks.

(2) To demonstrate the methodological convergences of the two fields, based on case studies in the non radiological domain.

The initial intention was to apply the methodological structure currently developed in the radiological field under the name of ALARA, to various non-radiological risk sectors with particular emphasize on chemical carcinogens and hazardous materials transportation.

II. Objectives for the reporting period:

Since this is the final contract report, these are identical to the objectives of the project itself, listed above.

III. Progress achieved:

1. Background

The main objective of the project was to compare the methodologies for risk management applied to nuclear and non nuclear activities, and to point out methodological convergences in the two fields. At the time the project started, the practicability and the efficiency of the ALARA (As Low AS Reasonably Achievable) approach had been recognized in the nuclear industry and it was logical to look at the potential for ALARA developments in the non-nuclear industry.

Apart from the specific studies realized under the present contract agreement, the project has benefited from other works performed at CEPN in parallel during the period like the NRPB-CEPN project on "Methods for the practical implementation of the ALARA principle" also supported by the CEC or, prior to the contract, studies on VCM and asbestos risk management.

The non-nuclear case studies have been performed in two main areas. First, the management of carcinogenic risks in the industry, where the nature of the risk (cancer induction), the environment (industrial facility) and the exposed populations (public, workers) combined themselves in a management situation which is very close from the radiation protection one in nuclear installations. Second, the management of the transportation of hazardous substances where pricing human life in safety investment decisions is a common practice, at least in France, which has some evident similarity with the ALARA approach. Moreover, the transportation of hazardous material was a good example to confront the ALARA framework to probabilistic events situations and to use models developed at CEPN to assess the risks associated with the transportation of radioactive materials.

Both methodological studies and case-studies have been conducted. Tables I and II below present lists of the works performed during the 1986-1989 contract period with corresponding references. Quite often methodological advances are embodied in the reports on case studies.

Table I : Methodological studies

Area	Subject	Publication
Risk assesment	- Carcinogenic potency ranking	Report [Ou88b]
	- Evaluation of risk (dose response) prediction methods	Thesis [Bo88a]
	- Statistical strategies for the search of TLVs	Paper [Ab88]
Risk management issues	- Economic analysis in carcinogen protection policies	Paper [Sc88]
	- TD50 and TLVs for carcinogenic substances	Paper [Bo88b]
	- Comparison of health significance of TLVs in different industries	Paper [Hu88]
Assessment of performances	- Decision aiding techniques applied to major hazards	Report [Lo89]
Overall survey	- Risk assessment and management for carcinogenic substances	Book [Ou88a]

Table II - Case studies

Main publication	Subject	Risk Identification	System's analysis	Risk Management	Performance Assessment	Cost effectiveness	Valuation consequences
Report [Lo88]	Nickel	x	x		x		
Report [Ou88c]	Benzene	x	x	x	x	(x)	
Report [Bo90] Report [Ba89]	PCB and 2.3.7.8 TCDD	x	x	x			
Report [Ou87b]	Chromium	x	x	x	x	(x)	
Report [Ou87b]	Acrylonitrile	x	x	x	x	x	
Report [De87] Report [Ez89]	Hazardous material routing in Lyon	x	x	x	x	x	x
Report [Br88]	Hazardous material risk in Grenoble	x	x	x	x		

2. Methodology

Industrial system's analysis : This aspect has been more specifically addressed in the nickel study [LG88]. Although the benzene study seems to be the most complex from this point of view [Ou88c], a great deal of effort has been spent on the subject in every case study. For hazardous material transportation, a standardized approach has been set up for the different case studies [De87][Br88].

Risk assessment : This step is usually quite simple when applying ALARA to radiation protection. For potentially carcinogenic substances, the question is much more complex. Fitting a dose response relationship has been dealt with in the nickel study (more precicely on some nickel compounds)[LG88], the chromium study [Ou87a] and the PCB study (for Dioxine) [Bo90]. The question here is the selection of a dose response relationship that can be of practical use in risk assessment and management, and that is infered from animal experiments or epidemiological surveys. The evaluation of risk prediction methods (eg. one hit, multihit, multistage, weibull, probit, logit) has been performed and applied on the TCDD 2.3.7.8 [Bo88a]. Also a methodology for assessing the carcinogenic potency from the TD 50 (the Toxic Dose 50 : a proposal of Peto, Pikes and Ames) has been looked after [Ou88a] and other strategies have been reviewed [Ab88]. The methodology of PRA applied to spatial analysis has been developed in the Lyons study [De87] and extended to environmental effects other than human health impact in the Grenoble study [Br88].

Risk management : This question has been specifically addressed when comparing the health significance of various Threshold Limit Values (TLVs) [Hu88] or looking at the practicability of grounding TLVs on TD 50 [Bo88b]. The introduction of economic analysis in the definition of protection policies against carcinogenic substances has also been discussed [Sc88]. For hazardous material transportation as the risk of accident is generally managed locally, the analysis have been performed on a geographical basis rather than on a sectorial one.

Assessment of performance : Methodological developments were mainly dealt with in the transportation studies with the adaptation of classic decision aiding tools to this complex decision making situation [Lo89].

Valuation of consequences : This question was not a major issue for carcinogens [Ou88a], but a key one in the transportation studies because of the heterogenety of detriments and the major hazard dimension [Hu89].

3. Results

The exploration of practices in non nuclear industries has revealed that optimisation is obviously not currently used outside the nuclear industry. As a philosophy it is sometimes mentioned about the definition of regulatory limits in the industry. For example regulations are often more stringent for new plants because design-based protection measures are likely to be more cost-effective (which is not always true - see table III). Also such an optimisation principle is mentioned in determining TLVs for non threshold products (cf [Ou88a]). However no practical applications have been observed in day to day protection, contrarily to what can be observed in protection against noise (cf [Sc89]).

However in the transportation risk area, cost-benefit is a rather familiar approach. In France the valuation of human life has been recommended in the early seventies and then applied as a "decision aiding indicator" for road safety. However this approach has never been applied for major hazards such as those associated with hazardous material transportations.

Having recognized this major discrepancy between nuclear and non nuclear activities, the project was basically focused on a comparison of implicit values of human life in various sectors including those resulting from previous works performed at CEPN on behalf of the CEC. The results (see Table III), do confirm a large similitude beween nuclear and non nuclear activities (see [Lo84]). Moreover many protection measures have been found to be cost saving.

Table III - Implicit values of human life

1. Implemented measures

Industries	Fields	Population at risk	Applications	(MF/h.l)	Reference
TR	Acc	P	Rerouting Hazardous Materials Shipments	2.6	De87
CH	Carc	O	Acrylonitrile	0.7	Ou87b
NU	Carc	O	Shieldings	11.0	Je84
NU	Carc	O	Design modification	135.0	Je84
NU	Carc	O	Mockup training	105.0	Je84
CH	Carc	P	New PVC plant	[2450-29580]	Ja83
CH	Carc	P	New MVC plant	[1940-23480]	Ja83
CH	Carc	P	Old PVC plant	[3-37]	Ja83
NU	Carc	P	R. effluents control systems	280.0	Lo82
NU	Carc	O	Uranium Mine	10.2	Ou83
H	Carc	O	Radiology Department	6.8	Ma82
CI	Carc	O	Asbestos (France)	[2.7-7.5]	Ro80
CI	Carc	O	Asbestos (UK)	[27.3-7.53]	Ro80
NU	Acc	P	R.Emergency Case Cooling System	0.35	Hu84
NU	Acc	P	R. Containment	11.0	Hu84

2. *Rejected measures*

Industries	Fields	Population at risk	Applications	(MF/h.l)	Reference
CH	Carc	P	Benzène (car)	500.0	Ou88c
TR	Acc	P	Chlorine	85.0	Hu84

Comments

Industries : *TR = transport ; CH = chemical industry ; NU = nuclear fuel cycle ; H = hospital ; CI = conventional industry*

Field : *Acc = accidental risk ; Carc = carcinogene, normal operation*

Population at risk : *P = public risk ; O = occupational risk*

Applications : *R = reactor ; SG = steam generator ;*

Values : ** = measure reducing both cost and risk*

MF/h.l : *Million French Francs per human life*

To enlarge the perspective to more general risk management rules, a comparison of the health significance of TLVs with the 0.05 Sv limit adopted in the radiological field have been performed. Results in Table IV shows that the differences are not so large, and that the radiological limit is not, as is often stated, more stringent than average except may be in the case of Monomere Vinyl Chloride.

Table IV - Risks associated with one year of exposure at the TLV (from [Hu88]).

Carcinogen	Annual TLV	Risk	Equivalent dose
Radiations	50 mSv	6.25 10-4	50 mSv
Acrylonitrile	2 ppm	14.26 10-4	114 mSv
Asbestos	1 fcc	[6.25 - 15.75] 10-4	[50 - 126] mSv
Benzene	5 ppm	[0.17 - 24.3] 10-4	[1.4 - 194] mSv
MVC	1 ppm	[0.08 - 0.96] 10-4	[0.64 - 7.68] mSv

For ionizing radiations, assessment of the effects is performed with the explicit purpose of protection on the basis of some consensus reached by scientific committees (US Academy of Science, UNSCEAR, ICRP...). Such an explicit decisionnal goal is not present for other carcinogens, nor are at work such institutions. IARC and EPA do not play in the scientific community nor with respect to the industry, a role that is comparable to the one of the previously mentioned institutions.

In the chemical field the emphasize is put on the establishment of TLVs and, should an ALARA philosophy be advocated for, it is at this level and not at the level of putting protection in operation. Both the linear hypothesis (or linear quadratic, which is quite close when fitted on present data sets) and the no threshold hypothesis are not accepted as a working basis. As a result, most of the discussion focusses on these matters while the quantification of the dose response relationship is secondary. Nowhere monitoring systems have been found that are comparable to the nuclear industry so that it is a task per se to identify exposed workers. Once workers at risk are identified, dose assessment results only

from specific surveys and modelling because no monitoring is performed except sometimes for the measurement of body burdens. The project allowed to develop a methodology to facilitate these tasks but nevertheless they remain time consuming.
For the transportation of hazardous materials, the ALARA procedure can be applied quite similarly to the nuclear field. The question of data collection is very similar, whatever the hazard and the modeling for risk assessment is often the same. The use of CCDF for the representation of a major hazard risk and the comparison of various safety strategies has been adopted for transportation studies with succes.

Finally, the main results can be summarized as follows :

- Formally the ALARA approach can be applied in the non nuclear field and it provides fruitfull informations.

- The content of an ALARA study is however quite different outside the radiation protection domain. A large amount of work is to be devoted to the first steps of the procedure i.e. risk and system identification.

- The risk management principles and practices are so different for carcinogenic substances that the meaningfullness of optimisation studies is not the same. Comparison, such as cost-effectiveness ratios are therefore difficult to interpret. The use of optimisation in installations is still a rather theoretical possibility in the general industry. However, first attempts in the case of deafness prevention, and to some extent, occupational accidents, have demonstrated possibilities of convergences [Sc89].

- On the contrary, the case of hazardous material transportation poses problem that are similar to radioactive and non nuclear commodities. In this field, where the implementation of ALARA is not straightforward when dealing with low probabilities/high consequences events, it is however possible to apply the generic ALARA framework to structure the assessment and management processes.

Besides answering the questions that where at the origin of the project, the research brought interesting outputs to improve risk assessment and management procedures that have found some positive applications. Methodologies for analysing industrial systems and uses of a given product have been elaborated. Possible statistic or biological models in different risk management strategies have been explored for carcinogenic substances. The French National Institute for Statistical and Medical Research (INSERM) has published a book about the results on this aspect to promote such approaches. Models for probabilistic risk assessment have been set up that are appropriate for risk associated with transportation of hazardous materials. The idea of combining spatial analyses (computing risk in small cells all over a geographical area) of major hazard analyses (classical CCDF) have been found to be interesting by the French Ministry of Environment and Transportation. The tools developed during the project period (eg. TRAMADAN) allowed to launch two studies at the national scale, for ammonia and propane, the results of which (risks, costs) must feed the french policy in the area [Ca89] [Ve90].

4. Perspectives

The project also allowed to better define some questions and openened new perspectives for research in risk management.

First, the comparative approach appears to be at the same time more complex but also more fruitfull than it was thought at the origin. Very quickly it appeared that the confrontation led to the improvement of methods, because of technology transfer, but also because the attempts to apply the ALARA methodology in other fields demonstrated that many questions have been artificially made sector dependant while they should be dealt with more

globally. A great deal of present researches such as risk management within institutions, human reliability, decision aiding methods, major hazards management will probably become developing areas in the future with large intersectorial applications.

Secondly, the analysis of the status and insertion of ALARA in risk management systems (either nuclear or non nuclear) allowed to better delineate the difference between a regulatory perspective aiming at setting limits and constraints for regulation and a management perspective to integrate protection and safety within industrial systems at both design and operation stages. This second perspective will certainly become dominant in the future.

Two directions arise from this analysis of potential ALARA developments for a more rationally grounded risk management. The first is the development of systems that allow a good feedback for data collection in a proper format. A great deal of work is to be done in this field, even in the nuclear industry where the needs increase as practices develop. The other is the development of assessment tools, which implies a double shift, from models to practical tools, from pure descriptive models towards decision oriented models.

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

V. Publications:

[Ab88] L. Abenhaim, Y. Bonvalot, F. Fagnani, A. Oudiz. "The value of probabilistic modelling for the estimation of carcinogenic risk at low doses. Application to 2,3,7,8 TCDD", in Seminar on Applications, Perspectives and Limitations of Comparative Risk Assessment and Risk Management, Nice, Sept. 26-30, 1988.

[Bo88a] Y. Bonvalot. "Evaluation des différentes méthodes de prédiction du risque dû à l'exposition à un cancérogène chimique". Application au problème de la 2.3.7.8 TCDD". Mémoire de DEA soutenu le 27 Octobre 1987. CEPN Paris, 1988.

[Bo88b] Y. Bonvalot, A. Oudiz, P. Hubert, L. Abenhaim. "Determination of carcinogenic threshold limit values using the tumorogenic dose rate 50 % (TD 50 %), in Seminar on Applications, Perspectives and Limitations of Comparative Risk Assessment and Risk Management, Nice, Sept. 26-30, 1988.

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Fontenay-aux-Roses
B.P. n° 6
F-92265 Fontenay-aux-Roses

Contract no.: BI6-F-115-F

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

Dr. G. Madelaine
IPSN
CEA-CEN de Fontenay-aux-Roses
B.P. n° 6
F-92265 Fontenay-aux-Roses

Telephone number: (1) 654.71.36

Title of the research contract:

Characterization of radon daughters and carcinogenesis.

List of projects:

1. Characterization of radon daughters and carcinogenesis.

Title of the project no.:

Caractérisation de la granulométrie et des propriétés électriques des descendants du radon en relation avec l'étude de la carcinogenèse pulmonaire chez le rat

Head(s) of project:

Dr. G. MADELAINE, chef des Laboratoires d'Etudes des Pollutions Atmosphériques, DPT/SPIN, Centre d'Etudes Nucléaires de Fontenay-aux-Roses, BP 6, 92265 FONTENAY AUX ROSES CEDEX

Scientific staff:

Dr. D. BOULAUD, chef du Laboratoire de Physique et Métrologie des Aérosols, DPT/SPIN/LEPA, Centre d'Etudes Nucléaires de Fontenay-aux-Roses, BP 6, 92265 FONTENAY AUX ROSES CEDEX

I. Objectives of the project:

Caractérisation de la répartition en dimension et de l'état de charge électrique des descendants du radon inhalés par le rat dans la chambre d'exposition du CEA à Razès, Division Minière de la COGEMA. Ces paramètres influent sur la dose reçue par les cellules des différents compartiments pulmonaires.

II. Objectives for the reporting period:

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.

III. Progress achieved:

Pour évaluer les doses inhalées par des rats placés dans une chambre d'exposition, il nous faut connaître la granulométrie et sa modification spatio-temporelle, des descendants à vie courte du radon. Pour ce faire, nous nous sommes attachés à mettre au point un dispositif original permettant d'accéder à la répartition en dimension et à étudier la fixation de ces descendants sur les aérosols présents. Le dispositif a été qualifié et étalonné dans diverses conditions avant d'effectuer des séries d'expérimentations en présence et en absence d'animaux pour détecter d'éventuelles modifications dans le comportement des aérosols "radioactifs".

III.1. METROLOGIE DES DESCENDANTS DU RADON 222

III.1.1. Justification théorique

Toutes les études montrent que la répartition en dimension des descendants du radon, hors conditions exceptionnelles, se situe en première approximation dans le domaine submicronique.

Deux types de mesures sont particulièrement adaptées à cette catégorie d'aérosols : les méthodes électriques, les méthodes diffusionnelles.

Ce sont ces dernières que nous avons particulièrement retenues en s'attachant plus particulièrement aux batteries de diffusion à grilles et à un nouveau dispositif mis au point appelé Spectromètre Diffusionnel et Inertiel (SDI 2000).

Le premier type de batterie est particulièrement adapté pour appréhender la fraction non recombinée du dépôt actif. La théorie et notamment la méthode d'inversion permettant à partir de mesures expérimentales, de remonter à la distribution granulométrique a été faite pour l'utilisation directe in situ dans la chambre d'exposition et dans une mine d'uranium. Ce type de batteries particulièrement onéreux servira de système de mesure de référence et/ou de comparaison. Son utilisation s'avère toutefois délicate en présence de concentration importante d'aérosols (colmatage rapide des grilles) et donne des résultats imprécis vers les dimensions les plus élevées (environ 1 μm).

Le SDI 2000 que nous avons étudié et mis au point permet pour un prix de revient et une fiabilité plus grande de pallier les inconvénients cités ci-dessus.

L'originalité de ce dispositif est d'avoir associé deux techniques séparatives jusqu'alors utilisées indépendamment. La première, classe les particules les plus grosses en fonction de leur diamètre aérodynamique : c'est un impacteur en cascade classique. La seconde, sélectionne les particules submicroniques

en fonction de leur diamètre de diffusion. Il couvre ainsi un domaine de dimension compris entre 5.10^{-3} µm et 10 µm.

La partie diffusionnelle, originale, est composée de cinq conduits cylindriques en parallèles contenant des lits de sphères de verre de dimension et de profondeur variables.

Un étalonnage de ce dispositif a été réalisé avec un aérosol monodispersé de ClNa dans le domaine 0,008 µm - 0,3 µm. On a pu montrer que l'efficacité d'un tel lit de billes, dans un régime purement diffusionnel est représentée par la loi suivante :

$$E_D = 13,8\ Pe^{-0,63}$$

Pe étant le nombre de Peclet ayant pour valeur Pe = Ø V/D avec Ø diamètre des sphères, V vitesse frontale et D coefficient de diffusion des aérosols. Ce traitement théorique donne une loi puissance divergeant faiblement de la loi expérimentale seulement au niveau du coefficient numérique.

Le traitement théorique des données montre que l'on obtient une bonne sensibilité et une bonne restitution de la granulométrie même pour des distributions bi-modales.

L'utilisation pratique d'un tel système revient à effectuer après prélèvement des comptages radioactifs des différents étages de l'impacteur et des filtres membranes absolues placés à la sortie des canaux de la batterie de diffusion. A partir des mesures obtenues sous forme d'activité, un programme de calcul sur micro-ordinateur permet alors d'obtenir le spectre granulométrique de l'aérosol à étudier. Ce programme est basé sur un traitement mathématique d'inversion de l'équation de Fredhlom utilisant une méthode d'itération non linéaire. Le logiciel, écrit en langage basic, est utilisable sur tous types de calculateurs de laboratoire.

III.1.2. Résultats expérimentaux et discussion

De nombreuses expérimentations ont été menées pour qualifier le dispositif avec des aérosols radioactifs ou non :

- la qualification du SDI 2000 pour la mesure de la distribution en activité à partir de la fixation des descendants du radon a été effectuée avec trois types d'aérosols introduits dans une enceinte de confinement à atmosphère contrôlée. Ils sont comparés avec l'analyse obtenue par une batterie de diffusion à grille ;
- Aérosol ultrafin produit par radiolyse et par conversion gaz-particules.
- Aérosol constitué par de la fumée de cigarettes.
- Aérosol de combustion.

Les résultats obtenus dans les deux premiers cas sont comparables ; dans le cas de l'aérosol de combustion les résultas sont sensiblement différents, mettant en évidence la fraction de la radioactivité fixée sur les plus grosses particules. On peut en déduire que le SDI 2000 permet une meilleure détermination de la granulométrie des descendants du radon fixés éventuellement sur les particules de la partie supérieure du spectre granulométrique.

Les diamètres médians en activité obtenus après traitement des données sont respectivement de 0,015, 0,316 et 0,870 μm.

Il semble donc, que le dispositif étudié et mis au point réponde bien à l'objectif fixé.

Pour préciser encore le domaine d'utilisation du SDI 2000 une série d'expérimentations a été menée à l'intérieur même d'une mine d'uranium en comparant de façon systématique les résultats obtenus avec un analyseur différentiel de mobilité électrique. Les résultats sont transcrits dans le tableau ci-après.

DMN* en μm	DMAt** en μm	DMAc*** en μm
$8{,}96.10^{-2}$	0,165	0,186
$7{,}95.10^{-2}$	0,154	0,165
10^{-1}	0,205	0,195
$8{,}56.10^{-2}$	0,143	0,180

* Diamètre médian numérique mesuré à l'analyseur électrique
** Diamètre médian en activé calculé
*** Diamètre médian en activé obtenu avec le SDI 2000

La figure 1 montre au cours du temps les variations des diamètres médians définis ci-dessus.
On peut voir que les résultats sont très comparables et que le SDI 2000 est un dispositif de mesure bien adapté pour l'étude des aérosols radioactifs.

La figure 2 donne un exemple des spectres granulométriques obtenus.

Des expérimentations avec des aérosols non radioactifs et notamment des aérosols émis par les véhicules diesel utilisés dans certaines mines d'uranium ont montré que le dispositif permettait d'obtenir une distribution granulométrique en masse de ces particules dont le diamètre moyen se situe aux environs de 0,1 μm.

III.2. CARACTERISATION DE L'ATMOSPHERE DE LA SALLE D'EXPOSITION

Ayant montré théoriquement et expérimentalement le bon fonctionnement et la reproductibilité du dispositif SDI 2000, nous avons procédé à des mesures systématiques de la répartition de la radioactivité des descendants du Rn222 dans la chambre d'exposition en présence ou non de rats pour tenter de mettre en évidence une évolution spatio-temporelle de cette répartition.

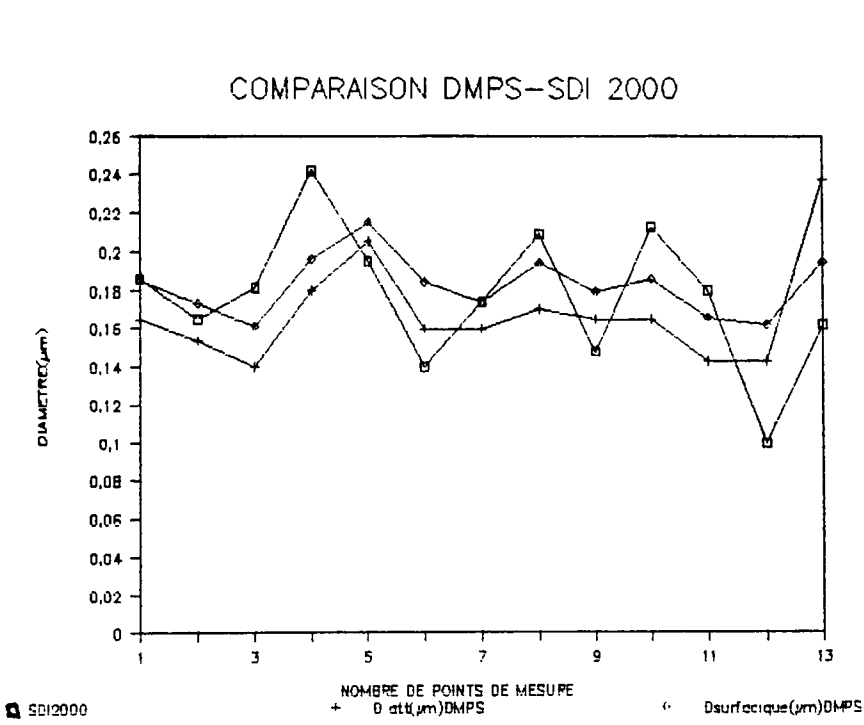
Le radon était introduit dans la chambre après renouvellement de son atmosphère par de l'air atmosphérique non filtré.

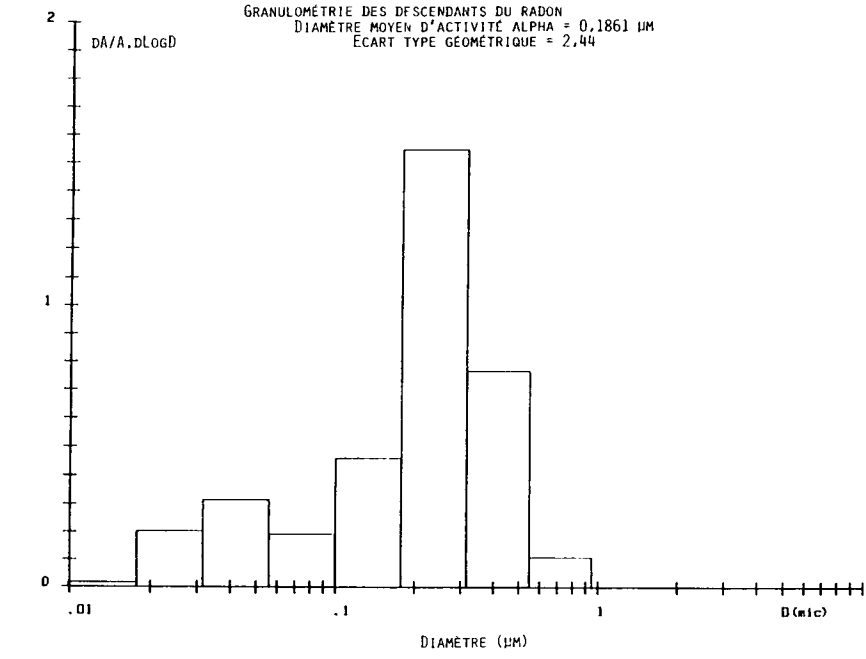
En l'absence d'animaux les résultats trouvés, moyenne de 10 expériences, donnaient un diamètre médian en activité de 0,26 μm avec un σg de 2,9. La présence de 50 rats répartis en 10 cages faisait passer ce diamètre à une valeur de 0,35 μm avec un σg pratiquement identique. Il semble donc que la présence d'animaux soit par émission de particules, soit par captation des plus fines particules par diffusion brownienne modifie sensiblement le spectre en dimension des aérosols et, par suite, change la dose inhalée. En faisant varier la concentration en radon et en aérosol dans l'enceinte, certaines anomalies sont apparues et nous ont conduit à préciser le comportement des étages inertiels vis-à-vis des aérosols submicroniques. On a pu ainsi mesurer à l'aide d'aérosols monodispersés que dans la gamme de dimension 0,05-0,1 μm environ 10 % de la radioactivité pouvaient être répartis sur les étages inférieurs de l'impacteur.

De plus, il est apparu que si l'on utilise un média filtrant pour collecter des aérosols sur les étages d'impaction une captation parasite de particules pouvait s'effectuer par filtration tangentielle et pénétration du jet d'air à travers une très faible épaisseur du filtre.

III.3. CONCLUSION

Au cours de ce travail nous avons mis au point une méthode peu onéreuse et facile d'utilisation pour appréhender la granulométrie des descendants du radon 222. Cette technique a été qualifiée expérimentalement et semble donner des résultats quantitatifs et reproductibles dans différentes conditions (concentration en aérosols, niveau d'activité, ...). Ces expérimentations de mise au point menées dans la chambre d'exposition permettent de conclure qu'une utilisation en routine de cette technique par les biologistes devrait caractériser de façon satisfaisante les doses inhalées par les animaux placés en expérimentation.


COMPARAISON DMPS–SDI 2000
DIAMETRE(µm)
0,26
0,24
0,22
0,2
0,18
0,16
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
1
3
5
7
9
11
13
NOMBRE DE POINTS DE MESURE
SDI2000
D att(µm)DMPS
Dsurfacique(µm)DMPS


GRANULOMÉTRIE DES DESCENDANTS DU RADON
DIAMÈTRE MOYEN D'ACTIVITÉ ALPHA = 0,1861 µM
ÉCART TYPE GÉOMÉTRIQUE = 2,44
DA/A.DLOGD
2
1
0
.01
.1
1
D(mic)
DIAMÈTRE (µM)

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

V. Publications:

/ 1 / M. DIOURI
Contribution à l'étude du comportement aérodynamique des aérosols. Mise au point d'un spectromètre diffusionnel et inertiel
Thèse Doctorat, Paris 1987

/ 2 / M. DIOURI, D. BOULAUD et G. MADELAINE
Collection of fine particles by granular bed. Application to the size distribution measurement of ultrafine aerosol
Proc. of the 2nd Int. Aerosol Conference, Berlin 1986

/ 3 / D. BOULAUD, G. MADELAINE
Nouveau Spectromètre Diffusionnel et Inertiel
3ème journée d'Etudes sur les aérosols, COFERA, Paris 1986

/ 4 / D. BOULAUD, M. DIOURI, G. MADELAINE, A. RENOUX
Nouveau dispositif de mesure des aérosols
Pollution Atmosphérique, (114), avril 1987

/ 5 / D. BOULAUD
Intercomparaison Radon 1989.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: University College Dublin
Belfield
IRL- Dublin 4

Contract no.: BI6-F-117-IRL

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

Dr. J.P. McLaughlin
Physics Department
University College Dublin
Belfield
IRL- Dublin 4

Telephone number: 69.32.44

Title of the research contract:

Assessment of the population dose indoors from natural radiation in Ireland with particular emphasis on radon daughter properties and behaviour.

List of projects:

1. Assessment of the population dose indoors from natural radiation in Ireland.
2. Investigations on the plateout velocities and removal rates of airborne Radon daughters.

Title of the project no.: (1) Assessment of the population dose indoors from natural radiation in Ireland.

Head(s) of project: Dr. J.P. McLaughlin
Physics Department
University College Dublin

Scientific staff: P. Wasiolek
B. Fitzgerald

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 radon concentrations and associated lung doses.

II. Objectives for the reporting period:

The analysis of the indoor radon survey data.

The initiation of a regional survey in the west of Ireland in support of the Nuclear Energy Board.

The completion of a field intercomparison of radon detectors in houses in France, Ireland and Italy.

III. Progress achieved:

INTRODUCTION

Following on an earlier pilot study by University College Dublin of indoor radon in 220 Irish dwellings (CEC Contract BI0-F-517-83-EIR) the national survey of indoor radon concentrations in Ireland commenced towards the end of 1985. One of the principal aims of the survey was to assess the radon exposure of the general population from which estimates of doses and associated risks could be made. The other principal aim was to try to identify factors in the Irish environment such as rock and soil characteristics, building practices etc. which are most likely to give rise to elevated indoor radon levels.

SURVEY DESIGN AND EXECUTION

Selection of Dwellings: Because the survey was concerned with radon exposure of the general public the principal sampling strategy used was to try to select dwellings for radon measurement on a population weighted random basis. The selection of participating households was made with the help of AFF (An Foras Forbartha: The National Institute for Physical Planning and Construction Design) during the period AFF was carrying out a nationwide study of Irish household energy consumption practices. Households were chosen by random selection from the national Electoral Register which is the best available sampling frame of general population in Ireland. This register, which is renewed each April, lists the names and addresses of all those eligible to vote in parliamentary and local government elections. Random samples were drawn from the Electoral Register using a computer based system called RANSAM, which was developed by the Economic and Social Research Institute in Dublin. This is a two stage equal selection probability method, stratified by geographical area. In order to reduce the risk of multiple sampling from selected households and to reduce the bias towards larger households a sample larger than necessary was drawn and from this only the first listed member of any given household was selected. A total of 3200 addresses were selected and an interviewer from AFF visited each address to explain the nature of the surveys and to invite participation. By the end of 1985 a total of 1427 households had agreed to participate in the radon survey out of a national housing stock of about 875000.

Detector Type: During the course of the national survey and smaller surveys conducted in specific parts of Ireland radon detection was carried out by the use of closed passive alpha track plastic radon detectors. In the initial phase of the work detectors based on the use of Kodak Pathe LR115 cellulose nitrate were used but as the surveys progressed the use of this plastic was phased out in favour of CR-39 because of its superior alpha detection characteristics. Both plastic types were chemically etched and track counting was by means of optical microscopy. In all cases the alpha sensitive plastic was mounted inside the lid of a small closed cylindrical plastic container. Containers of two sizes were used: Type A (LR 115 and CR-39) with a sensing volume of height = 6.9cm and radius = 2.6cm; and Type B (CR-39 only) with a sensing volume of height = 4.4cm and radius = 1.05cm. During the project period the laboratory participated in the ongoing series of CEC/OECD radon

intercomparisons held at the NRPB (UK). The LR 115 based detector was found to have a radon sensitivity of 2.9 tracks cm^{-2}. $kBq^{-1}.m^3.hr^{-1}$ while for CR-39 Type B geometry the radon sensitivity is 3.1 tracks $cm^{-2}.kBq^{-1}.m^3.hr^{-1}$. The almost identical radon sensitivities of the two different detectors is a coincidence arising from the combined effects of geometry and alpha energy response of the detecting media.

In addition to participation in the intercomparisons at the NRPB (UK) quality assurance of radon measurement was also verified by participation in field intercomparisons in dwellings in Brittany (1986, 1988/89) and in Italy 1988/89 in collaboration with the Commissariat a l'Energie Atomique/Universite de Bretagne Occidentale (Brest) and with E.N.E.A. (Roma). Details will be reported elsewhere.

Survey Methodology: Using the postal services surveys were carried out in three phases between 1985 and 1989. A participating household was sent one radon detector together with a letter of introduction containing instructions regarding the use of the detector and general information on radon. Participants were instructed to place the detector in a well used part of the dwelling (preferably the principal bedroom). Having regard to Irish social factors and limitations of survey manpower six months was considered as being close to the practical optimum exposure period for a detector in a household. During the random phase of the national survey a total of 1427 detectors were sent to households and eventually a total of 1259 detectors were successfully recovered and processed. This represents a successful detector recovery rate of 88%. This very high recovery rate is considered to be largely due to the fact that all 1427 participating households had prior to the survey given their agreement to participate to AFF interviewers. Of the 1259 households which returned exposed detectors 25% did not return completed questionnaires. Analysis of questionnaire data could thus only be carried out for 943 households.

About 3% of the detectors sent to households were returned within a few days by the postal services due principally to difficulties with addressee identification in some rural areas with a predominance of certain surnames. These detectors were immediately processed and together with normal laboratory background controls were used for survey alpha track background purposes. The 1259 successfully recovered detectors represents an achieved mean nationwide sampling rate of 1 per approximately 675 households.

On the basis of the information in the 943 completed questionnaires a number of simple statistical tests were applied in an attempt to determine if the sampled households were representative of the national household stock. Comparisions were made with national data compiled from the Census of Population 1981. Tables 1 and 2 show two such comparisons which are those of most relevance to radiation protection considerations. These clearly show that the surveyed households were quite representative of the general situation in Ireland. As can be seen from Table 2 households containing only one occupant were undersampled significantly. This was not unexpected as it was an almost unavoidable outcome of the use of the Electoral Register as a sampling frame. Unlike questions relating to age and number of persons

in a household answers to questions relating to social class in the questionnaire could not be taken as reliable. In these cases again however the sampled households and the national data gave a degree of agreement similar to those shown in Table 1 and 2. On the basis of analysis of the questionnaires it appears that the households surveyed do not significantly deviate in character from the national stock.

Table 1: AGE DISTRIBUTION IN SURVEYED DWELLINGS.

AGE GROUP	Over 65	18-65	5-18	under 5
SURVEY	10.1%	52.9%	28.8%	8.2%
IRELAND*	10.7%	49.5%	29.5%	10.3%

* Source: Central Statistics Office

Table 2: PERCENTAGE OF OCCUPANTS IN DWELLINGS

OCCUPANTS	1	2	3	4	5	6	7	≥8
SURVEY	8.5%	18.0%	12.0%	19.7%	17.9%	11.8%	5.5%	6.6%
IRELAND*	16.8%	20.2%	14.8%	15.4%	12.0%	8.9%	5.1%	5.5%

* Source: Central Statistics Office

SURVEY RESULTS

The distribution of measured values of indoor radon in 1259 Irish dwellings are presented in histogram form in Figure 1 and as a cumulative frequency distribution in Figure 2. Median, geometric mean and arithmetic mean values of 34, 36 and 60 Bq/m^3 were obtained for these distributions. In view of the sensitivity of the arithmetic mean value to the presence of even a small number of very high values in the distribution it was considered that the median value of 34 Bq/m^3 is a value that may be seen to be "typical" of Irish dwellings. A reasonable correspondence of the measured distribution to that of a log-normal distribution (dotted line) is indicated in Figure 1 and is also indicated by the closeness of the values of the median and geometric mean values. The indoor radon concentrations measured during the national survey period were found to range from a minimum of 2 Bq/m^3 to a maximum of 1740 Bq/m^3. Table 3 gives the fractile values for the 1259 set as measured and as predicted on the basis of a log-normal distribution. Good agreement was obtained for lower fractiles but for fractiles >400 Bq/m^3 and >1000 Bq/m^3 the observed values are much greater than those predicted from a log-normal model.

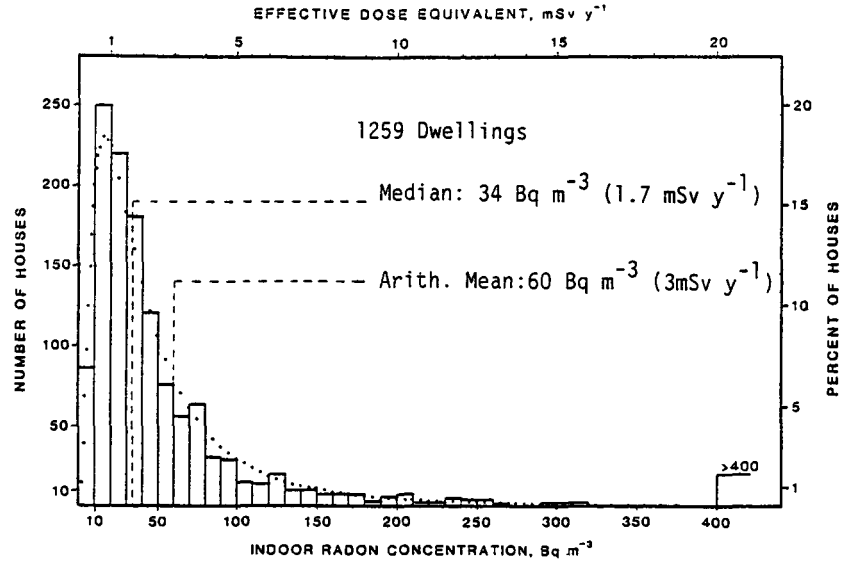


Figure 1: DISTRIBUTION OF INDOOR RADON AND ESTIMATED DOSES.

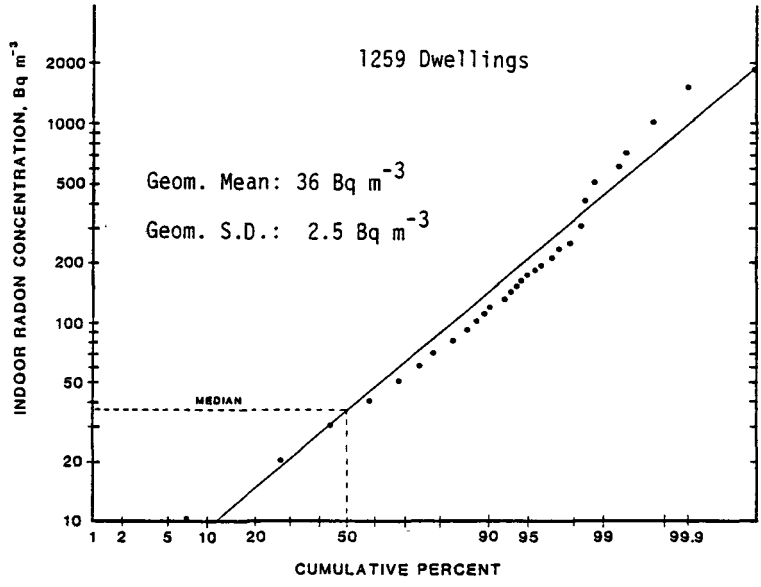


Figure 2: CUMULATIVE FREQUENCY DISTRIBUTION OF INDOOR RADON.

Table 3:

FRACTILE [Bq/m^3]	>100	>200	>400	>1000
PERCENT MEASURED	11.9	3.8	1.6	0.3
PERCENT PREDICTED	12.9	2.9	0.4	0.01

The survey results are presented in Table 4 on the basis of the twenty-six counties into which the Republic of Ireland is divided. Here a county is taken to mean the total geographical extent of the named county inclusive of both urban and rural areas. Where no entry is made for a fractile this indicates that in the surveyed houses of a particular county no house was found to have a radon level in the particular fractile.

Table 4: INDOOR RADON IN IRISH COUNTIES

COUNTY	POPULATION (thousands)	PERCENT OF INVESTIGATED DWELLINGS >100	>200	>400 [Bq/m^3]
Carlow	41	15	-	-
Cavan	54	-	-	-
Clare	91	40	13	10
Cork	413	13	4	2
Donegal	129	6	1	-
Dublin	1021	4	1	-
Galway	178	15	7	2
Kerry	124	22	4	4
Kildare	116	5	3	-
Kilkenny	73	4	4	-
Laois	53	-	-	-
Leitrim	27	14	7	-
Limerick	164	13	4	-
Longford	31	-	-	-
Louth	92	8	4	-
Mayo	115	19	14	7
Meath	104	11	-	-
Monaghan	52	-	-	-
Offaly	60	-	-	-
Roscommon	55	40	10	10
Sligo	56	28	16	4
Tipperary	137	28	10	-
Waterford	91	21	12	6
Westmeath	63	25	6	-
Wexford	102	7	-	-
Wicklow	94	21	-	-
Total	3536	11.9	3.8	1.6

It should be noted while averaged over all the counties the mean sampling ratio was 1 dwelling in 675 that in two counties (Monaghan and Laois) for a variety of reasons sampling was as low as 1 dwelling in 2500 while in the case of Carlow approximately 1 dwelling in 250 was sampled. Notwithstanding the statistical implications of this it is obvious from Table 3 that in some counties a significant percentage of surveyed houses were found to be in the two upper fractiles (i.e. >200 and > 400 Bq/m^3). These fractiles it should be noted cover the range of most of the recommended international limits or action levels for continuous exposure of the general population. In Northern Ireland since January 1990 the Action Level recommended by the U.K. National Radiological Protection Board is 200 Bq/m^3. As a result of the developing data base from the national random survey as summarised in the foregoing figures and table a number of smaller targeted random local surveys in some parts of Ireland commenced in 1987 and continued into 1989. These were mainly in the western counties of Galway, Mayo and Sligo. Typically these surveys consisted of indoor radon measurements in 20 to 40 dwellings in parts of a county where one or more dwellings in the national survey were found to exceed 400 Bq/m^3. About 150 dwellings were thus investigated usually with the enthusiastic assistance of a local doctor, pharmacist, schoolteacher or scientist. One such local survey of particular interest was carried out in County Galway where indoor radon was measured in 40 dwellings of which half were built on the Murvey granite formation in Connemara and the other half were built on the adjacent and abutting limestone formation around the village of Moycullen. Contrary to expectations indoor radon levels in the limestone area were found to be significantly greater than those in the granite area as shown in the following table:

Table 5:

ROCK TYPE	PERCENTAGE OF HOUSES			
	>100	>200	>400	[Bq/m^3]
GRANITE	18	-	-	
LIMESTONE	61	44	17	

Mainly as a result of the findings of the national survey and the later local surveys a decision was made by the Nuclear Energy Board (Dublin) to carry out a regional survey in the west of Ireland. The radon group of University College Dublin participated fully in the planning, execution and measurement phases of this regional survey which commenced in 1989. The survey area (~9000 km^2) consisted chiefly of the central and eastern parts of Counties Mayo, Galway and Clare which is largely carboniferous limestone. Small adjacent parts parts of Counties Sligo and Roscommon were also included. A total of 600 volunteer households were chosen in the region with the assistance of local Environmental Health Officers. By the end of 1989 approximately 550 measurements of indoor radon were made using CR-39 alpha track detectors identical to the type used in the national survey. Initial analysis shows that approximately 2.8% of the dwellings surveyed had indoor levels above 400 Bq/m^3

and about 9% above 200 Bq/^{3} which is in broad agreement with the national survey data for the region. It is expected that detailed follow-up studies of this regional survey will take place in 1990.

GEOLOGICAL CONSIDERATIONS

Most Irish building materials do not contain elevated concentrations of radium-226 and as such make a minor contribution to indoor radon levels in the majority of dwellings. Where high indoor levels are encountered it is considered that the principal source of this radon is in the soil and rock immediately subjacent to the dwelling. It was therefore expected on the basis of surface radiometric measurements of terrestrial radiation made by the Geological Survey of Ireland and mineral exploration companies that formations such as the granites of Leinster, Galway and Donegal might be areas of elevated indoor radon. The earlier pilot study of indoor radon in 1983-85 did find some elevated indoor radon levels on the Leinster granites.

High indoor radon levels over granites was not verified by the surveys in this project, in which most elevated indoor radon levels were found to occur on limestone formations near the boundary of formations such as granites known to contain high uranium and radium concentrations. The data in Table 5 is an excellent example of this type of observation. Discussions on these findings with both government and academic geologists has led to the development of a hypothesis that in the most affected western regions elevated indoor radon levels arise due to hydrogeological effects combined with the release of radon in fractured and permeable limestone or karstic structures. The radon in such formations may migrate more efficiently to the surface than it can through granite or other low permeability rocks. The radon may either be brought directly into the limestone in solution in water or may be produced from radium salts leached from the adjacent source rock (i.e. granites etc.) and precipitated in the limestone. In support of this latter possibility it is noted that recent studies by Dr. McAulay (Trinity College Dublin) have independently shown that the western region of elevated indoor radon also has soils with radium-226 contents 3 to 8 times the national average value of about 25 Bq/m^3. Other formations in Ireland found to have associated high indoor radon levels are the phosphatic shales of Clare and the Kiltorcan old red sandstone formation of Munster.

DOSE ASSESSMENT

The estimation of doses arising from radon exposure of the general population is a topic which has been under active review in recent years both by the ICRP and other bodies. Having regard to the great variability in exposure parameters such as occupancy factor, equilibrium factor, unattached fraction etc. it is not surprising that unanimity in the choice of an appropriate exposure to dose coefficient has not yet been reached. It must also be emphasised that ultimately in radiation protection one is concerned with risks arising from an exposure.

For a variety of reasons the past ten to fifteen years has witnessed over a three fold change in estimated risk factors to the exposed individual from 1.25×10^{-2} Sv^{-1} to as high as 4.5×10^{-2} Sv^{-1}. In the same period the consensus view on the value of the radon exposure to dose conversion coefficient has changed by a factor of about two to the present operational value of 20 Bq/m^3 per mSv y^{-1} between the time average activity concentration of radon gas and the annual effective dose equivalent for members of the general population in the indoor environment. This value which was recommended by a Working Party of the Euratom Treaty Article 31 Group of Experts has been used in assessing the doses from radon exposure in Ireland. On this basis it can be seen in Figure 1 that the estimated median dose in Ireland arising from indoor radon is about 1.7 mSv/y. This is about 50% of the presently estimated total annual average dose to the population from all sources of about 3.5 mSv/y. If a lifetime risk factor for the general population of 3.3×10^{-2} Sv^{-1} (1 in 30 Sv^{-1}) which is compatible with the risk analysis given in ICRP Publication No. 50. 1987, is used with the radon fractile data from the survey as presented in Table 3 the following estimates of the contemporary risk from indoor radon in Ireland may be obtained.

TABLE 6:

RADON FRACTILE (Bq/m^3)	>100	>200	>400	>1000
*DOSES (mSv/y)	>5	>10	>20	>50
*LIFETIME RISK	>1.1%	>2.3%	>4.6%	>11.7%
*APPROX. NUMBER OF PERSONS AFFECTED	416500	133000	56000	10500

*(Estimates)

It cannot be overemphasised that the estimated risks, doses and sizes of population groups presented in Table 6 are based on a series of assumptions each with its own uncertainty. Nevertheless the information in the table clearly indicates that in the light of present knowledge exposure to radon for some tens of thousands of the population may be giving rise to unacceptable but as yet unverified risks. For the purposes of comparison it should be noted that the lifetime risk of lung cancer in Ireland is at present about 3% (45×10^{-5}/y) and the lifetime risk of a fatality arising from a road traffic accident is about 1% (14×10^{-5}/y). In relation to doses to the population as presented in Table 6 it is of interest to note that the annual average dose arising from artificial radionuclides ingested by consumption of fish from the Irish Sea is approximately 0.0015 mSv/y and that from the consumption of foodstuffs contaminated by Chernobyl fallout is presently estimated to be 0.01 mSv/y while in 1986/87 the dose was about 0.1 – 0.15 mSv/y.

CONCLUSION

With the support of the CEC Radiation Protection Programme 1985-89 the project was carried out in which indoor radon levels were measured in approximately 2000 dwellings in the Republic of Ireland. Surveys of three types were carried out: a national population weighted random survey of approximately 1300 dwellings, a follow up regional survey with the Nuclear Energy Board of approximately 550 dwellings in a 9000 km^2 region of the west of Ireland and various small local surveys. The national survey results presented here show that the median radon level in Ireland is about 34 Bq/m^3 (estimated associated dose 1.7 mSv/y) while 3.8% of dwellings had radon in excess of 200 Bq/m^3 (10 mSv/y) and about 1.6% were in excess of 400 Bq/m^3 (20 mSv/y).

Analysis of the survey data found no strong correlation between dwelling construction or age and indoor radon levels. Detached houses about 50% of the sample, had however a slightly higher median value (40 Bq/m^3) compared to other housing types such as semi-detached etc. with a median value of about 28 Bq/m^3. The strongest influence on indoor radon in Ireland appears to be geological with the most elevated levels so far detected to be in limestone formations in western counties close to primary sources such as granite. Partially as a result of the findings of these surveys a recommended action level of 200 Bq/m^3 for all dwellings in Ireland is being seriously considered by the Irish government agencies with a responsibility for radiation protection. It should be noted that the estimated risk at this level (2.3%) is about two-thirds of the national risk of contracting lung cancer. As most dwellings in Ireland have radon levels well below the probable action level of 200 Bq $/m^3$ the overall radiological health significance of indoor radon may perhaps best be gauged from considering the estimated risk at the median value of 34 Bq/m^3. This is about 0.4% compared to a national lung cancer liftetime tisk of about 3% which implies that up to about 1 in 8 lung cancers in Ireland may be due to exposures arising from radon in the home. Similar estimates have been made by radiation protection agencies in recent years in countries such as the U.S.A. and the U.K. While these estimated risks are based on many assumptions, with their associated uncertainties, it is now clear that radiation doses and risks from radon are no longer "background" phenomena and are of a radiological health significance to the general population far in excess of those arising from artificial radionuclide contamination of the environment.

While epidemiological studies, over the past forty years, on the effects of radon to occupational groups such as miners have established that radon decay products are carcinogenic it is only in the last few years that properly designed epidemiological studies on its effects on the general population have commenced in countries such as the U.S.A., the U.K. and Scandinavia. In the case of Ireland the population estimated to be at high risk from radon exposure is very small in absolute terms. For this and other reasons it would be very difficult to carry out a satisfactory epidemiological study in Ireland.

IV. **Other research group(s) collaborating actively on this project [name(s) and address(es)]:** Department of Pure and Applied Physics, Trinity College Dublin, Ireland. (Dr. I.R. McAulay).

Commissariat a L'Energie Atomique, Fontenay aux Roses, France (Dr. A. Rannou).

Nuclear Energy Board, Dublin (Dr. N.V. Nowlan).

E.N.E.A. Rome Italy (Dr. L. Tommasino).

V. **Publications:**

I.R. McAulay and J.P. McLaughlin, "Indoor Radiation Levels in Ireland ". Science of the Total Environment, 45, 319-325, (1985).

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" 24, 383, Radiation Protection Dosimetry 1988.

Tymen, G., Mouden, A., McLaughlin J.P., Wasiolek, P and Rannou, A. "Comparison of the Exposure Response of French and Irish Radon detectors during Field Measurements in Brittany". Radiation Protection Dosimetry, 24, 371, 1988.

Wasiolek, P. "Indoor Radon in Ireland: Exposures and Laboratory Dose Reduction Studies". Ph.D. Thesis National University of Ireland. August 1989.

Title of the project no.: (2) Investigations on the plateout velocities and removal rates of airborne radon duaghters.

Head(s) of project: Dr. J.P. McLaughlin
Physics Department
University College Dublin (IRL)

Scientific staff: P. Wasiolek

I. **Objectives of the project:** To investigate the behaviour of radon daughters in an experimental room with particular emphasis on plateout onto surfaces. With the aid of electric fields and unipolar ion generation to investigate the feasability of reducing the potential alpha energy concentrations and consequently the expected lung dose due to radon daughters in the room.

II. **Objectives for the reporting period:** To make measurements of the deposition of radon daughters within an experimental room with and without field-ion treatments.
To develop a new technique for measuring radon daughter plateout onto surfaces.

III. Progress achieved:

INTRODUCTION

In recent years a number of techniques have been developed aimed at the direct reduction of the potential alpha energy concentration (PAEC) and associated doses of radon decay products (Rn-d) in indoor air. In this report an account is given of studies on the efficiency of a PAEC reduction method based on electric fields both with and without unipolar ion production to remove Rn-d. An account is also given of measurements of the plateout behaviour and characteristics of Rn-d. An outline description of a new technique to measure the equilibrium plateout Rn-d activity on surfaces is also presented.

PAEC REDUCTION IN INDOOR AIR

Methodology

The investigations were carried out in a laboratory room of volume 44m^3 with an internal surface area of 76m^2. A room was chosen for these investigations in preference to a chamber in order to test the techniques in conditions similar to those in a real dwelling. When the room was sealed the exchange rate of the room air with its surroundings was typically in the 0.3 to 0.4 hr^{-1} range as determined by the rate of disappearance of radon released from a dry radium-226 source in the room. Sampling of Rn-d and radon gas activity in the room air either close to the walls or in the room centre was made from outside the room via sampling ports. Alpha spectroscopic analysis of filter collected Rn-d activity was made in vacuo using a 450mm^2 passivated implanted planar silicon detector (Canberra PIPS type) coupled to a multichannel analyser. Energy resolution of the system was 90 keV FWHM which for the 6 MeV (Po-218) and 7.68 MeV (Po-214) alpha peaks was more than adequate. Open face filters were used to collect and measure total airborne activity while unattached Rn-d activities could be determined by mounting fine stainless steel meshes in front of the filters during sampling. Determinations of plateout of Rn-d activity on room surfaces were made using bare alpha track detectors (CR-39 and LR-115) mounted on the surfaces. Radon gas concentrations in the room air up to about 50,000 Bq/m^3 could be obtained by passing air over a 5.5 MBq dry source of radium-226. Generally however a steady state radon concentration between 3000 and 6000 Bq/m^3 was used. Continuous monitoring of the radon was carried out by using a flow through Lucas cell mounted on a photomultiplier tube. Grab samples of room air were also taken using calibrated Lucas cells. Time integrated mean radon concentrations in the room were made using closed passive alpha track detectors (CR-39 type). A Nolan-Pollak condensation nucleus counter was used to determine aerosol concentrations in the room. The production of ions and electric fields in the room was achieved by means of a centrally mounted fine stainless point driven by a stabilized 0 to ± 30 kV variable D.C. supply. Unipolar ion production of either polarity could be achieved by changing the polarity of the point. The ion current typically ranged from 0.3 to 2.5 microamps. It was also possible to produce an electric field with no measurable ion current by covering the high potential point with a blunt polythene sheath thereby reducing the field gradient at the point to a sub-corona discharge configuration.

Modifications of the general room field configuration could be achieved by placing grounded sheets of aluminium foil on the room walls but the inevitable presence of metal in the form of conduits, piping, radiators etc. meant that the field configuration could not be accurately determined.

Experimental Results

In all experiments to determine the reduction of Rn-d PAEC filter samples were always taken before the application of the electric fields or ion production air treatments to ensure that approximately steady state levels in airborne activity had been achieved in the room air. The percentage reduction in PAEC at any time during the air treatment was determined from the ratio between the PAEC at that time and that prior to the treatment. The results of a series of experiments are shown in Figures 1 and 2. It can be seen that the greatest reduction (94%) in PAEC was achieved by having the point at a positive potential of 30 kV with positive ions being produced. It was found for any potential level if the discharge was driven by a positive potential on the point that the reduction was substantially more effective than for a negative potential and negative ions. As can be seen in Figure 1 the reduction in PAEC due to negative ions and field was only marginally more effective than that of the field only situation with the point at a positive potential. It is also shown in Figures 1 and 2 that for a particular potential the time taken to achieve maximum PAEC reduction decreased with increasing potential. For example as shown in Figure 2 at +30 kV the final steady state PAEC level is reached in about 1.5 hours while at + 5 kV the time to reach steady state is about 4.5 hours. Some tests were also carried out to determine the PAEC reduction capabilities of small inexpensive commercially available "clean air ion generators". It was found that one of these of Danish design could reduce the PAEC in the room air to 20% of its original value in 2 hours.

In addition to reducing PAEC it was observed that substantial changes in both the equilibrium factor F (with respect to PAEC) and the unattached fractions of Rn-d occurred as a result of the application of the field and ion air treatment. As a result of the air treatment the F factor could drop from its typical pre-treatment value of about 0.5 to as low as 0.05 to 0.1. This reflects the significant removal of airborne Rn-d activity to the room surfaces. While the total PAEC was always reduced the unattached fraction of Rn-d was always found to increase due to air treatment from about 0.1 to 0.3 thus reflecting the overall reduction in the total aerosol concentration in the room air. In the case of individual Rn-d activities this relative change before and after air treatment to increased unattached fractions while at the same time the total PAEC was reduced is illustrated in Table 1.

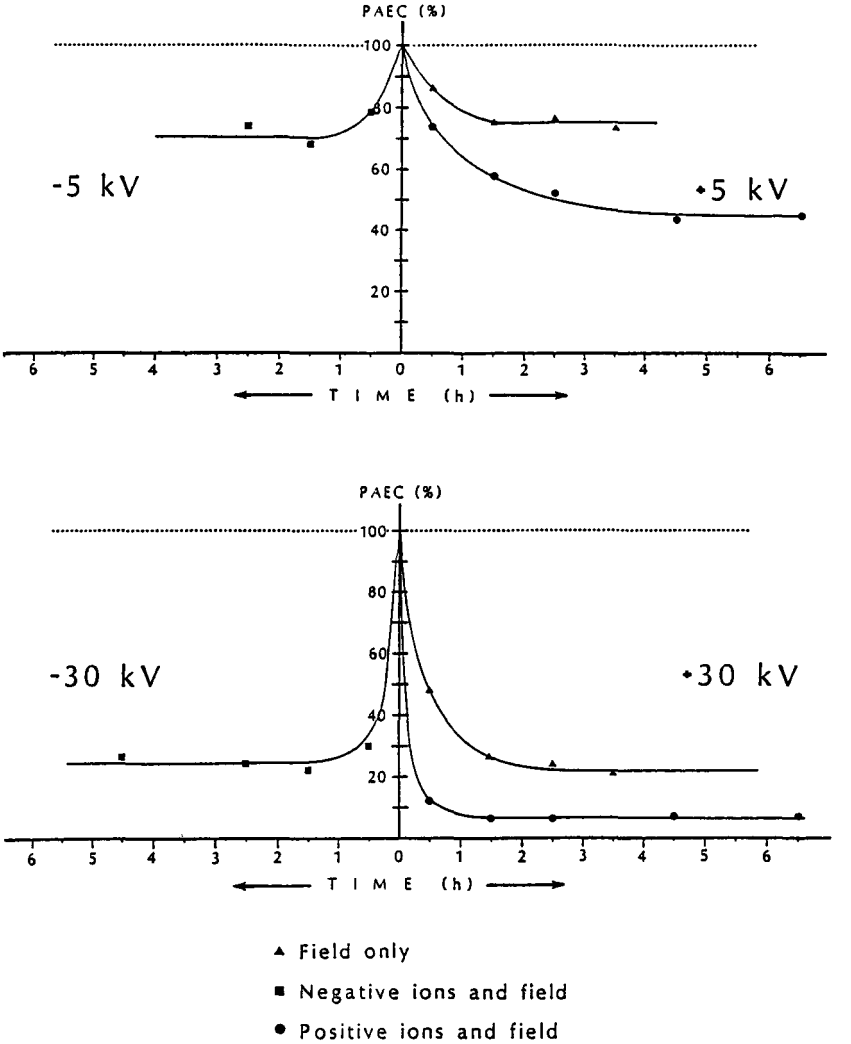


Figure 1: Reduction in Rn-d PAEC by fields and ions.

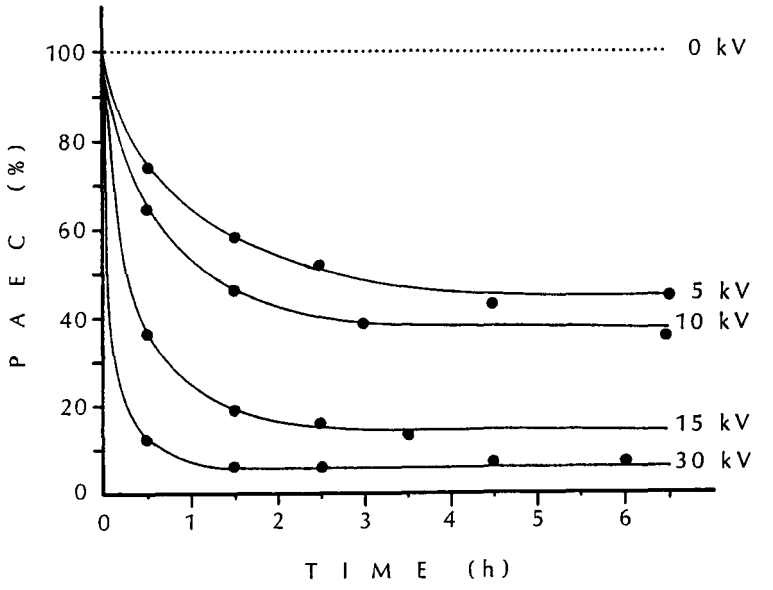


Figure 2: Reductions in PAEC by positive fields and ions.

Table 1

Radon Decay Product	Attached : Unattached Proportions	
	Before	After
Po-218 (RaA)	0.73 : 0.27	0.23 : 0.77
Pb-214 (RaB)	0.93 : 0.07	0.43 : 0.57
Bi-214 (RaC)	0.92 : 0.08	0.40 : 0.60

One of the difficulties with experiments of the type described here is to know if the technique can be effectively and predictively applied in the rooms and airspaces of real dwellings. Some attempts have been made in recent years to develop models to explain the mechanism by which the combined effect of electric fields and unipolar ion production result in substantial reduction in PAEC. These models may be used to describe experiments carried out in well controlled conditions in environmental chambers but at present are unlikely to be applicable to rooms in real dwellings where the very variable geometrical distribution of metal objects within rooms will make the achievement of a desired field configuration almost an impossibility.

A second difficulty with the air treatments described here is that a reduction in PAEC in a room may not necessarily result in a corresponding reduction in lung dose to a room occupant.

The crux of the matter in regard to lung dose reduction is that current lung dose models ascribe a much greater dose per unit exposure to inhaled unattached Rn-d activity than to the attached component. In a recent review of current lung dose models James has shown that the estimated lung dose per unit exposure due to unattached Rn-d activity (approx. size 1-10 nm) may be greater by a factor of 10 or more than that due to the attached component (approx. size 0.05-0.2 micrometers). On this basis a large reduction of PAEC due to these electric field and ion air treatments may be considerably offset by a relative dose increase as a consequence of the observed increase in the unattached fraction. This possibility, like the models on which it is based, has not yet been experimentally confirmed or refuted. For the air treatments described in this work the application of current lung dose models suggests that the estimated percentage reduction in lung dose is about half of the experimentally determined percentage reduction in PAEC. Notwithstanding these considerations the present work clearly demonstrates that substantial reductions in Rn-d PAEC can be achieved in rooms by means of electric fields and ion production. These techniques can be considered as supplementary to primary methods of reducing doses arising from radon reduction in which radon entry to the dwelling is prevented or minimized.

PLATEOUT OF RADON DECAY PRODUCTS

Radon decay products (Rn-d) in either the unattached or attached state may deposit on surfaces thereby becoming removed from the air space and being no longer available for inhalation. In a room of volume V and surface area S under well-mixed conditions the rate of removal of the airborne activity of the ith decay product of concentration C_i is given by $\lambda_p C_i$ where the plateout rate constant $\lambda_p = \upsilon_p S V^{-1}$ and υ_p is the plateout velocity. The value of υ_p will depend on the size of particle containing the activity of the ith decay product and on conditions in the room such as air movement, electric fields etc. In the initial part of the project work Rn-d plateout rates were derived via room model equations from measurements of radon and airborne activities (both attached and unattached) in the air of the sealed room. Aerososl (condensation nuclei) concentrations Z from as low as $5x10^{+3}$ cm^{-3} to greater than $10^{+6}cm^{-3}$ could be produced in the room by electrically heated nichrome or by gas combustion so that a wide range of plateout rates could be achieved. At the highest Z values the percentage of unattached Po-218 was found to be almost zero while at the lowest Z values it was as high as 33%. Plateout velocities for unattached Po-218 were calculated to range from 0.7 $m.hr^{-1}$ to almost 11 mhr^{-1}.

Because the application of electric fields combined with unipolar ion production reduces Rn-d PAEC by causing enhanced plateout of the airborne activity it was decided to investigate the spatial variation of plateout and airborne activity in the room both without and during the application of electric field based air treatments. This was carried out by placing passive alpha track detectors in various surfaces in the room to detect both plateout and airborne alpha activity. Two types of alpha track detectors with quite different alpha energy responses were used i.e. CR-39 and LR-115. The CR-39 is capable of producing an alpha track over the full alpha energy

range of the Rn-d activity (i.e. up to 7.68 MeV). The LR-115 Type II as used and processed in this work in effect produces a measurable track for particles less than 4 MeV. These differences in alpha particle registration characteristics were exploited to advantage. A bare piece of LR-115 mounted on a room surface effectively "sees" only alpha particles emitted within a small air volume some centimetres away from its surface and gives no record of the 6.00 and 7.68 MeV alpha particles emitted by plateout activity on its surface. The LR-115 so used behaves as an excellent detector of airborne Rn-d activity and of radon gas. A piece of bare CR-39 will on the other hand produce an alpha track both for all airborne alpha emitters within alpha range and will also produce tracks from plateout Rn-d alpha emitters decaying on its surface.

Bare alpha track detectors of both types were mounted at various positions on walls, floors and ceiling both under normal (no treatment) conditions and also when various electric fields and ion currents were used. Figure 3 shows the results of such measurements. In what is termed "modified field" conditions a grounded aluminium foil sheet was mounted high on a wall in an attempt to change the field configuration to cause increased field/ion driven plateout onto upper surfaces such as the ceiling or high on walls. In Figure 3 for each experiment the alpha track densities measured by optical microscopy were normalised for each experiment and are presented as track production rates as recorded on the surface of the plastic detectors per unit activity of radon gas in the room. The radon gas concentration was measured by closed CR-39 calibrated passive radon detectors.

An examination of the LR-115 measurements presented in Figure 3 shows that during all conditions the total airborne activity (from radon gas and Rn-d) was fairly constant at all measuring points thus indicating good mixing of airborne activity throughout the room. The decrease in LR-115 track production rate shown then the fields were used is interpreted as being due to the removal by field enhanced plateout of alpha activity from the air. The remaining airborne activity during field conditions appears to be also constant throughout the room. Some additional decrease in the track production rate for LR-115 was expected in some parts of the room when the modified was used but this did not occur. A possible explanation is that LR-115 is a material that easily becomes electrostatically charged. It is thus likely that its own field combined with the regular field may have caused an almost complete local depletion of charged Rn-d within the small detecting air volume close to the plastic such that no further decrease in airborne activity close to the LR-115 occurred when the field was modified. Further work is needed to verify this hypothesis.

In the case of CR-39 mounted on surfaces the strong effects of the fields on enhancing plateout are very clearly shown in Figure 3. It should also be noted in Figure 3 that when the ordinate scales of the two plastics are compared the greater sensitivity of CR-39 than LR-115 for alpha detection is apparent. With no field treatment a low but fairly constant track production rate is found throughout the room thus reinforcing the observation form the LR-115 data that good mixing of radon

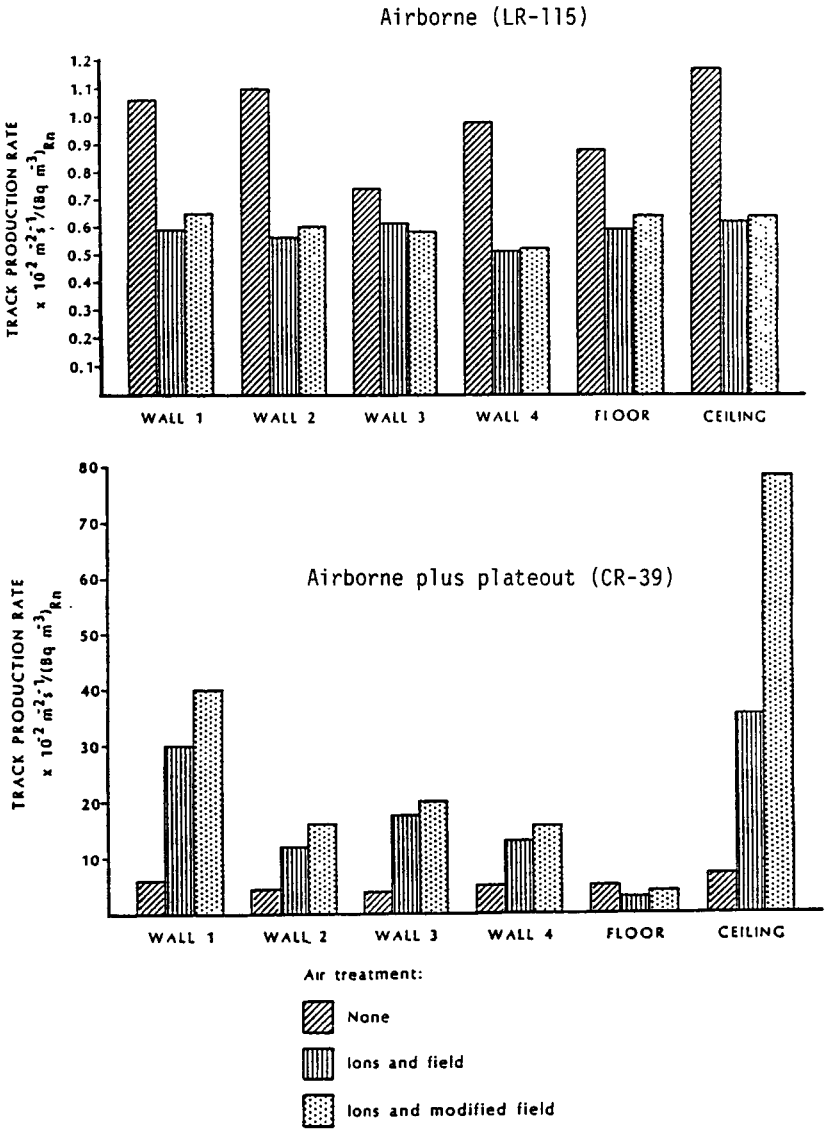


Figure 3: Spatial distribution of Airborne and Plateout activity for various air treatments.

exists in the room. When a field with ions is used substantial increases in track production rates were recorded, when a field combined with ion production was used, for all surfaces except the floor where even a slight decrease was noted. This spatial pattern of track production rates suggests that in the room used the unmodified field configuration established by a single steel point at a high potential in the presence of the regular complement of room metal fittings is such to cause Rn-d activity to plateout preferentially towards walls and ceiling and away from the floor. When the modified field is used this tendency is reinforced with a dramatic increase in plateout occurring in particular on the ceilings. This again is interpreted for this particular room as being due to the new field configuration being such as to concentrate field enhanced plateout activity towards the upper surfaces of the room. In rooms in houses with the inevitable presence of metal objects and such devices as televisions it is almost impossible to predict the spatial deposition with and without additional fields. The experiments described however give tentative evidence that plateout patterns in homes may be measured and in principle successfully modified by imposing configured electric fields.

A New Technique to Measure Rn-d Plateout

A common method used to measure Rn-d plateout on surfaces is by measuring the accumulated alpha activity on metal foils mounted on surfaces in rooms or chambers containing radon. As Rn-d effectively reach equilibrium on a surface after about four hours long term exposure of such a foil does not increase the sensitivity of the method. During the course of the project a new technique to measure plateout activity was invented which in principle is inherently more sensitive and accurate than the stationary metal foil method.

The new technique is very simple in concept. A small piece of alpha track plastic (CR-39) is driven slowly at constant speed and with precision over the surface of a smooth material such as glass or stainless steel onto which radon decay products are depositing and have established equilibrium activity. The detector to surface gap should ideally be zero (i.e. 2π counting geometry) but in practice a gap of 1 mm or slightly less is achievable. As the detector leading edge moves over a particular point of Rn-d activity on the surface all further plateout is stopped at that point and the activity decays with its characteristic half-life. The recorded alpha track density distribution on the underside of the CR-39 thus becomes a time record of the decaying Rn-d surface activity. When the point on the surface re-emerges from beneath the trailing edge of the CR-39 plateout re-commences and the surface Rn-d activity grows back to its original equilibrium value. If the dimensions and speed of the detector are accurately known the time distributed alpha track record on the CR-39 may be analysed using a modification of some gross alpha activity analysis procedure (i.e. Thomas method) to determine the equilibrium activities of the individual radon decay products deposited per unit area of the glass surface. From these values plateout velocities can be determined. A number of simple prototypes of devices using this technique were made and used. The simplest of these used as a driving motor an electric clock with the CR-39 mounted on the hour

arm. As the transit time of the detector (21 mm x 21 mm) was half an hour by the time it returned to cover the same spot on a glass surface a time interval of 12 hours had elapsed and the surface Rn-d activity was back at its original value. The first clock mechanism was operated at 220 V.A.C. and the glass surface showed an activity up to three times that measured when the mechanism was clockwork or a low voltage D.C. quartz clock mechanism was used. Although the method is intrisically correct many technical difficulties were encountered with mounting and maintaining the detector continuously parallel with a 1 mm (or less) gap above a glass or metal surface. Towards the end of project it was decided to build a device using a high precision stepping motor mounted on a heavy steel base in which the detector moving linearly across the glass surface and its distance to the surface can be maintained accurately. Stationary pieces of plastics mounted above the glass are being used as controls and for the purpose of quantifying alpha detecting geometry edge effects. It is intended that devices of this type will be used in actual dwellings. In particular they will be used in a planned project with the University of Lund on the retrospective assessment of radon exposure from long-lived Po-210 activity resulting from the decay of deposited Rn-d on glass in dwellings. The new plateout devices will determine if contemporary plateout rates in these dwellings are compatible with the measured Po-210 activities in the glass which is important in assessing if the historic and comtemporary radon levels in these houses are similar. Until the new plateout devices are throughly tested it is premature to quote with confidence the actual values of plateout velocities determined by this new technique except to state that they were found to be in the range from 1 to 10 $m.hr^{-1}$.

CONCLUSIONS

Substantial reductions of Rn-d PAEC in an experimental room by means of electric fields with and without unipolar ion production was demonstrated. It was further shown by modifying the configuration of the fields that the plateout activity removed from the air space could be concentrated if required onto specific surfaces in the room such as the ceiling and away from the floor. The electric field air treatments described here were found to increase the unattached fraction of Rn-d which on the bases of current lung dose models may lessen their effectiveness in reducing lung dose. In view of the developmental nature of these yet to be experimentally verified lung dose models this aspect of the field based air treatments remains an open question. By means of a new technique invented during the project it will in future work be possible to make more accurate measurements of Rn-d plateout than heretofore.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]: Dr. Niels Jonassen, Technical University of Denmark Laboratory of Applied Physics 1. Lyngby, Denmark.

V. Publications: McLaughlin, J.P. and Wasiolek, P. "Experimental Room Studies on the Reduction of Radon Progeny Concentrations using Unipolar Ions and Electric Fields".
Proceedings of EPA Symposium on Radon and Radon Reduction Technology, Denver, Colorado. October 1988.
Wasiolek, P. "Indoor Radon in Ireland: Exposures and Laboratory Dose Reduction Studies". Ph.D. Thesis. National University of Ireland. August 1989.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Risø National Laboratory
P.O. Box 49
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Contract no.: BI6-F-296-DK

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Title of the research contract:

Validation experiments for near-site region atmospheric dispersion models.

List of projects:

1. Validation experiments for near-site region atmospheric dispersion models.

Title of the project no.: BI6-F-296-DK

VALIDATION EXPERIMENTS FOR NEAR-SITE REGION ATMOSPHERIC DISPERSION MODELS.

Head(s) of project:
Torben Mikkelsen

Scientific staff:
Leif Kristensen, Søren Thykier-Nielsen
Hans L. Pécseli, Hans E. Jørgensen (Ph.D - student)

I. Objectives of the project:
From full-scale diffusion experiments to establish reference data sets of instantaneous crosswind concentration profiles, from which uncertainties in atmospheric dispersion models, used in near-site probabilistic accident consequence codes, can be assessed.

II. Objectives for the reporting period:
Starting in 1988, the first year was devoted to preparation and field testing of a new Mini-LIDAR system for remote measurements of instantaneous concentration profiles inside smoke and aerosol plumes.
Upon improvements of the LIDAR-system, including its data processing and interpretation programs, the second year of research was allocated for the conduction of full-scale experimental campaigns, - and to process and quality assure the measurements of crosswind plume concentration profiles. They in turn should be statistically analyzed and classified, together with simultaneously measurements of turbulence and mean meteorological data, - into a series of well-defined diffusion experiments, suitable for intercomparison, validation and uncertainty studies for near-site atmospheric dispersion models.

III. Progress Achieved:

1. INTRODUCTION

A full-scale experimental study of the natural variability and fluctuations in dispersing aerosol plumes have been conducted in the atmospheric surface layer, using LIDAR for remote sensing of instantaneous concentration profiles in cross-sections of a plume, released from a ground-level point source.

Measurements of the near-ground, cross-wind, concentration profiles were obtained at ranges of between 100 m and 1000 m with high spatial (1.5 m) and temporal (3 sec) resolution during three experimental campaigns: BOREX '86, '88, '89. The experimental campaigns have to date resulted in a total of more then 30 individual, multi-hour lasting trials, conducted under a variety of different meteorological situations.

The dispersion experiments have been supported by detailed measurements of wind and turbulence from sonic anemometers, meteorological tower data, and from intensive launching of radio-sonde balloons.

The objective of our study has been to establish a data base, consisting of series of instantaneous cross-wind concentration profiles, from which fundamental plume statistics, - relating to the inherent uncertainties in performance with radiological atmospheric dispersion modelling, can be revealed.

2. EXPERIMENTS

The BOREX dispersion experiments took place over flat and homogeneously vegetated terrain: First, in August 1984, as a feasibility study at the Borris Moors south of the Danish town Borris in western Jutland, - from where the campaigns still carry their names (BORris EXperiments).

Subsequently, in August 1986, in October 1988 and again in August 1989, experiments took place at the Meppen proving grounds in the north-western part of the Federal Republic of Germany (BOREX '86, BOREX '88 and BOREX '89).

These sites were chosen as ideal for micro-meteorological dispersion experiments due to their flatness and homogeneity. The vegetation consisted of natural growing grasses and heather plants, which produced a homogeneously distributed roughness over the terrain of the order of 1 cm.

The general outline and set-up for the experiments are shown in Fig. 1

2.1 Meteorological Instrumentation

Conventional, but also rather sophisticated meteorological

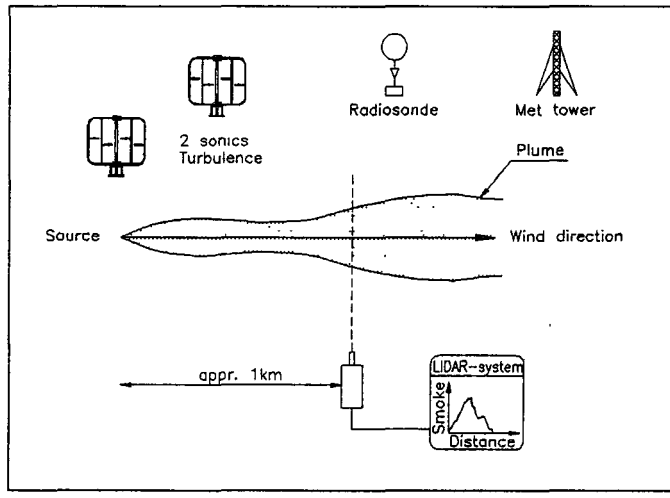

Fig. 1 Schematic experimental setup for the BOREX experiments.

instrumentation were committed to support the plume dispersion experiments. Meteorological data from here accompanies the concentration-profile data-base for the evaluation study of plume statistics.

Mean wind and turbulence, including atmospheric stability, were monitored with two 3-axis acoustic sonic anemometer-thermometers (Kaijo Denki type DAT 310) situated on 10 m high meteorological masts immediately upwind of the aerosol-generator. Radio-sondes were launched as often as every third hour to monitor upper-air winds and the inversion height. A nearby 100 m high meteorological tower was in routine operation and measured 10-min averages of the vertical mean wind and temperature profiles.

2.2 Generation and Detection of Aerosol-plumes

2.2.1 Source

A powerful and sturdy aerosol-generator, designed and constructed by Dynamit-Nobel, FRG, was used to produce continuous surface releases of sub-micron aerosols plumes consisting of conglomerated SiO_2 and NH_4CL, which could easily be detected by the LIDAR. The aerosols were formed by mixing the liquids $SiCL_4$ and a 25% water-solution of NH_4OH in the neutralizing stoichiometric ratio (1:3.2) into a strong-wind venture. Depending on the atmospheric stability, and the flow-rate of the chemicals, this plume was visible kilometers downwind, and detectable even at further downwind distances by the LIDAR.

2.2.2 Aerosol measurements

Particle number concentrations and particle size distributions were measured at several downwind distances, both inside and in the margins of the meandering plume, by means of a white light optical particle counter (type Polytec HC-15). This instrument covered a particle radii size range from 0.16 um to 5.4 um, with 126 sizing bins for small aerosol particles.

Fig. 2 shows five size distributions measured in the time between 13:02 and 13:05 on October 13. during BOREX '88. When size distributions with coincidence errors less than 10 % are compared, the shape and form of the distributions turned out to be remarkably consistent and reproducible.

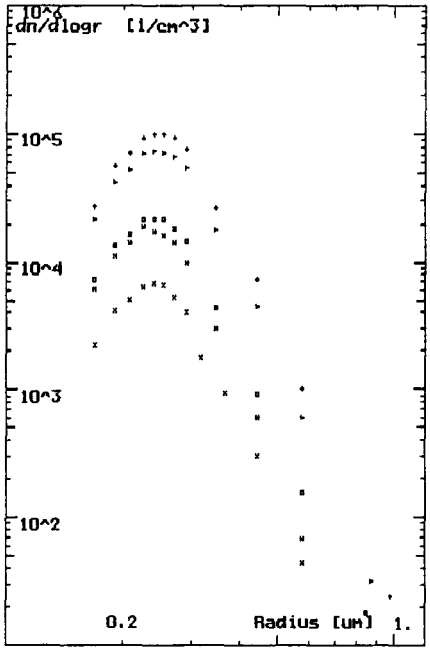

Fig. 2. The artificial aerosol-plume's size number distribution, dN/dlogr vs. radius r, measured during BOREX '88.

Five size distributions are shown, sampled consecutively over 30 sec's intervals on October 13. during Run 13/2.

Particle number concentration per cm^3, coincidence error, and position are from top down:

51826 (9%) plume center; 36806 (6%) margin; 10324 (2%) margin; 8053 (1%) almost outside; 3038 (1%) almost outside.

2.2.3 LIDAR

The very central instrument, used during the experiments for detection of instantaneous "snapshots" of the crosswind profiles of in-plume concentrations, was a new Mini-LIDAR system, originally designed by the German Aerospace Research Establishment, Department of Opto-electronics, and build under licence for our purpose by IBS GmbH, Grafrath (FRG). It consists of a pulsed laser (Nd:YAG 1064 nm), a telescope with build-in high-speed photo-detector and pre-amplifiers, a transient recorder, and a PC-controlled data storage and monitoring facility.

Its principle of operation is similar to the commonly known radar-principle of remote detection, only does the LIDAR use coherent light (from a laser) instead of microwaves: Pulsed laser radiation of very short duration (pulse length less than 1 m) is transmitted from the laser and horizontally through the artificially generated aerosol plume, where a small fraction, proportional to the number of aerosols in the measurement volume, is back-scattered into the telescope. The time (in nanoseconds) between transmission and reception of a light pulse indicates the range of the particles from the LIDAR. At the same time, the intensity of the signal is a measure of the particle concentration in the small volume occupied by the travelling light pulse.

Including the finite width of the laser pulse, the response time of the photo-detector and of the pre- and transient recorder amplifiers, the overall impulse response time was maintained as low as 10 nanoseconds, which corresponds to an effective distance-constant of the instrument equal to 1.5 m. The laser-beam divergence was 3 milli-radian, corresponding to a 3 m pulse beam-width at the 1 km range. Most plumes were measured from a distance perpendicular to the plume-axis of less than 300 meters whereby the effective volume of measurement was 1.5 m in length and of near one meter in diameter.

The photo-detector signals were sampled and digitized at 250 MHz, corresponding to one fixed-point measurement through the plume every 0.6 meter. With an effective distance-constant of the instrument equal to 1.5 meters, however, this corresponds to an over-sampling rate of 2.5.

The signal-to-noise ratio of a LIDAR-system deters partly with increasing band-width of the pre-amplifiers, but in particular with range (to the second power). Without artificial aerosol generation, an unity signal-to-noise ratio could be detected at ranges between 150 m and 300 m, depending on the background aerosol concentration (typically this was of the order of 100 particles per cm^3). During experiments, however, aerosol concentrations at the 100 m mark downwind distance from the source typically exceeded 100000 particles per cm^3 , yielding a plume centerline signal-to-noise ratio as high as 1000 (60 dB).

From a variety of different downwind positions, the mini-LIDAR was used to record instantaneous high-resolution "snapshots" of the crosswind concentration profile, with an effective spatial resolution of 1.5 m, and with a repetition cycle between shots as high as 1/3 Hz. Detailed information about the meandering and fluctuating plume, including its position, size and shape, was in this way acquired for a variety of different meteorological situations.

3. DATA REGISTRATION AND PROCESSING

3.1 Wind and Turbulence

Signals from the sonic anemometers were processed real-time during the experiments via an on-line, fast-scanning data acquisition system. This system provided consecutive 10-min averages of the mean wind speed and direction, in addition to the co-variance matrix of the fluctuating down-, cross-,and vertical wind components (u', v', w') and temperature (T'). Key parameters for classification of the meteorological conditions and atmospheric stability, including the turbulence intensities, surface-layer shear-stress (u^*), and the surface-layer heat flux (H_0), were in this way available already during the experiments.

In addition, analog-recording of the raw sonic-anemometer signals were performed for subsequent spectral analysis, and for use with dispersion simulation (plume advection) in real-time Lagrangian dispersion models and training systems.

3.2 LIDAR - Signals

Extensive data-processing of the measured (in-plume) backscatter profiles have been undertaken in order to correct the LIDAR measurements for range-dependance and for in-plume extinction. The so-called LIDAR-differential equation have been solved to yield volumetric backscatter coefficients as function of range by various techniques, including the Klett-method. Best results, however, were obtained with an iteration-method suggested by our collaborators at DLR, FRG. To compensate for the extinction (damping) of the light pulse as it travels through thick aerosol plumes, an estimation of the extinction-to-backscatter ratio (18.0), appropriate for the artificial aerosol plume, was used.

A background visibility of 20 km, corresponding to an extinction coefficient of 2E-4 [m^{-1}] was used as an initial estimate in the iteration procedure. The threshold value applied for clipping of background noise was 1.5 times this value. By inclusion of the system-constant for the mini-LIDAR in the iteration procedure, the photo-detector signals could be transformed into cross-section profiles of volumetric backscatter coefficients for the plume. The backscatter-coefficients in turn, we assume are proportional to the particle number concentration per unit volume, i.e. to the concentration. The constant of proportionality, however, must be calibrated with aerosol counter measurements taken from inside the plume from experiment to experiment.

4. RESULTS AND DISCUSSION

As a result of the BOREX experiments, there now exist high resolution meteorological and diffusion data sets composed of 29 multi-hour lasting aerosol plume trials, 25 days of surface and upper air meteorology and select periods of special measurements including aerosol size distributions, aerial photography, ground-level video recordings and photography, see Table 1.

Year (Period of Exp)	Day	Month	Measuring Period (MEZ)	Phase (Date of Exp.)
	06	10	15:49 - 18:00	06/3
	07	10	07:55 - 10:30	07/1
06 - 10 Oct 1986	08	10	07.12 - 09·48	08/1
	09	10	11:47 - 14.53	09/2
	09	10	16:49 - 18:04	09/3
	11	10	15:52 - 17.41	11/3
	12	10	11:58 - 13:12	12/1
	12	10	16:26 - 17.47	12/2
10 - 14 Oct 1988	13	10	09:33 - 10:50	13/1
	13	10	11:08 - 14:00	13/2
	13	10	15:30 - 17 42	13/3
	09	08	10:42 - 14:50	9/1
	09	08	15:33 - 16:37	9/2
	09	08	16:54 - 18:55	9/3
	10	08	09:10 - 09:14 10:35 - 13.48 13:56 - 14:10	10/1
	10	08	17.05 - 17:58	10/2
	10	08	18:40 - 19:30	10/3
08 - 18 Aug 1989	11	08	09:24 - 09:54 09·59 - 10:21	11/1
	11	08	10.35 - 11:02	11/2
	14	08	11:57 - 14:39 15:05 - 19:54	14/1
	15	08	14:20 - 17 24	15/1
	15	08	19:04 - 21 00	15/2
	17	08	09:30 - 10·57	17/1
	17	08	11:36 - 13·00	17/2
	17	08	16.00 - 20:30	17/3

Table 1: Borex 86/88/89 Measurements

As an example of the data base, measurements of meteorology and plume statistics is presented next for the unstable subset of concentration fluctuation profiles recorded between 16:29 and 17:45 during the BOREX '89 Run 17/3 experiment:

4.1 Meteorology

Thursday the 17. of August 1989 was a clear sunny day with few, scattered cumulus clouds riding on top of a 1200 m inversion cap. Winds came from the west with speeds between 4 to 6 m/s during the day. A transition from unstable to stable flow regime was experienced around sunset at 19:00.

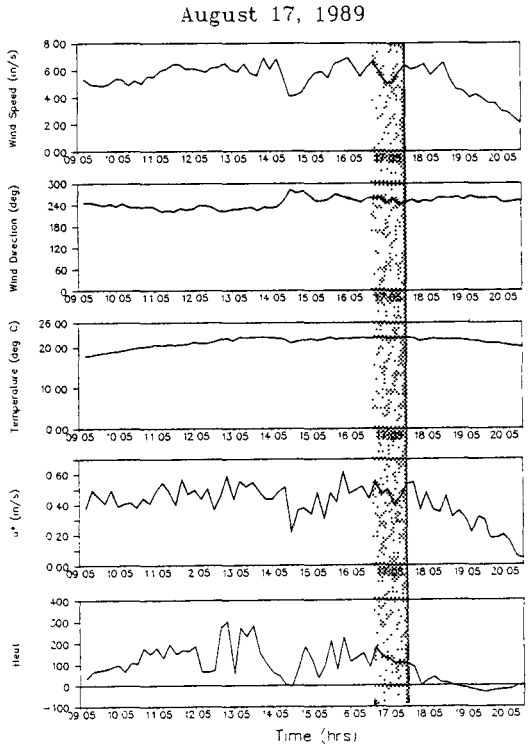

Fig. 3. 10-min averaged meteorological quantities, measured with sonic anemometer on August 17. during BOREX '89. The shaded period (16:29 to 17:45) marks the unstable experiment selected for analysis. Quantities shown are: Wind-speed, Direction, Temperature, Mechanically induced shear-stress (turbulence level), and turbulence-transported heatflux (stability measure).

Figure 3. shows profiles with time of the 10-min averaged quantities of wind speed and direction, temperature, mechanically induced shear-stress and surface heatflux as measured with the sonic anemometer upwind of the source location. The shading marks the time interval of the unstable subset (between 16:29 and 17:45) of Run 17/3 presented here.

Figure 4. shows the high-resolution (10 Hz) digitized time-series of the sonic's fluctuating velocity and temperature signals from the period between 14:51 and 17:39, and Figure 5 presents the turbulent energy spectra of the three velocity components (u', v', w'), calculated from the time-series in Fig. 4 between 16:40 and 17:35.

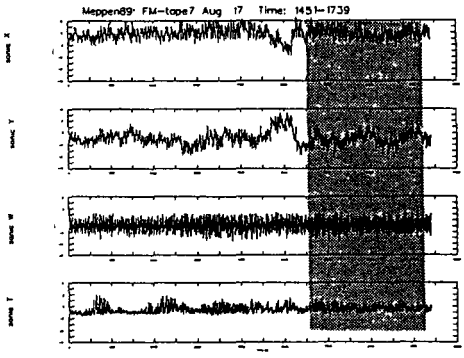

Fig. 4. High-resolution (10 Hz) time series of fluctuating wind components and temperature signal on August 17. The shaded period marks the interval between 16:40 and 17:35, selected for the spectral analysis.

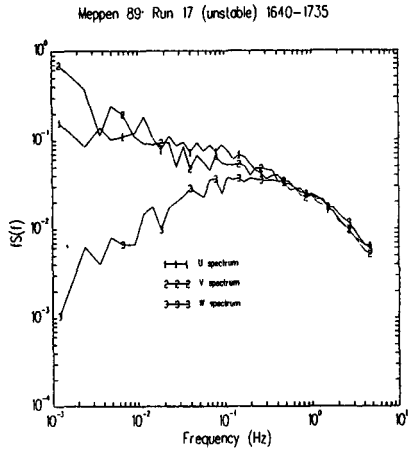

Fig. 5 Ensemble-average of 5 velocity spectra of the windcomponents u', v', w' calcu-lated from the shaded part of the time series in Fig. 4.

4.2 The Crosswind Plume Structure

In the time period of between 16:29 and 17:45, a series of 872 high-quality, near-ground, crosswind plume profiles were taken at right angles to the plume with the LIDAR-system positioned 160 m downwind of the source.

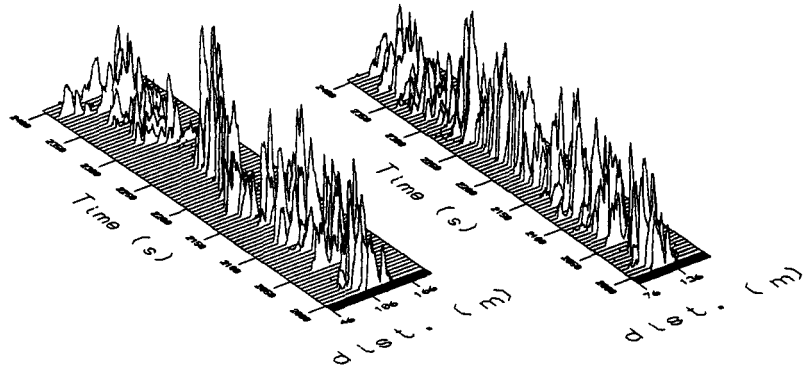

Fig. 6. Fixed-frame (left) and Moving-frame (right) profiles

Figure 6 shows a 400 sec long sequence of these data, holding some 80 measured profiles between 17:03 and 17:09. The profiles are presented in two different ways:

The "Fixed frame" (Fig. 6 left), presents the profiles as they are measured from the fixed position of the LIDAR.

The "Moving frame" (Fig. 6 right), however, shows the same data set, but with the plume-meander component removed. In this frame of reference, the profiles have been aligned in the cross-wind direction with respect to their individual center-of-mass positions, hence the larger scale turbulent eddies, responsible for the plume-meandering, have been removed. Measurements and calculations referred to in this frame are consequently of importance for "in-plume" statistics and for relative diffusion, including puff diffusion.

High-resolution LIDAR-statistics of the crosswind plume structure, based on the entire subset of 872 profiles, is next presented in Figure 7. The Figure again distinguishes between "fixed" (left) and "moving" (right) frame statistics. The Figure shows from top:

1) Mean-concentration profiles.
2) Fluctuation-concentration profiles (root-mean-square).
3) Intensity-profiles, defined as the ratio of 2) and 1).
4) Intermittency-profiles, defined as the time fraction of non-zero concentration values.

Significant differences is seen to exist between statistics calculated in the two frame of references. Where the mean- and fluctuation profiles in the fixed frame exhibits significant irregularities, the moving frame profiles becomes almost Gaussian in form. Notice also that the center-line peak concentration is about 5 times higher in the moving frame, and that the plume width (sigma-value) here is much smaller. The intensity-profiles exhibit the anticipated, characteristic U-shaped form often found from wind tunnel studies, but here with amazing symmetry and "height" for the moving frame case.

The profound difference in peak intermittency, exceeding the 99% level in the moving frame, and barely reaching the 50% level for the fixed frame case, confirms to full that we are dealing with in-plume statistics when meander has been removed.

4.3 The Concentration pdf

For fixed crosswind positions, also the entire probability density function (pdf) could be calculated from the 872 sequential concentration profile measurements. Figure 8 distinguish the pdf's between fixed (left) and moving frame (right) as before, but also divides between centerline (top) and plume margin (bottom).

The important scaling parameter is here again seen to be the intermittency factor. The shape and form of the 99% intermittent moving-frame centerline-pdf, differs from the three other pdf's with lower intermittency by having a non-zero modal value.

When compared with other experimentally determined pdf's, however, it is important to keep in mind, that our pdf's

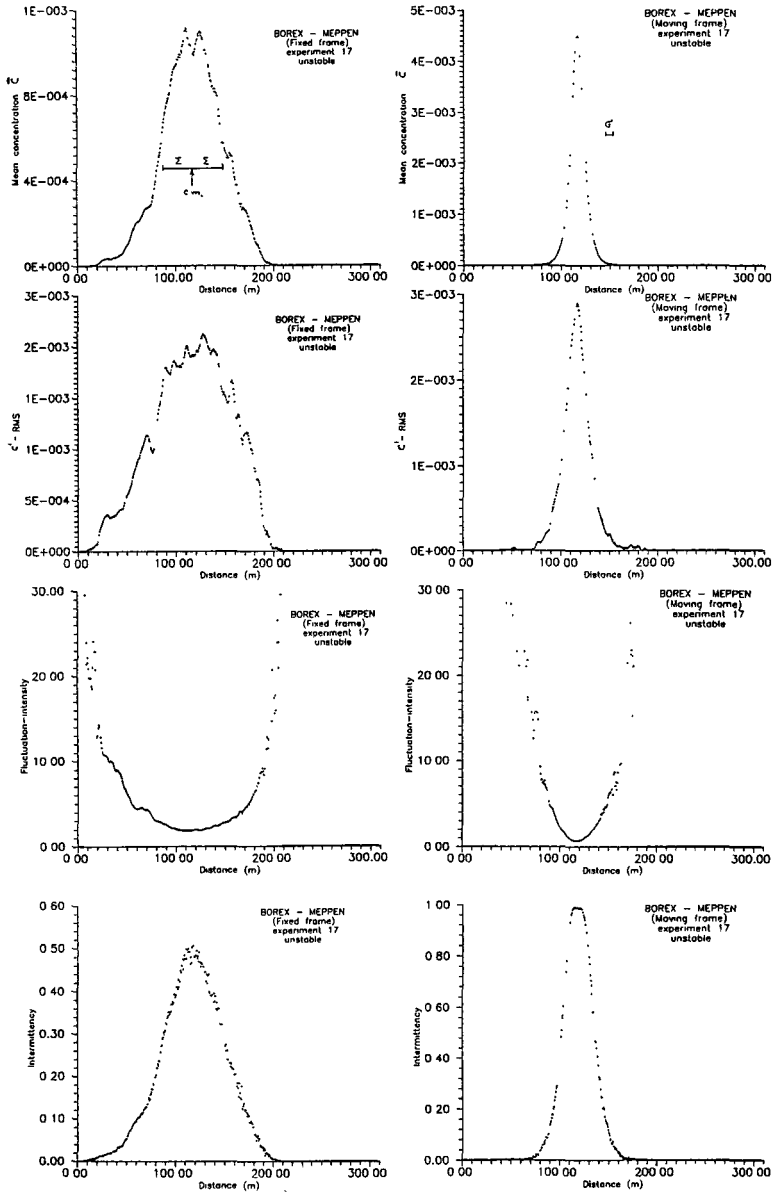

Fig. 7. Crosswind plume structure from unstable subset of Run 17/3. Shown for fixed (left) and moving (right) frames of reference are: 1) Mean-concentration profiles, 2) Fluctuation-concentration profiles, 3) Intensity-profiles and 4) Intermittency-profiles. Abscissae shows distance from the LIDAR, with measurements taken every 0.6 m. Distance from source 160 m. Concentrations are expressed in extinction-value above threshold (2E-4 m^{-1}). Plume standard deviations are: 29.6 m in fixed frame and 8.9 m in moving frame.

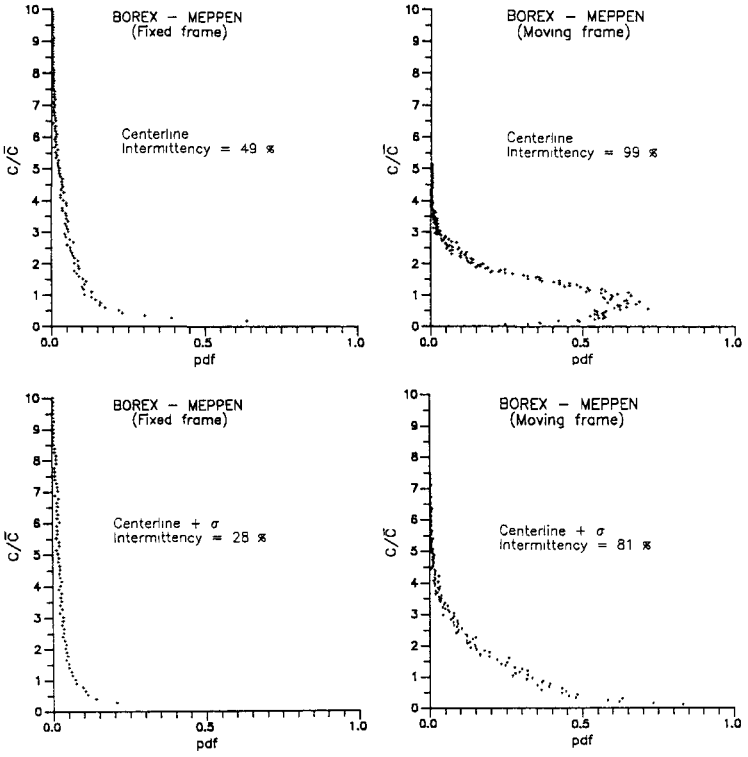

Fig. 8. Probability distribution functions from the unstable BOREX '89 experiment. The figure shows: Fixed frame (left) and moving frame (right) pdf's, at plume centerline (top) and at plume margin (bottom).

have been measured with an effective distance-constant (of the LIDAR-system) equal to 1.5 m.

5. SUMMARY

Full scale diffusion experiments with continuous, surface releases of sub-micron aerosol plumes were conducted. For a variety of different atmospheric stability and wind conditions, measurements were obtained of the ground-level, cross-wind plume-concentration profile, with high spatial (1.5 m) and temporal (3 sec) resolution using LIDAR. Statistics of the concentration fluctuations across the plume have been investigated. In particular, we have distinguished between fixed and moving frame statistics, in order to differentiate between absolute and relative type diffusion.

The BOREX data base provides the Radiation Protection Programme with a series of near-site reference dispersion experiments in which the inherent, naturally occurring fluctuation levels have been investigated. It establishes a reference for inter-comparison and evaluation of near-site atmospheric dispersion models.

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

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S. Borrmann, Institute of Meteorology, University of Mainz, FRG.

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V. Publications:

Weber, H., W. aufmKampe and T. Mikkelsen (1988): Concentration Fluctuations Measured in the Atmospheric Surface Layer. In: Eighth Symposium on Turbulence and Diffusion (Published by the American Meteorological Society, Boston, MA, USA).

Mikkelsen, T., H. E. Jørgensen, W. aufmKampe, H. Weber and S. Borrmann (1990): The Effect of Finite Sampling Volumes on Measured Concentration Probability-Density-Functions. In: Ninth Symposium on Turbulence and Diffusion (Published by the American Meteorological Society, Boston, MA, USA).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no : BI6-F-121-F

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Title of the research contract:

Comparative risk evaluation on a regional scale.

List of projects:

1. Comparative risk evaluation on a regional scale.

Title of the project no.: 1

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Scientific staff:

5 persons

I. Objectives of the project:

- The aim of this project is to evaluate the various types of nuisances (chemical and radiological) resulting from different sources (industrial, agricultural, domestic, natural,...) affecting the population of a region (South-East of France) in order to put them into perspective.
- The management of these nuisances does not allow to privilege one or the others of these exposition sources. First of all, we have to evaluate them in real conditions as in a specific area.
- In addition to this analysis, a reflection about the possibility to incorpore results obtained from accidental situations with those resulting from normal operation is conducted in order to give informations to the deciders. The decision making in matters of pollutions in France is also studied.

II. Objectives for the reporting period:

During the reporting period, the exposures resulting from heating (residential and tertiary industries) and indoor pollution were achieved. Results obtained early (1982) for electricity generating power were updated.

Finally, all results obtained during this project were described in several annexe reports, each of them being devoted to a specific subject, and the synthesis of the project was performed.

III. Progress achieved:

1/- INTRODUCTION

The aim of the "Greater Rhône Delta Programme", in progress at IPSN since 1981, consists in a comparative assessment of chemical and radiological nuisances to which the population of the South-East of France area is exposed, in order to determine the relative importance of the various sources (i.e. industrial, natural, domestic,...) for one or more pollutants and to manage them.

This programme was successively partly funded under two contracts with E.C.C. (DG XII)[1]. Results undermentioned, take up the main obtained data, those of the first contract have been updated when possible.

The different emission sources studied during normal operation of activities were:
- electricity generating cycles : coal, oil and uranium;
- use of energy (heating) in residential and tertiary industry sectors;
- specific industries according to the free use of the data;
- certain medical activities as radiological diagnosis;
- natural radiations;
- indoor: radon, consumer home products, combustion processes as open fire (fireplace, kitchen,...).

The objective of accidental risk assessment is to compare nuisances resulting from accidental and normally operating situations. The methods used for risk evaluation are described and incorporation of results resulting from accident with those relating to normal operations is assayed. A generic classic pragmatical viewpoint reliability of which the theories are indissociable from risk analysis, are developped.

Finally, management of pollutions leads to the study of the decision making in matters of pollutions in France and the possibility of taking into account such a study as the Greater Rhône Delta Programme by decision makers.

2/- THE GREATER RHONE DELTA AREA

The greater Rhône Delta area (figure 1) covers approximately the south-east segment of France given one fift of the national territory and one fift of the population. Geographical, geological, hydrological and climatological data obtained allowed us to place human in his environment and to build up databanks which were used during the evolution of the study.

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[1] contracts BIO.F.320.81.F (1981-1985) and BI6.F.121.F (1985-1989) between the E.C.C. (DG XII) and "le Centre de Développement des Etudes et Applications en Hygiène et Sécurité (C.E.D.H.Y.S).

3/- EMISSIONS AND EXPOSURE SOURCES

3.1. Electricity generating cycles

The coal, oil and uranium cycle plants were situated on figure 2. The contribution of the area to the national electricity production reaches 40% at least. The part of the nuclear cycle to this production reached 70% in 1987.

The emission rates of electricity generating cycle plants are provided from plant managers or ministerial departments, from measurements in this plants or from general literature when there are some problems of confidentiality.

3.1.1. Atmospheric releases

The local and regional exposure resulting from each source was evaluated for the reference group (maximum individual exposure) and for the population being within a given radius or in the whole area studied (collective exposure). These exposures were evaluated using the atmospheric concentrations of the main pollutants emitted. These concentrations were calculated using an atmospheric dispersion gaussian model taking annual mean weather conditions into account.

The distribution of the calculated concentrations is given either in polar coordinates, in 18 sectors opening at 20° around the emission points, or in orthogonal Lambert coordinates using a grid system 100 times smaller (100 km^2) than the european grid.

In each square, the concentration is considered uniform and equal to that of the geometrical centre. The exposure for people being in each square is evaluated for each pollutant and each main route of exposure (inhaling, external exposure, ingestion) taking into account demographic and agricultural data specific to the area studied. The distribution of the concentrations calculated are illustrated in figures 3 (SO_2) and 4 (U-238).

The individual exposure can be calculated for the reference group but also for the neighbouring groups in order to characterize the exposure profile along two directions from the emission point: in the axis of maximum according to the distance from the source point and as a fonction of the sector for the maximum concentration radius. This phenomena is illustrated on figure 5 for SO_2 and its transformation product SO_4^- released by coal and oil fired power plants. This figure shows a clearer picture of the amount of pollution in the sector concerned.

3.1.2. Liquid releases

Only liquid releases affecting the Rhône river are take into account since hydrological data on other rivers are still inadequate. It should be pointed out that the most recorded releases of the electricity generating cycle plants take place in the Rhône for the area studied.

In the hydrological model used, the Rhône is divided into 9 segments in which the hydrodynamic characteristics are considered as constant. Taking into

account data about eating habits, agricultural productions, irrigation, fishing and sediment transports, the individual and collective exposures were calculated for the main pathways to man: drinking water, consumption of fishes and irrigated products, external irradiation.

For these evaluation, we made an hypothesis: all the agricultural and piscicultural production is consumed by the population, regardless of its geographical distribution. Under these conditions, about 60% of the collective exposure (2 to 3 man.Sv) due to liquid releases is the result of the irrigated product intake.

3.2. Use of energy: residential and tertiary industry heating

As regards yearly district fuel consumptions (coal, wood, oils, gas), the emission factors of six pollutants were determined for each square (100 km^2). For residential heating this determination was performed by taking into account the population, the dwelling types (individual and collective house) and the heating systems (single or multi-user central heating and separate heating appliances). For tertiary industry heating, we assumed that these tertiary activities are performed in the urban squares.

Each square is considered as a source and a receptor. We have applied a simplified model featuring gaussian bursts and straight flight paths, in which settling phenomena and physico-chemical transformations are neglected. Concentrations in each sqare are thus obtained through summation of contributions from all sources, and for the three representatives wind velocities, namely 1, 3 and 5 m/s.

Figure 6 shows cumulative graphs of atmospheric concentrations for the six pollutants considered. For identical fuel consumptions, pollutant concentrations in the environment as a consequence of residential heating are generally higher than those caused by tertiary industry heating or power generation.

3.3. Not generating electricity industries

Data about atmospheric and liquid releases of industrial sector are described. When a release is known for a specific plant, it is possible to evaluate the exposure resulting from this release as described above.

3.4. Natural background

The results of a national study on natural background exposure are recorded as regards south-east area. The measurements concerned:
- *external irradiation* outside and inside dwellings, using radiothermoluminescent dosimeters in 11 of the 19 districts making up the area studied, with about 200 measuring points in each district;
- *internal irradiation* due to the inhalation of radon and its short-lived decay products, by means of passive dosimeters fitted in a certain number of dwellings.

The results obtained were used to map out the exposure distribution in the area. Outdoor, the telluric irradiation was lower than 1 mGy/y. Indoor, external irradiation was greater than 1 mGy/y for one of the eleven districts measured. Indoor concentrations of radon ranged between 4 to 900 Bq/m^3 according to the

area studied in the south-east of France.

3.5 Radiodiagnostic examinations

Exposure to ionising radiation for medical purposes is now the main non-natural source of irradiation of the population. Radiodiagnostic examinations alone can even exceed the natural radiation level in developed countries.

In the present study, the following data relative to the south-east of France are compared with results obtained for the country as a whole:
- ♦ total number of examinations and distribution in terms of examination category;
- ♦ evaluation of collective exposure due to radiodiagnostic activities;
- ♦ estimation of the per-caput dose in each district of the area studies.

Collective exposure for the south-east of France averaged 3,500 man.Sv. The individual mean exposure is 0.3 mSv/y, as the national mean value.

3.6. Indoor activities

People in industrial countries spend at least 90% of their time in confined spaces (at home, in travel, at work, during leisure time,...). On the other hand, the energy crisis of 1973 provided an incentive to reduce heat losses in dwellings. The result was a significant reduction in ventilation rates, causing a rise in pollutant concentrations. Rises of radon concentration were observed indoors. It was the same for the usual pollutants emitted by combustion (heating, cooking, smoking). Further, technological advances have created new materials and chemicals for housing construction and decoration, as well as new household products of which the emissions rates of volatiles organic compounds (VOC) cannot be neglected.

Evaluations of VOC emissions from construction and decoration materials and from household products (house cleaning, do it yourself, gardening, people comfort) were mainly conducted in Nordic countries and in the United States. They relate to specific living conditions. We therefore felt it appropriate to collect a national data bank relative to the emissions from the various household product types and frequency of usage in order to determine the exposures resulting from their use and to compare them with the indoor exposure to radon.

3.6.1. The household products

♦ *Data bank*
Informations were collected using first, surveys in supermarkets, specialized stores (do it yourself, gardening) or not, since they ensure 50 to 60% of the French sales or more and, secondly, consumer association reports and marketing publications. For each class of products, we list the following features: names of the products and their manufacturers, conditionning form and associated volums, qualitative or quantitative composition when they were displayed on the container, yearly consumptions, most widely-used products and channel distributions.

♦ *VOC emission rates*
The household products are conditionned under different forms: spray, solid, liquid, paste and colloid. A specific methodology has been developed for the spray

cans in order to determine the composition of their propellent phase (figure 7) and the main componants of the liquide phase. The emission rates of products under liquid, solid, colloid or paste form were investigated in normal using conditions (figure 8).

♦ *Exposure evaluation*
A dispersion model is developped for a typical house. It calculates the concentrations inside the different rooms of the house according to their ventilation rates. The concentrations calculated can reach high values as working levels even maximal values for certain activities performed in these rooms (painting, using glues for covering,..) or utilization of usual products (room desodorizers for exemple).

3.6.2. Wood combustion in a fireplace

Experimentations were carried out in order to determine the main VOC and PAH (polycyclic aromatic hydrocarbons) released during wood combustion in a fireplace. During the kindling period, high concentrations of benzene, furfural, phenol and mutagenic PAH as benzo(a)pyrene, benzo(b)fluoranthene and benzo(k)fluoranthene were measured in the room near the fire according to the draught of the chimney. During the cinders period, acid and ester compounds are produced. The resulting exposures are calculated as described above.

3.6.3. Radon

Distribution of indoor radon levels in area studied are provided. Equilibrium factor value according to aerosol particle sizes is investigated.

4/- ACCIDENTAL RISK

In the framework of this programme, risk assessment methodology is mainly devoted to put in perspective accidental risk with normal operation. As previously, the evaluation process is carried out using 3 steps:
- risk identification;
- determination of exposure indices;
- risk quantification.

Results obtained could be applied on large industrial or urban areas, which represent the only relevant scale to quantify such risks assuming that economical and political constraints are supported.

At regional scale as Greater Rhône Delta area, the proposed schemes could be used as guidelines but do not constitue a realistic objective for decision in a near future. Nevertheless, identification of sources and targets improves our knowledge which would be of great interest in the global risk analysis and management.

5/- POLLUTION MANAGEMENT AND DECISION MAKING

The comparative nuisance analysis would allow the decision makers to optimize their choices. Neglecting the imperfection of this analysis which is not totally objective, we studied how does work the decision making in matters of pollutions in France (figure 9).

Our observations in the field of the practical realities of decision making confirm that the decision can be interpreted as the result of conflictual interactions between different interest groups: the State and the authorities, industry, the public, the experts. The decision is a process of reciprocal influence between interdependent forces.

The negociated process of decision presented permits solutions to be arrived at despite the scientific uncertainties. The interests of society are not necessarily compatible with technical or scientific logic.

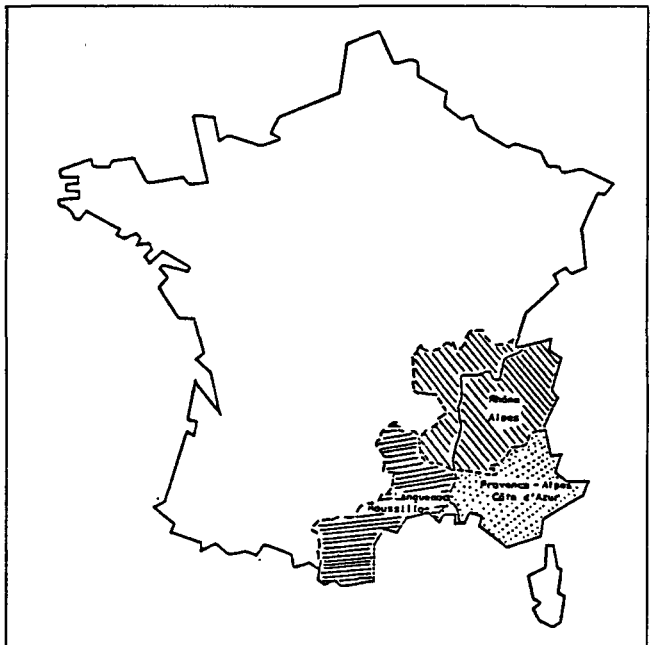

FIGURE 1 : The area studied: the South-East of France

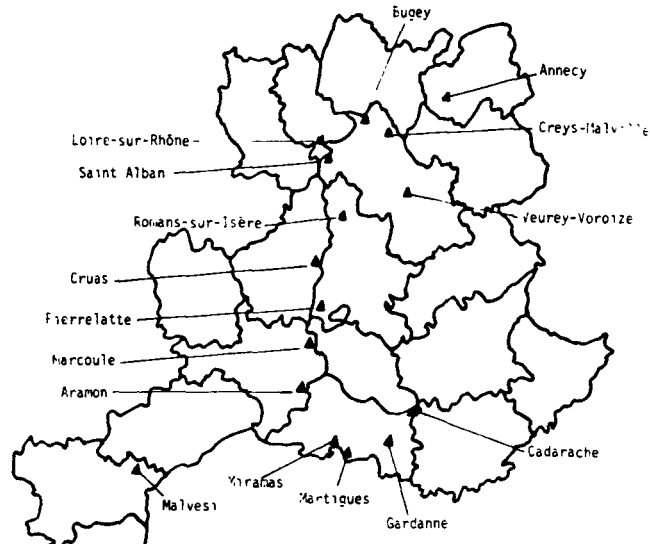

FIGURE 2: The emission points of the electricity generating cycles within the area studied

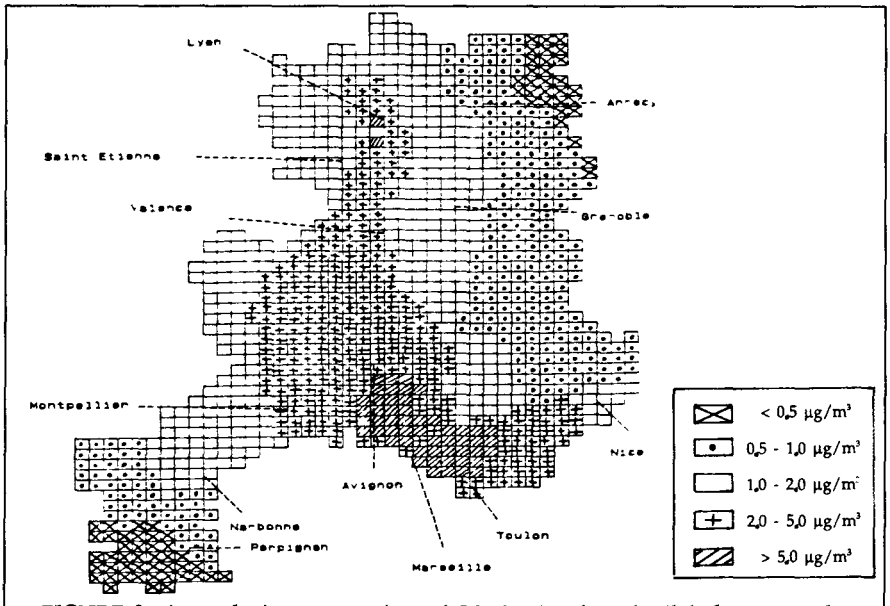

FIGURE 3: Atmospheric concentrations of SO_2 issuing from fossil fuel power cycles

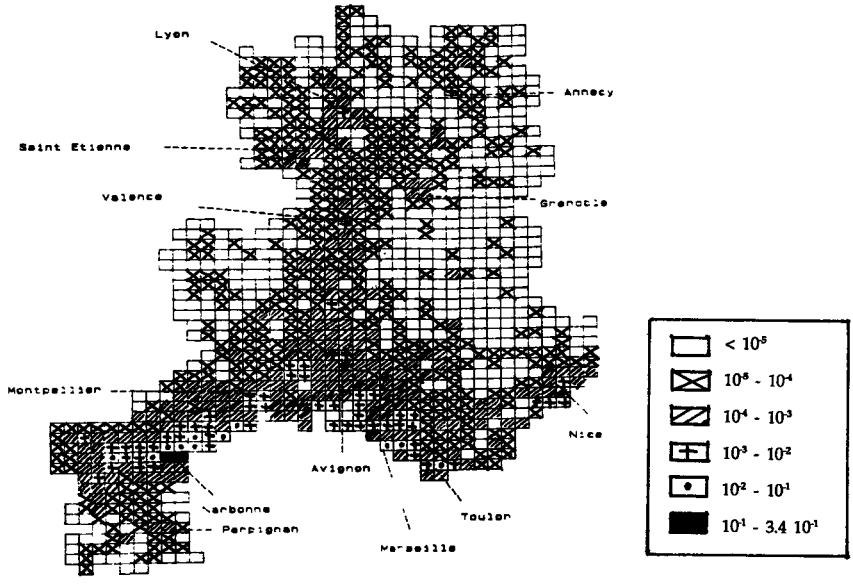

FIGURE 4: Collective exposure (man.Sv/y) of U-238 issuing from uranium power cycle

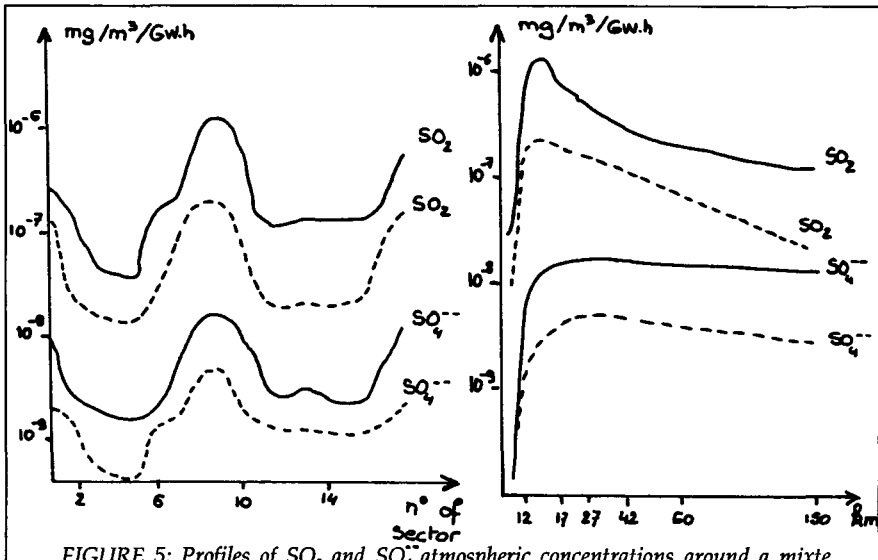

FIGURE 5: Profiles of SO_2 and SO_4^{--} atmospheric concentrations around a mixte power plant: coal (——) and fuel oil (---)

A/- as a fonction of the sector for the maximum concentration radius
B/- in the axis of maximum according to the distance from the source point

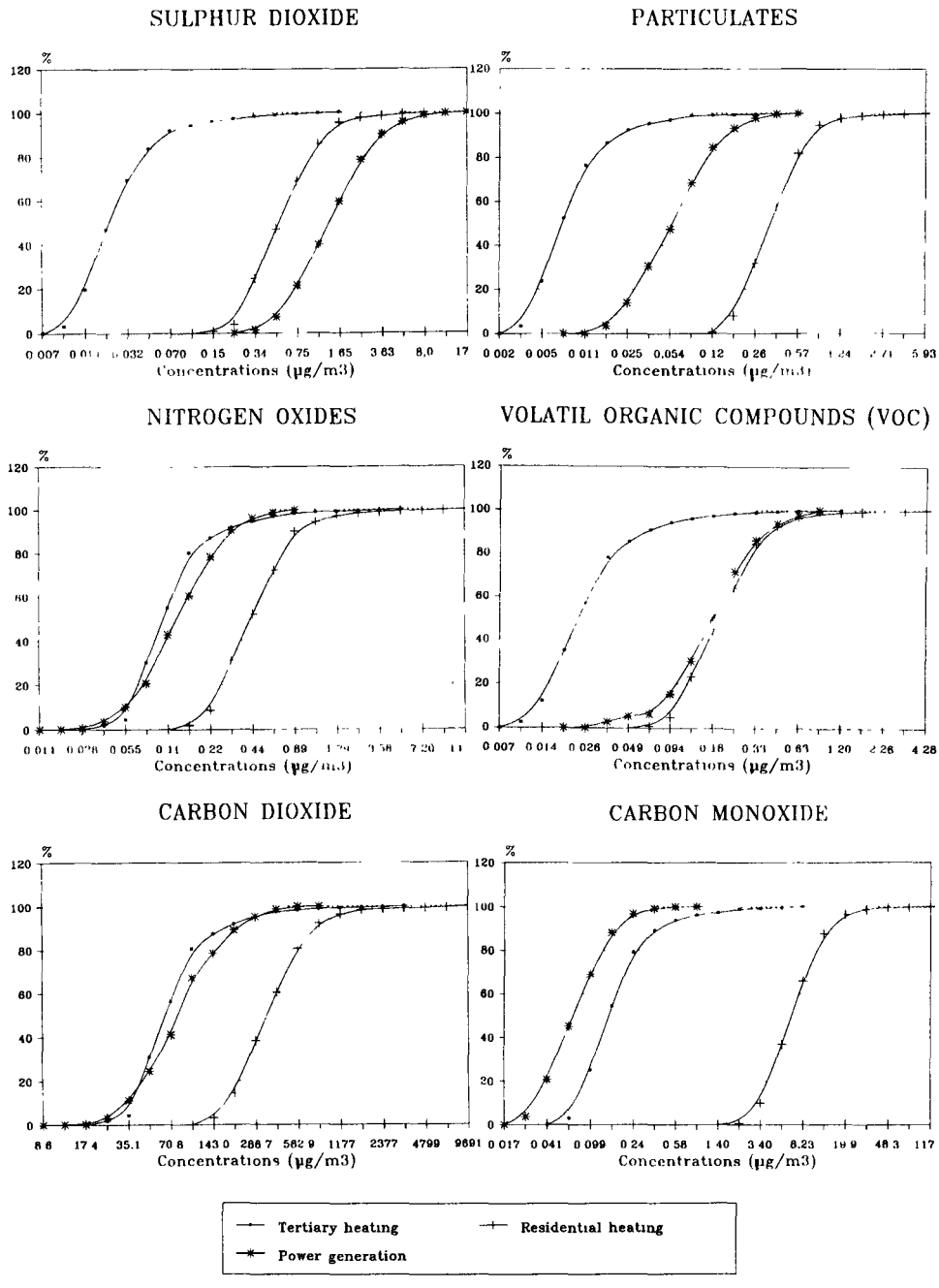

FIGURE 6: Cumulative atmospheric concentration graphs, computed for the 1,133 squares covering the region of interest.

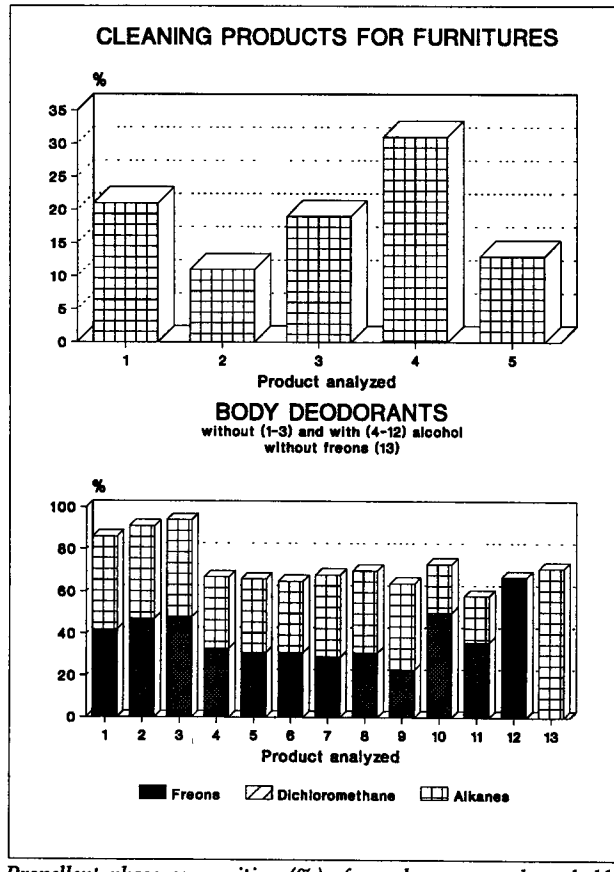

FIGURE 7: Propellent phase composition (%) of usual spray can household products

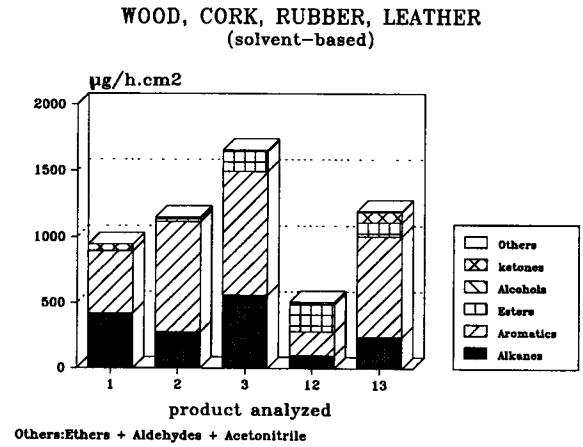

FIGURE 8: VOC emission rate of five solvent-based glues

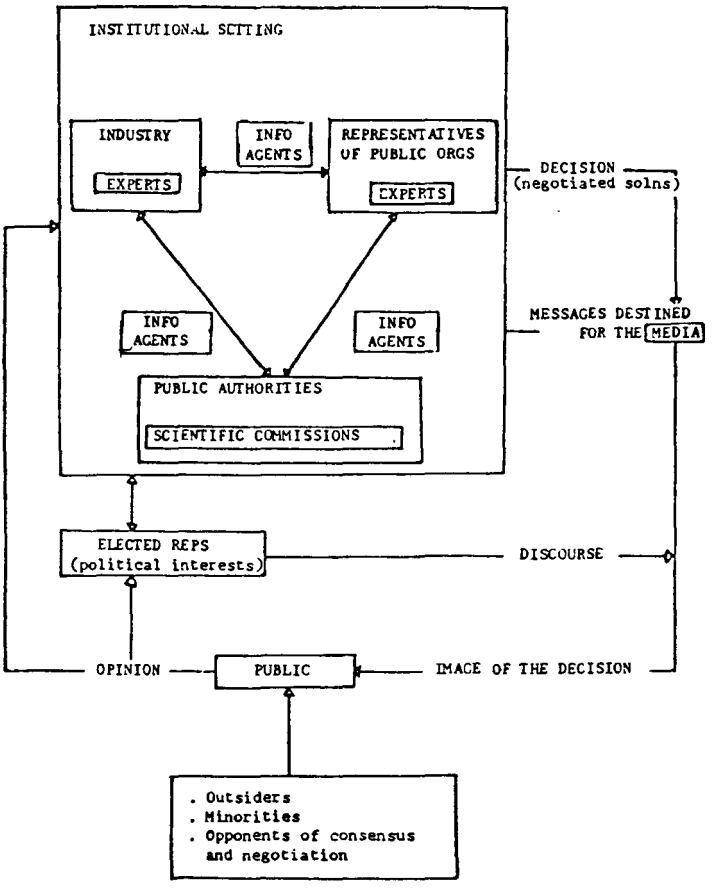

FIGURE 9: Decision making in matters of pollution: positions of particpants

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

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V. Publications:

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Nashville, Tennessee, USA, June 22-26, 1981. Eds IAEA : IAEA-SM-254/53 (1981)
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♦ AIGUEPERSE J., ANGUENOT F., PERSON A., LAURENT A.M., LOUIS-GAVET M.C., FESTY B.
The contribution of indoor pollution to the contamination level on a regional basis (Greater Rhône Delta Programme)
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♦ GALLE M.
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♦ AIGUEPERSE J., ANGUENOT F., PERSON A., LAURENT A.M., LOUIS-GAVET M.C., FESTY B.
La pollution a l'interieur des habitations : role des produits a usage domestique
Man and his Ecosystem - Proceedings of the 8th World Clean Air Congress 1989 -The Hague, The Nederlands, 11-15 September 1989, Vol. I pp. 387-392. Eds : L.J. BRASSER and W.C. MOLDER (1989)

RADIATION PROTECTION PROGRAMME
Final Report

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)]:

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Miss F.A. Fry
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Chilton, Didcot
GB- Oxon OX11 ORQ

Telephone number: (235) 83.16.00

Title of the research contract:

Impact assessment of artificial and enhanced natural radioactivity in the outdoor and indoor environment.

List of projects:

1. Characterization of radioactive aerosols near a coastal nuclear facility and assessment of their impact.
2. Study of methods for remedying and preventing high radon levels in dwellings.

Title of the project no.: 1

Characterisation of radioactive aerosols near a coastal nuclear facility and assessment of their impact.

Head(s) of project:

F A Fry

Scientific staff:

M R Bailey, N J Dodd, G Etherington, N Green, A C James, J C Strong, S P Stewart

I. Objectives of the project:

The objectives of this project are 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:

(1) to identify the information required for a comprehensive evaluation of the significance of airborne radionuclides

(2) to review existing sampling techniques

(3) to design and test a prototype air sampler

(4) to measure activity concentrations in air with an existing air sampler.

III. Progress achieved:

1. Introduction

The nuclear fuel reprocessing plant at Sellafield on the north-west coast of England discharges radionuclides both to sea and directly to atmosphere. Previous investigations of airborne radionuclides in the area indicated that there are two characteristic components to the radioactive content of the aerosol. Fission products originally discharged to the atmosphere are attached to particles with activity median aerodynamic diameters (AMAD) in the range 1 to 3 μm, while actinides initially discharged to the sea are attached to aerosols of marine origin with a much larger AMAD. Particles with aerodynamic diameters (d_{ae}) up to about 100 μm can readily enter the respiratory tract. The aspiration efficiency (the ratio of the concentration entering, to that in the ambient air) of the human respiratory tract, averaged over all orientations and a range of wind speeds, is about 50% for d_{ae} 30-100 μm. Although doses from inhalation of these large particles are likely to be small, the maritime phenomenon justifies further investigation because of the implications for the disposal of transuranic waste into coastal waters.

A comprehensive evaluation of the radiological significance of the marine resuspension and inland transport of aerosols requires the following information: (i) the activity concentration versus size distribution of the aerosol under a variety of meteorological conditions; (ii) the lung solubility classification, for inhalation dose assessment; (iii) the physico-chemical properties of the particles to which radionuclides are attached, to predict the behaviour of radionuclides following deposition in the terrestrial environment.

Particles with d_{ae} <5 μm are adequately sampled by conventional techniques, such as the high volume air sampler which has been operated by NRPB at Seascale near Sellafield since 1980. Particles with d_{ae} over 5 μm are not representatively sampled by techniques currently in use. The first requirement of the project was therefore to devise an improved method for sampling particles with d_{ae} up to 100 μm.

2. Methodology

2.1 Design of a large particle sampler

Two approaches to sampler design were considered. One was to use a sampler with the same aspiration efficiency as a human. The other was to sample representatively the aerosol over the full particle size range, and also determine the size distribution. The latter was preferred, because it was considered that it would be difficult to develop a sampler that would match human aspiration efficiency at all particle sizes and wind speeds. Furthermore, measurements of the total aerosol would be more useful for considering environmental transport and deposition of the aerosol.

Techniques for sampling the total aerosol were therefore examined. For this application, three specific requirements were identified: (i) representative sampling of particles with d_{ae} up to 100 μm, with an aspiration efficiency independent of wind speed and direction; (ii) sampling of a sufficient volume of air: since the limits of detection for the radionuclides of interest with the techniques available are about 2 mBq, and air concentrations are of the order of 10^{-6} Bq m^{-3}, the volume sampled and the sampling rate should be about 10^{4} m^{3} and 1 m^{3} min^{-1} respectively; (iii) ability to withstand severe weather conditions. Two types of total aerosol sampler with the potential for size fractionation were identified, the Rotary Impactor and the Wide Range Aerosol Classifier (WRAC).

The Rotary Impactor consists of adhesive-coated plates, rotating at high speed about a vertical axis. Collection efficiency depends on d_{ae}, rotation velocity and plate diameter. Thus several plates in parallel will provide size fractionation. Its advantages are that it is simple, relatively inexpensive, does not require an air inlet as do conventional air samplers, and is compact and easily tested in a wind tunnel. Its disadvantages are its low collection capacity, and the difficulty of making it weather-proof, particularly preventing wash-off by rain.

The Wide Range Aerosol Classifier (WRAC) has been developed by

groups in the US and West Germany. The design (Figure 1) has three main features: (i) an upward facing inlet to avoid dependence on wind direction; (ii) a cylindrical wind shield surrounding the inlet, to create relatively calm air conditions within the shield, and so limit the effect of wind speed on the sampler's aspiration efficiency; (iii) a 'rain cap' placed over the wind shield. In the original US design, 40 m^3 min^{-1} of air is sampled through an inlet 0.6 m in diameter (dimension d in Figure 1), with aerosols collected by parallel impactors, each at a rate of 1.5 m^3 min^{-1}.

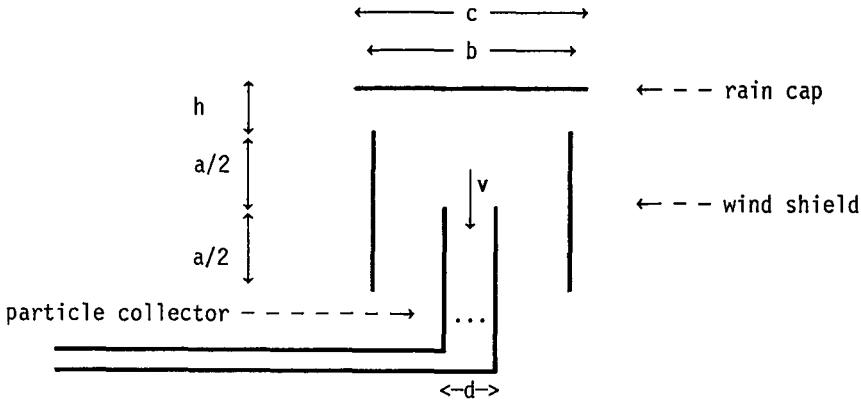


Figure 1. Schematic side view of WRAC large particle sampler

Advantages of the WRAC are that its sampling rate is high, and that it is robust enough for field use. Effort was therefore concentrated on its development. The main disadvantages of existing designs were their great size and cost. Impactors and conventional filters present relatively high impedance to air flow, requiring powerful air moving equipment to sample at high flow rates. However, if small particles (<5 μm d_{ae}) do not need to be collected, open pore polyester foams, which have very low impedance, can be used as the filtration medium.

Preliminary design work was carried out in consultation with scientists at the Institute of Occupational Medicine (IOM) in Edinburgh, who then produced a detailed design for a prototype. In this design study, considerations of particle losses on the external surface of the wind shield and of the sampling efficiency of an upward facing inlet in calm air conditions inside the wind shield were used to determine optimum values for the dimensions of the sampler. The height (a) and diameter (b) of the wind shield were chosen to be large enough to limit losses due to impaction. For a and b both 1.7 m, losses were predicted to be <10%. The requirement for representative sampling with d_{ae} up to 100 μm sets a lower limit on the diameter of the inlet (d), and that chosen (0.3 m) was significantly greater than this limit. The inlet air velocity specified was 2.4 ms^{-1} (see section 3.1), giving a volume sampling rate of 10 m^3 min^{-1}. Dimensions for the rain cap were c=1.9 m, and h=0.15 m.

2.2 Porous foam filter characterisation

Porous foams form an interconnected array of fibres which, when suitably coated, collect particles above a certain size efficiently by impaction. They are characterised by specifying the number of 'pores per inch' (ppi), which is correlated with the fibre diameter. To investigate the range of particle sizes collected and the potential for particle size fractionation, equipment was set up to measure the collection efficiency as a function of particle size for various grades of foam. Graded fused alumina dusts were dispersed into an air flow using a Small-Scale Powder Disperser (Thermo Systems Inc.) and the collection efficiency of each foam sample was then determined from the relative weights of powder collected on the foam and on a glass fibre backing filter.

2.3 Tests on the prototype large particle sampler

Following the design study, an approximately half-size prototype of the sampler (a=b=0.6 m; d=0.15 m) was constructed, this being the largest suitable for testing in the IOM wind tunnel. The dimensions

were chosen so that the the aspiration efficiency characteristics of the half-size sampler were as far as possible the same as for the full-size version. In particular, the inlet velocity of 2.4 ms^{-1} was kept constant. The one important difference was that losses due to impaction on the smaller windshield were expected to increase from <10% to about 30%. The performance of the prototype was tested in the IOM wind tunnel. To optimise use of the time available, measurements were concentrated on the most severe test conditions that could be arranged. This was achieved by using the highest wind speed available, 9 ms^{-1}, and the largest particles, graded fused alumina dust with a dae of 75 μm. Measurements were made without the raincap fitted. The sampler aspiration efficiency was determined gravimetrically.

2.4 Concentrations of actinides in air with particle sizes < 5 μm

The Seascale air sampler consists of an upward-facing open face filter through which air is drawn at about 1 m^3 min^{-1}. It is 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. The air filters were combined into monthly samples and then ashed. After radiochemical separation, actinide activities were determined by alpha spectrometry.

3. Results

3.1 Porous foam filter characterisation

Investigations of the range of particle sizes collected showed that a 50 mm thick 30 ppi foam collects virtually all particles with diameters > 5 μm at an inlet velocity of 2.4 ms^{-1}, and this grade and thickness of foam was therefore used in the sampler tests. For practical reasons, 2.4 ms^{-1} was the highest face velocity (i.e. the velocity at the surface of the foam) at which foams could be tested, and the design inlet velocity was therefore limited to this value, since total particle collection could not be guaranteed at higher inlet velocities. Measurements of air flow through a 50 mm thick 30 ppi foam showed that it presented a negligible impedance at a face velocity of 2.4 ms^{-1}.

The use of foams for size classification would require a multi-layer foam filter with a relatively coarse filter in the uppermost layer to collect most of the larger particles while allowing smaller particles to penetrate. Progressively finer foams in the lower levels would collect the smaller particles. Theoretical calculations indicate that, while the cut-off in the collection efficiency versus particle size curve is not sharp, a suitable choice of foam thicknesses and pore sizes would enable particles to be classified into four size fractions in the 5 - 100 μm range. Measurements on 20 ppi foams showed a fall-off in efficiency below d_{ae} of about 12 μm, i.e. at a higher particle size than for 30 ppi foams. This tends to confirm the expected dependence of the cut-off particle size on foam coarseness.

3.2 Tests on the prototype large particle sampler

The measured aspiration efficiency of the half-size sampler for a particle size of 75 μm at a wind speed of 9 ms^{-1} was 8%, which should be compared with a theoretically predicted value of at least 70%. Measurements at a lower wind speed of 3 ms^{-1} gave a higher aspiration efficiency, as expected.

3.3 Concentrations of actinides in air with particle sizes < 5 μm

Figure 2 shows the monthly air concentrations of ^{238}Pu, $^{239/240}Pu$, and ^{241}Am measured using the Seascale air sampler since the beginning of 1980.

4. Discussion

Measurements on open pore foams showed that they are a practical, low impedance air filtration medium capable of sampling virtually all particles with d_{ae} greater than 5 μm. Their inability to sample smaller particles is not a serious drawback because conventional sampling techniques can be used for them. The measurements also showed the potential for size fractionation in the 5 - 100 μm range, of multi-layer open pore foams using 10 ppi or coarser foams for the initial size selection.

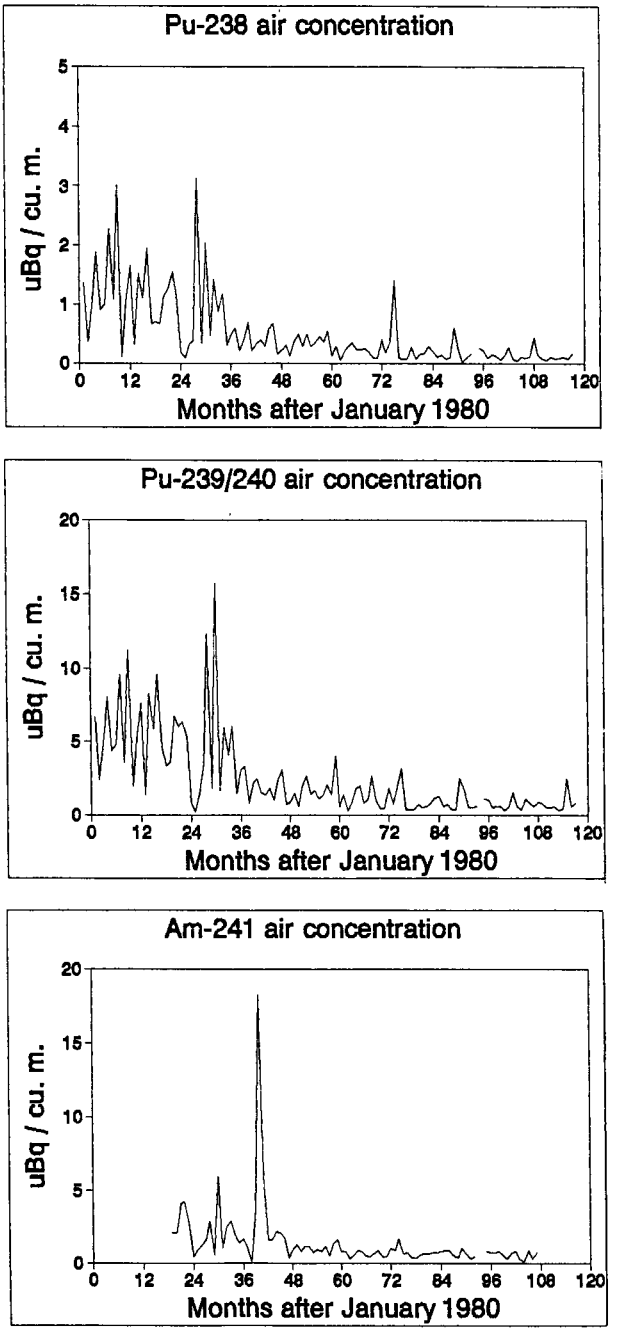


Figure 2. Pu-238, Pu-239/240 and Am-241 air concentrations measured by the high volume air sampler at Seascale, since 1980.

Although the aspiration efficiency of the prototype sampler is expected to increase for lower wind speeds and particle sizes, an efficiency of 8% at a wind speed of 9 ms^{-1} and a d_{ae} of 75 μm was not considered acceptable. To understand the reasons for the low measured efficiency, investigations of the air flow over the sampler were carried out. It was found that the air flow diverged from the horizontal at an angle of about 20° on encountering the wind shield, an effect which had not been anticipated. Particles thus have a net vertical velocity away from the inlet which must be overcome before they can be sampled. Simple kinetic arguments show that, with the volume sampling rate used, the angle of vertical divergence would need to be reduced to approximately 2°.

Recommendations for further work on the development of the sampler can be made on the basis of the wind tunnel tests. Firstly, it is clear that the established theories of air sampling are not sophisticated enough to predict the behaviour of the type of sampler being developed. Theory can aid design work, but the aspiration efficiency must be determined empirically. Secondly, the volume sampling rate, and therefore the velocity of air into the wind shield, must be increased to help produce a net particle velocity into the wind shield. This would require the determination of the particle collection efficiency of porous foams for the higher consequent face velocities. For the same reason, modified windshields which present a smaller or more streamlined cross-section to the wind need to be tested. Lastly, further work is required on the use of coarse foams for size fractionation.

Figure 2 shows that measured air concentrations of actinides at the sampling location have been on a long-term downward trend since the beginning of 1983, when a rapid decrease took place. The average level of ^{238}Pu, ^{239}Pu, ^{240}Pu and ^{241}Am in 1988/89 was approximately 2 μBq m^{-3}.

It is acknowledged that the objectives of the project have been only partially achieved. Work has concentrated on the development and testing of suitable sampling techniques, which were considered to be pre-requisites for field measurements additional to those using the

conventional sampler. Useful progress has been made, although much development work remains to be done. The work reported here has enabled practical and specific recommendations to be made on how work in this area could continue.

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

D Mark, J H Vincent, Institute of Occupational Medicine, Roxburgh Place, Edinburgh, UK.

V. Publications:

Title of the project no.: 2

Study of methods for remedying and preventing high radon levels in dwellings.

Head(s) of project:

J C H Miles

Scientific staff:

K D Cliff, J C Strong, R A Algar, P R Lomas

I. Objectives of the project:

To test potential methods for reducing the concentration of radon and its decay products in indoor air and to prevent the occurrence of high radon concentrations in dwellings.

II. Objectives for the reporting period:

To investigate the parameters affecting radon entry into two structures built on phosphate sand, one with a monolithic concrete slab floor, the other having a suspended timber floor. To determine the efficacy of remedial measures installed in a variety of buildings. To optimise the configuration of remedial measures where possible.

III. Progress achieved:

Exposure to radon in indoor air accounts for nearly half the average annual effective dose equivalent received by the UK population[1]: in Cornwall, it accounts for some 80%. Such exposures are controllable to a large degree and some persons receive doses that are by any standard unacceptable. Acting on the advice of the National Radiological Protection Board[2], the UK Government advised that high radon levels in dwellings should be reduced and that new homes in areas where indoor radon might be a problem should be designed so that high indoor radon concentrations were unlikely to occur[3]. Whereas limitation of radon exposures in dwellings is advisory, in workplaces it is subject to the legal requirements of the Ionising Radiation Regulations 1985, which apply[4] to all places of work, above and below ground. In areas where high radon concentrations have been found in dwellings, similar levels may be found in workplaces.

The development of cost-effective and durable techniques for the reduction of indoor radon in buildings of the diverse structural styles found in the UK needed to be developed. It was necessary also to devise details of floor construction that would limit the ingress into new buildings of soil gas containing high concentrations of radon. The work described here was aimed at satisfying these requirements and proceeded on three fronts: an experimental programme using small structures built on a soil with a high production rate for radon; the formulation of advice for new construction and the testing of its efficacy; attempts to reduce radon levels in existing buildings, both dwellings and workplaces. This work was carried out with the cooperation of the Building Research Establishment (BRE).

Experimental programme

A radon test site was established on BRE land. A pit of surface plan 9 m by 15 m, having maximum depth of 4 m with sides graded at 45° nominally, was excavated and filled with phosphate sand. The phosphate sand had a ^{226}Ra concentration of 1.6 kBq kg^{-1} and a radon emanation rate of 0.15 mBq kg^{-1} s^{-1}. The radon concentration in the interstitial pores of the phosphate sand at depth exceeds 10^5 Bq m^{-3}. Two structures, each of plan 3 m by 3 m and separated from each other by 3 m, were erected on the sand. One structure had a monolithic concrete floor slab with the supporting walls built off this slab; the other had a ring-beam foundation on which were built the brick supporting walls. Each structure was of wood and was clamped to the supporting walls. A gasket between the brick supporting walls and the wooden superstructure ensured that the complete structure had a very low infiltration rate, namely, less than 0.01 h^{-1}.

Each structure was fitted with a variable speed centrifugal fan enabling the air pressure in the structure to be increased or decreased relative to ambient. Variable ventilation openings were provided also; ventilation and relative pressure could be controlled independently to a fair degree. Transducers for the measurement of pressure differences across the components of the structures, temperature internal to each structure and in the outside air, and absolute

atmospheric pressure were each installed together with continuous monitors for radon concentration. A weather station was erected at the site to record wind speed and direction. All parameters monitored were logged at 0.5 h intervals and recorded on magnetic tape, but for data analysis, 4 h averages were used in most cases.

It was originally intended to use the structure with the concrete floor slab as a reference structure to determine changes due solely to environmental conditions, and to use the other structure with a sequence of floor types to study factors determining the ingress of radon from the underlying sand and thus investigate methods of restricting radon entry for each floor type. The construction of the test facilties presented some difficulties, and the complete instrumentation was not effected until late 1988. This necessitated a revision of the plan of work at the site: each structure was studied independently with a suspended timber floor installed in that with the ring-beam foundation. Full analysis of the results obtained for the suspended timber floor and a concrete slab floor are reported elsewhere[5]: salient features are reported here.

The concrete slab foundation was initially installed so that it was some 500 mm below the level of the sand. The sand covered the slab-to-wall joints and the first four courses of brick-work. With this configuration, the rate of radon entry into the structure was typically 12 Bq m^{-3} h^{-1} when the differential pressure across the fabric of the structure was less than 1 Pa; radon entry here is predominantly by diffusion. Reducing the pressure inside the structure to 12 Pa below ambient increased the radon entry rate to 19 Bq m^{-3} h^{-1}, the additional ingress being due to pressure-driven flow of soil gas into the structure. The radon exhalation rate from the concrete floor slab was found by measurement to be less than 0.2 mBq m^{-2} h^{-1}: this rate is typical of that expected from the radium content of concrete and indicates that diffusion of radon through the floor slab from the underlying sand is negligible.

A trench was excavated round this structure to expose the floor slab, and all the sand was removed from the floor-to-wall joint and from the brickwork and mortar joints of the retaining walls that had been covered. The radon entry rate was reduced below 0.5 Bq m^{-3} h^{-1}, not significantly different from that expected from the exhalation of radon from the concrete floor and supporting walls of brick. There was no discernible increase in the radon entry rate for underpressures of as much as 22 Pa in this structure (the measurement limit of the transducers being $\pm$ 25 Pa). One parameter logged for this structure was the pressure difference across the floor slab: this was done by sealing a metal sampling tube in a hole drilled through the centre of the floor. The sampling tube has been in position for over a year but the seal is still intact, demonstrating incidentally that adequate sealing around metallic service pipes penetrating concrete floors is possible.

The results for the solid floor demonstrate that significant radon entry is likely for buildings where the soil is allowed to cover wall-to-floor joints. A monolithic floor structure with walls built off the floor results in little radon entry, providing service penetrations are adequately sealed.

Initial measurements in the structure with the ring-beam foundation were carried out without a floor installed. For a ventilation rate below $0.2\ h^{-1}$, the steady-state radon concentration in this structure was about $6\ 10^{4}$ Bq m^{-3}, caused predominantly by diffusion from the sand. When the air pressure inside the structure was reduced to 5 Pa below ambient, pressure-driven flow of radon from the sand into the structure was a factor of four greater than that due to diffusion. During installation of the suspended timber floor, three air-bricks were installed in each of the front and rear walls to provide natural ventilation of the underfloor space. Each air-brick was ceramic with four rows and nine columns of openings giving a total open area of $0.026\ m^2$. The floor was of tongue-and-groove construction with substantial and variable gaps at the floor edges.

With the maximum open area of air-brick and with natural ventilation only of the underfloor space, the radon concentration under the floor was nearly two orders of magnitude lower, on average, than that obtaining in the structure before the floor was installed, but was subject to wide variations caused by wind speed and direction. The radon level under the floor was at least an order of magnitude lower than that previously existing in the structure even when the total open area of air-brick was reduced to $0.0014\ m^2$ equally distributed between the two walls. These results reflect the small floor area and are unlikely to be repeated in buildings of conventional size, but they do suggest that adequate ventilation of underfloor spaces can reduce radon concentrations substantially.

In housing, the ventilation provided beneath timber floors is designed to prevent rot from moisture and is not adequate to reduce the very high radon concentrations that may occur in radon-affected areas. Initial guidance provided for householders[6] suggests that where high indoor radon levels exist in homes with suspended timber floors, an extract fan should be fitted to one air-brick and that all other air-bricks should be blocked. At the test site, it was found that for a given fan power, a more effective reduction in underfloor radon concentration was achieved when a number of small openings were distributed in the wall opposite the fan than when all air-bricks, save that carrying the fan, were occluded. This results from the fact that, with all air-bricks closed, the fan causes the rate of entry of soil gas, and hence radon, into the underfloor space to increase.

Work at the test site continues in cooperation with BRE, with initial emphasis on the sealing of timber floors. Suspended concrete floors and floors constructed by pouring concrete between walls are also to be studied.

New buildings

As a result of collaboration with BRE, interim guidance[7] on anti-radon construction in areas where high indoor radon levels are likely was issued by the UK Department of the Environment. A major construction company has several major sites in radon-affected areas where building had commenced before the interim guidance was issued. More recent building on these sites has incorporated

features to limit radon entry from the ground. A study of homes on these sites, with and without anti-radon measures, has commenced with the aim of demonstrating the effectiveness of the radon protective designs. This is being done through a statistical analysis of the results of comparative radon measurements with an examination of factors that cause failures. The results of this study are being accumulated.

Existing buildings

A device for reducing radon concentration, which pumps air from the loft space into the living space, has been tested in a conventional house with radon concentration of 600 Bq m^{-3}. Various measurements were carried out over a two-month period using a cycle of three days with the device operating and three days with it turned off: there was a reduction in radon level by a factor close to two during periods of operation. Initially, studies of infiltration at this house indicated that the reductions in radon level were due solely to increases in infiltration (R Stephen, BRE, Personal Communication). More detailed analysis of the results indicates a reduction in radon level in excess of that explained by increased infiltration alone. The house was of reasonably tight construction with a low infiltration rate: in these circumstances, this type of device may have a role in radon reduction, by altering the pressure differential, where the reduction required is moderate.

Occupational premises

Adventitious occupational exposure to high levels of radon and its daughters has been demonstrated at many workplaces in areas where high radon exposures occur in dwellings. In particular, a policy has been pursued with Cornwall County Council (CCC) to investigate radon levels in buildings for which it is responsible. Where high levels have been found, work has been undertaken to reduce levels so that buildings such as schools and libraries are not subject to the statutory regulations[4].

In buildings with solid concrete floors, remedial measures involving underfloor suction have been highly successful[8]: reductions in radon levels by at least 95% have been achieved in many cases. Suspended timber floors have proven more difficult, successful reduction in levels being achieved in some instances, but with failures in others of similar configuration. NRPB, in conjunction with CCC, is studying the causes of failure, and the indications are that an effective remedial strategy for such cases can be implemented.

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

Building Research Establishment
Garston
Watford
WD2 7JR

Dr S J Wozniak, Mr R Stephen

V. Publications:

1. Clarke R H and Southwood T R E. Risks from ionizing radiation. Nature, 338, 197-198 (1989).

2. NRPB. ASP 10. Exposure to radon daughters in dwellings. Chilton (1987).

3. Parliamentary Debates (Hansard). House of Commons Official Report, 27 January 1987, cols. 189-90.

4. Statutory Instruments 1985 No. 1333. The Ionising Radiation Regulations 1985. HMSO, London, 1985.

5. Cliff K D and Algar R A. Studies of radon entry into structures having different floor constructions. In preparation.

6. Department of the Environment. The householders' guide to radon. London, DOE (1988).

7. Department of the Environment. Building Regulations 1985 - Part C: Interim guidance on construction of new dwellings. London, DOE (1988).

8. Cliff K D, Britten J R, Gardner P H and Richardson P A T. Radon control and remedies in buildings. Radiat. Prot. Dosim., 24 (1-4), 531-535 (1988).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

CEN-IPSN de Fontenay-aux-Roses
B.P. n° 6
F-92265 Fontenay-aux-Roses

Contract no.: BI6-F-119-F

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

Dr. N. Parmentier
CEN-IPSN de Fontenay-aux-Roses
B.P. n° 6
F-92265 Fontenay-aux-Roses

Telephone number: (1) 46.54.83.35

Title of the research contract:

Assessment of population doses from accidental releases of radioactivity and socio-economic cost of evacuation.

List of projects:

1. 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.
2. Evaluation of population dose preceding or following accidents involving releases of gases and radioactive aerosols into the atmosphere.

Title of the project no.:

Evaluation of absorbed doses due to external exposure to photons emitted by a radioactive cloud and assessment of the protection of dwellings

Head(s) of project:

J. LE GRAND

Scientific staff:

J. LE GRAND, Y. ROUX, N. PARMENTIER, V.D. NGUYEN, C. MADELMONT, P. BOUISSET, D. ROBEAU, G. KERLAU

I. Objectives of the project:

En cas d'accident radiologique, les mesures de protection des populations reposent sur :
- **l'estimation prévisionnelle des doses reçues par un individu à l'air libre en terrain dégagé ;**
- **l'estimation de facteurs de protection, exprimés en terme de réduction de la dose prévisible si une mesure de confinement dans les habitations est considérée comme devant être prise. Ceci en réalité correspond à un essai de quantification de l'efficacité de cette mesure de protection.**

Ces deux estimations ne sont pas indépendantes et la même méthode de calcul doit être appliquée dans les deux cas.

Une enquête sur les caractéristiques de l'habitat autour des sites nucléaires français sera menée.

Des expériences de validation des codes de calcul utilisés pour déterminer les facteurs de protection seront effectuées.

II. Objectives for the reporting period:

III. Progress achieved:

1 - Introduction

Lors d'un accident dans une centrale nucléaire, des produits radioactifs peuvent être rejetés dans l'atmosphère. L'évaluation de l'exposition de la population - tant à l'extérieur que dans les habitations - aux rayonnements photoniques émis dans le nuage a été menée en quatre étapes :

- une enquête statistique sur les caractéristiques de l'habitat au voisinage des centrales nucléaires françaises,
- le développement de codes de calcul utilisant les techniques de Monte-Carlo pour le calcul des doses reçues tant à l'extérieur que dans les habitations,
- des expériences de validation des codes de calcul,
- la détermination et l'étude des distributions de facteurs de protection dans des échantillons d'immeubles représentatifs de l'habitat.

2 - Méthode générale

2.1 - Enquête sur l'habitat et échantillon d'immeubles représentatifs [1, 2]

Les caractéristiques de l'habitat, susceptibles d'influencer sur son rôle de protection en cas de rejets atmosphériques de radionucléides à la suite d'un accident dans une centrale nucléaire, sont celles qui, d'un point de vue général, déterminent le potentiel d'échanges avec l'extérieur. Il s'agit :

- d'une part, des données géométriques qui qualifient le volume habité et ses surfaces exposées, notamment les éléments les plus perméables : ouvertures et toitures,
- d'autre part, des données qui concernent les matériaux de construction, c'est-à-dire les probabilités des diverses techniques qui peuvent avoir été mises en oeuvre pour les parois, les toitures et les sols.

Afin d'obtenir des résultats réalistes assez rapidement et au moindre coût, on a opté pour une méthodologie historique et quantitative. L'esprit de la démarche consiste à considérer le patrimoine de logements comme la somme de strates historiques successives dont on évalue les caractéristiques.

La structure des sources a contribué à définir l'échelle géographique de l'analyse. Pour représenter l'environnement des centrales nucléaires, le **département** a été retenu.

En conformité avec la connaissance générale des différenciations typologiques, l'étude du contenu des sources a conduit à répartir le parc de logements départementaux en quatre catégories de base qui résultent du croisement de deux dualités : - l'ancien et le moderne ; - l'individuel et le collectif.

Compte tenu des disparités régionales et dans l'esprit d'en rester à deux classes temporelles, la date de 1948 a été retenue comme discriminant de l'ancien et du moderne. De même, en vue de simplifier le modèle d'analyse, l'individuel comprend les immeubles de 1 ou 2 logements, et le collectif comprend les immeubles de 3 logements ou plus.

Les principales caractéristiques qui sont utilisées pour définir les immeubles sont : - la surface au sol, le nombre de niveaux, la fréquence de mitoyenneté, les profondeurs entre façades principales et les surfaces d'ouvertures.

Pour chacune des quatre catégories de base, la volumétrie des immeubles est ramenée à un modèle moyen. C'est un parallélépipède dont la surface au sol est déterminée par celle du logement moyen et le nombre moyen de logements par immeuble. La profondeur entre façades principales a été, autant que possible, estimée à l'occasion des études sur cadastre. La hauteur de l'immeuble dépend naturellement du nombre de niveaux, de la hauteur moyenne de plafond et des sols intermédiaires. Les toitures triangulées sont représentées par une section de parallélépipède de surface exposée équivalente. Le modèle volumétrique est complété par une estimation de la densité de cloisonnement intérieur faisant la part des gros murs et des cloisons légères.

Pour décrire les diverses variantes caractéristiques d'un site, on considère ensuite les technologies et les matériaux susceptibles d'avoir été employés pour les deux principales surfaces en contact avec l'extérieur : les parois verticales et les toitures.

Le cas de la maison individuelle est certainement celui qui justifie l'approche la plus fine, en raison à la fois des effectifs en cause et de l'importance des surfaces exposées. En effet, la surface de parois apparentes est déterminée notamment par le caractère mitoyen ou non, tandis que la variation du nombre de niveaux (généralement 1 ou 2, parfois 3, exceptionnement plus) a, par ailleurs, une incidence sérieuse sur l'exposition des volumes habitables à travers les toitures.

En vue de classer les différentes variantes d'un immeuble par ordre de probabilité décroissante, la probabilité associée à chacune d'entre elles est calculée.

L'échantillon d'immeubles représentatifs dans un département est composé des habitations dont la probabilité est supérieure à 0,01 c'est à dire celles qui représentent au moins 1 % du parc de logements du département.

2.2 - Méthode d'évaluation de l'exposition de la population [1-3-4-5]

Dès le début de l'étude, il est apparu que le nombre d'immeubles ou de maisons qui seraient retenus pour représenter la diversité de l'habitat autour des centrales nucléaires françaises, serait grand. Par ailleurs les raies photoniques, émises par les radionucléides qui peuvent être rejetés dans l'atmosphère au cours d'un accident, recouvrent une large gamme d'énergie. En conséquence, un grand nombre de problèmes devront être traités pour déterminer les facteurs de protection apportés par les habitations au voisinage des centrales nucléaires françaises. Afin de limiter le nombre de problèmes à traiter ainsi que l'encombrement des codes sur l'ordinateur Hewlett Packard HP 1000, les calculs ont été menés en deux étapes :

- la première traite du transport des photons dans l'atmosphère et le sol afin de déterminer d'une part l'exposition d'un individu à l'air libre en terrain dégagé et d'autre part les caractéristiques du champ de photons auquel est exposée une habitation (code SUIF) ;

- la seconde détermine l'exposition d'un individu vivant normalement dans son logement (c'est-à-dire qu'il peut se déplacer sur toute la surface habitable du logement), en simulant le champ de photons sur les faces exposées de l'immeuble, à partir de ses caractéristiques calculées dans la première étape (code BILL).

La source de rayonnement considérée est un nuage semi-infini de concentration uniforme, limité par un milieu semi-infini absorbant, représentant le sol. Les deux milieux sont séparés par une surface plane infinie. Ces hypothèses présentent l'avantage de décrire une situation physique bien définie qui est identique tant pour l'exposition d'un individu à l'air libre que pour celle des immeubles.

L'exposition de l'individu à l'air libre est caractérisée par le kerma, à 1 m au-dessus du sol, tandis que celle du second individu est le kerma moyen dans le logement évalué à partir du

flux de photons entrant dans chaque pièce. En raison de la diversité des types de logement et dans un souci de simplification, la notion de logement est remplacée par le niveau dans l'habitat collectif et par l'ensemble de la maison dans l'individuel.

Le facteur de protection est le quotient du kerma moyen dans le logement par le kerma à 1 m au-dessus du sol.

2.3 - Expériences de validation [6]

Naturellement, pour des raisons évidentes, il ne peut être question de valider expérimentalement la première étape du calcul (source semi-infinie). Par contre, la seconde étape - détermination du kerma moyen dans un logement - peut être effectuée si les conditions d'exposition de la maison et les caractéristiques du champ de rayonnement émis par la source utilisée ne sont pas trop éloignées de celles pour lesquelles le code BILL a été mis au point.

Parallèlement, d'autres codes ont été développés, notamment l'un d'entre eux calcule la répartition du débit de kerma autour d'une source de gammagraphie industrielle placée dans un environnement complexe. La source peut être ponctuelle, surfacique ou volumique. Le code calcule, dans des mailles cubiques, soit le débit du kerma, soit les caractéristiques du champ de photons (flux, spectre d'énergie, distributions angulaires). Tous ces codes ont une partie commune qui détermine l'histoire de chaque photon émis par la source.

Les expériences ont donc été conçues pour :

- vérifier dans quelles conditions les débits de kerma, calculés dans des mailles cubiques de dimension donnée, sont comparables aux mesures faites par des dosimètres thermoluminescents quasi-ponctuels,
- vérifier les méthodes de détermination du kerma moyen dans un logement, utilisé par le code BILL,
- enfin, comparer les spectres d'énergie des photons mesurés - dans les cas où cela est possible - aux spectres obtenus dans les mailles cubiques.

Ces expériences ont été menées en collaboration avec le "G.S.F." - Institut für Stahlenschutz, Neuherberg (R.F.A.), qui s'est chargé des mesures de spectres d'énergie des photons.

2.4 - Distribution des facteurs de protection

Pour chaque département, les facteurs de protection dans les logements de l'échantillon d'immeubles représentatifs sont calculés pour six valeurs de l'énergie initiale des photons.

La distribution de facteurs de protection a été étudiée, en fonction des caractéristiques de l'habitat du département, et de l'énergie initiale des photons. Elle peut être résumée par une valeur prudente de la moyenne et par l'étendue de la distribution.

La valeur prudente de la moyenne est la plus défavorable parmi les trois valeurs suivantes :

- le facteur de protection moyen par immeuble de l'échantillon,
- le facteur de protection moyen de l'habitat moderne (individuel et collectif) lorsqu'il dépasse 50 pour cent des logements,
- le facteur de protection moyen de l'habitat individuel (ancien et moderne), lorsqu'il dépasse 50 pour cent des logements.

L'étendue de la distribution est, naturellement, égale à la différence entre le facteur de protection du logement le moins protégé et celui du logement le plus protégé de l'échantillon.

3 - Les résultats

3.1 - Les caractéristiques de l'habitat [1 - 2]

L'enquête a fourni la proportion de logements associée à chacun des quatre types d'habitat : individuel ancien, collectif ancien, individuel moderne et collectif moderne. L'importance relative de ces catégories dans les 31 départements étudiés a permi de les classer en trois groupes.

Le premier, qui est le plus nombreux, regroupe les départements où l'habitat indivuel (ancien et moderne) est largement dominant. Dans le second groupe, un peu moins important, l'habitat moderne, et plus particulièrement le collectif moderne, voit sa fréquence s'accroître au détriment de l'indivuel ancien. Enfin, dans le troisième qui ne comprend que certains départements de la Vallée du Rhône, l'habitat collectif et particulièrement le collectif moderne est prédominant.

Les murs peuvent être classés en trois catégories en fonction de leur qualité d'écran vis-à-vis des rayonnements :

- les matériaux lourds qui représentent un excellement écran du fait de leur épaisseur : la pierre et le pisé,
- les matériaux lourds qui constituent un moins bon écran du fait de leur épaisseur moindre : le béton et la brique pleine,
- les matériaux légers, assez perméables aux rayonnements : le parpaing, la brique creuse et le bois (terre et pans de bois).

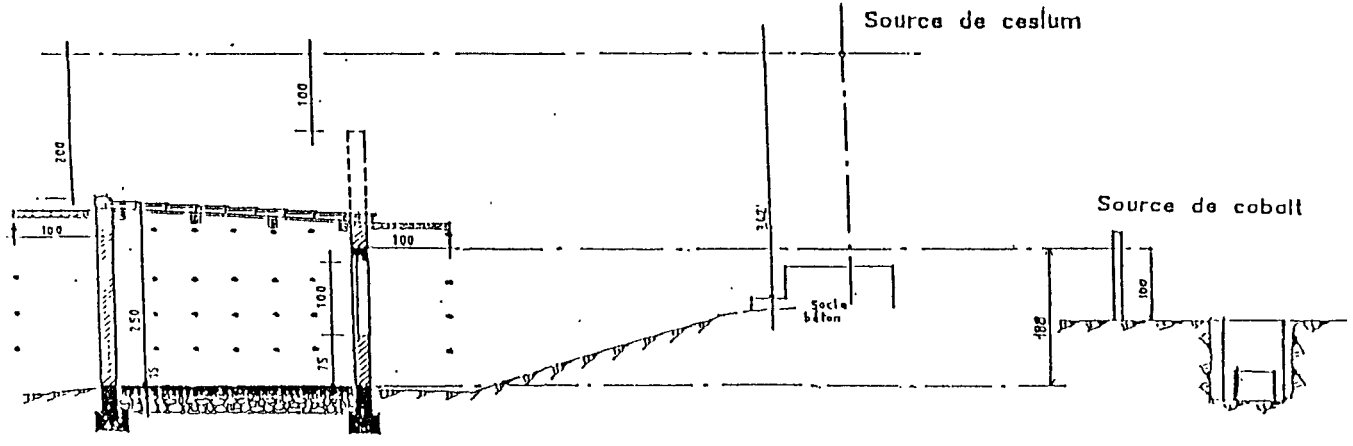
Dans l'habitat ancien, le matériau qui est présent en toutes régions est la pierre. Il est largement dominant dans la plupart des départements, tant dans l'individuel que dans le collectif. Cependant, d'autres technologies anciennes ont, dans certaines régions, une fréquence équivalente voire supérieure à celle de la pierre. Ce sont :

. le pisé dans le nord de la Vallée du Rhône,
. le bois en Alsace et en Seine-Maritime,
. la brique pleine, surtout dans le Nord et le Pas-de-Calais où celle-ci est dominante.

Dans l'habitat moderne, c'est évidemment le béton sous ses diverses formes : parpaings (blocs creux) ou panneaux pleins, qui représente la solution la plus fréquente. Néanmoins, la présence de la brique, souvent devenue creuse, se confirme dans les régions où le matériau était d'usage traditionnel. Les matériaux légers sont presque partout dominants, même dans le collectif moderne.

Les couvertures sont sujettes à des transformations fréquentes. Le cycle de 30 ans qui correspond généralement à la durée de vie des matériaux de couverture a autorisé des interversions entre des matériaux compatibles avec le support. Il faut donc admettre la présence non négligeable de matériaux modernes dans le parc ancien. Dans l'ensemble, c'est encore la tuile de terre cuite qui reste le matériau dominant. Les exceptions concernent les zones de production ou de commercialisation de l'ardoise qui se sont restreintes au cours du temps par le déclin de la production (Savoie et Ardennes).

Sur la base des résultats de l'enquête sur l'habitat, la méthode décrite au paragraphe 2.1. a permis de définir, dans chaque département, un échantillon d'une vingtaine d'immeubles représentatifs, soit au total 647 immeubles pour les 31 départements étudiés. En général, la proportion de logements représentés par l'ensemble des immeubles de l'échantillon dépasse 80 % du parc du département. Le plus souvent, seules les combinaisons comprenant des technologies marginales qui ne représentent que quelques pour cent de logements du type, sont omises.



Vue en élévation de la maison, de l'irradiateur (source de céslum) et de la source de cobalt.

3.2 - Les expériences de validation [6]

Deux campagnes expérimentales ont été effectuées :

- la première, du 9 au 18 septembre 1987, avait pour objectif principal la vérification de la méthode d'évaluation du kerma moyen dans un logement,

- la seconde, du 19 au 28 octobre 1987, a été effectuée en collaboration avec le G.S.F. qui s'est chargé de la mesure des spectres d'énergie des photons.

3.2.1 - Le site et les sources

Afin d'étudier l'influence d'une irradiation gamma chronique sur un écosystème, une source de césium 137 a été implantée en 1969, dans l'enceinte du Centre d'Etudes Nucléaires de Cadarache. La zone irradiée, d'une superficie de 7 ha, occupe un petit vallon boisé dans les collines de la partie nord du Centre de Cadarache. L'irradiateur est implanté dans une clairière près d'un chemin forestier.

La source de césium coulisse dans un tuge de guidage vertical, entre un point situé à 3,50 m au-dessus du sol et un château de plomb souterrain.

Durant la première campagne d'expérimentation, son activité était donc de 28,12 TBq (760 Ci). Dans le creux du vallon, en contrebas de l'irradiateur, une maison a été construite. Lorsque la source est en position haute, le toit et la façade avant sont exposés aux rayonnements venant de la source.

La source de césium étant trop intense pour les mesures spectrométriques, une source de cobalt 60 a été implantée sur le site, durant la seconde campagne. Son activité à cette date était de 4,33 GBq (117 mCi).

3.2.2. La première campagne

La maison de 12 m2 de surface habitable a des murs en parpaing et une toiture en tuiles. Sa façade comporte une fenêtre et une porte.

Durant la première campagne, quatre configurations de la maison ont été considérées pour étudier l'influence des ouvertures (fenêtre obturée dans les expériences 2, 3 et 4), l'influence du cloisonnement (la pièce est divisée en trois petits volumes habitables par une cloison légère dans l'expérience 3 et 4), et la façade est surélevée dans l'expérience 4 pour protéger la toiture.

Les mesures de dosimétrie ont été réalisées, à l'aide de dosimètres thermoluminescents Panasonic sous forme de badges. Des badges, composés de quatre dépôts minces de $^{n}Li_2{}^{n}B_4O_7Cu$ sous écran de plastique, ont été utilisés pour les quatre configurations de la maison (source en position haute) ainsi que pour l'expérience 1 de 5 montées et de 5 descentes de source. Des badges, composés de 3 dépôts minces de $CaSO_4$, Tm sous écran de plomb, ont été utilisés pour les expériences 2, 3, 4 de 3 montées et de 3 descentes de source. Tous ces badges sont triés à $\pm$ 7,5 % et donnent des mesures à $\pm$ 10 %, tous facteurs de réponses en dose, en énergie et de fading compris.

Dans chacune des quatre expériences de la première campagne, des TLD ont été disposés en une cinquantaine d'emplacements décrivant tout le volume habitable de la maison. La comparaison des mesures avec les calculs a permis d'améliorer la méthode d'évaluation du kerma

moyen dans un logement utilisé dans le code BILL. Les facteurs de protection moyens dans le volume habitable de la maison sont respectivement de 0,49 - 0,42 - 0,42 - 0,33 dans les expériences 1, 2, 3 et 4.

3.2.3. La seconde campagne

Durant la seconde campagne, les kerma ont été mesurés en quelques points avec des TLD pour une configuration de la maison (avec fenêtre), tandis que les spectres d'énergie aux mêmes emplacements ont été mesurés dans la maison avec et sans fenêtre.

Les dosimètres thermoluminescents, dans leur badge, ont été sélectionnés et regroupés par 6, dans un étui en plastique, afin d'obtenir la répartition de la dose dans un carré vertical de 5 cm de côté environ.

L'interprétation des résultats et surtout l'intercomparaison des mesures et des calculs ne sont pas terminées. Cependant, il semble qu'il y ait un relativement bon agrément d'une part, entre les mesures et les calculs tant du kerma que des spectres d'énergies, et d'autre part entre les résultats obtenus respectivement par le GSF et le CEA.

3.3 - Distribution des facteurs de protection

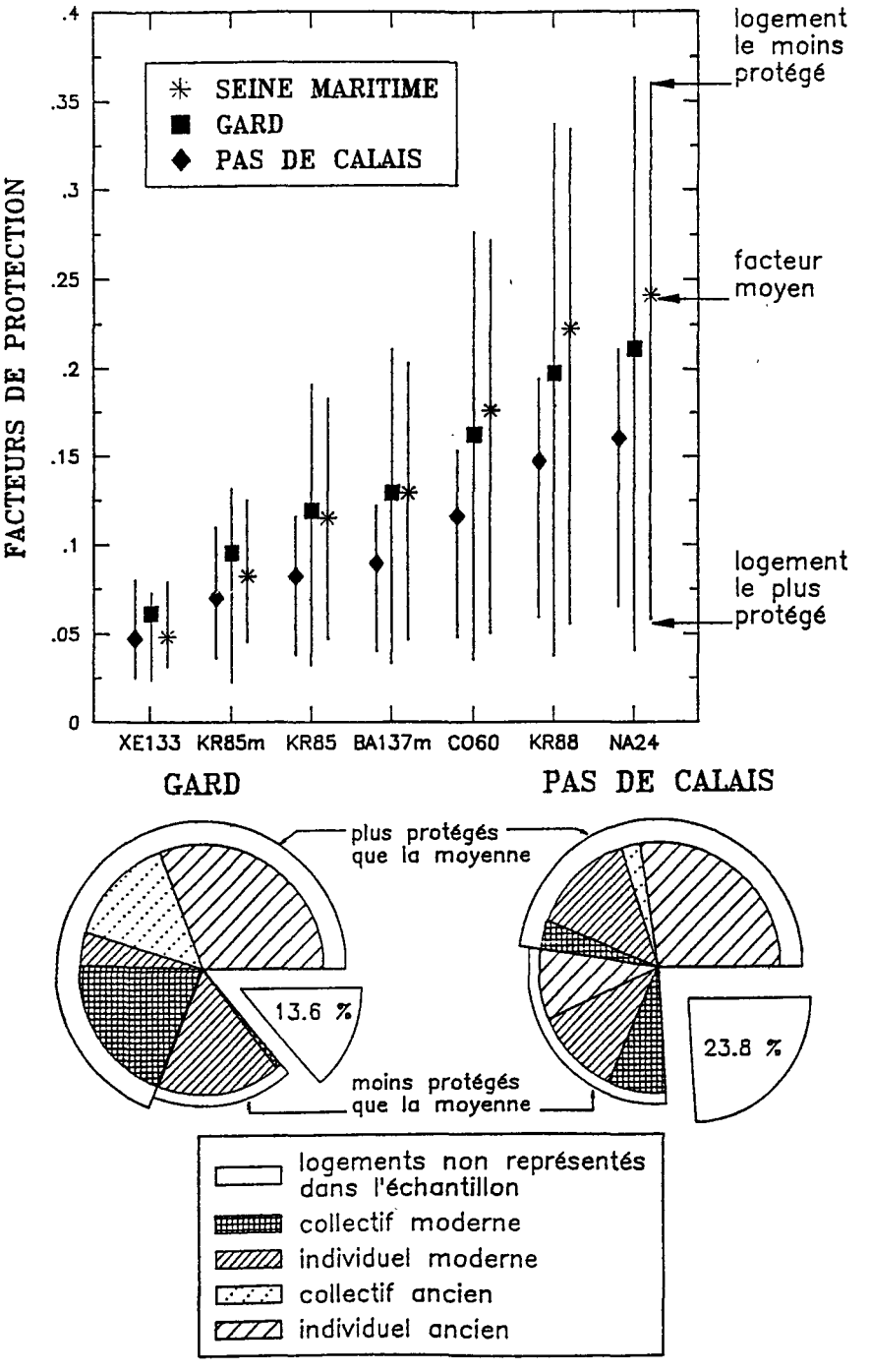
Dans un premier temps, les débits de kerma pour une concentration atmosphérique unitaire ont été déterminés au-dessus de différents types de sol [5 - 7] ainsi que les doses absorbées à l'organisme entier. Dans un deuxième temps, les facteurs de protection ont été calculés dans 647 immeubles représentatifs des départements étudiés, pour six valeurs de l'énergie initiale des photons (0,1 - 0,2 - 0,5 - 1 - 2 et 5 MeV). La base de données ainsi établie comprend les facteurs de protection par logement et les facteurs moyens par immeuble.

Dans chaque département, les distributions de facteur de protection ont été étudiées en fonction des caractéristiques de l'habitat et de l'énergie initiale des photons. Ensuite, les facteurs de protection, par département et par nucléide, ont été calculés en fonction de l'énergie des raies et de leur fréquence d'émission.

Les figures ci-dessous présentent les résultats obtenus dans trois départements pour sept radionucléides, choisis afin d'illustrer l'influence de l'énergie sur les facteurs de protection. Ce sont, par ordre d'énergie croissante, le xénon 133, le krypton 85m, le krypton 85, le baryum 137m, le cobalt 60, le krypton 88 et le sodium 24.

Le département du Pas-de-Calais présente un habitat très homogène, dominé cependant par les maisons individuelles mitoyennes de deux niveaux. Les immeubles collectifs y sont peu élevés. Enfin, la technologie la plus fréquemment utilisée dans la construction des murs est la brique pleine. En conséquence, la moyenne adoptée est le facteur de protection moyen par immeuble, et l'écart entre le facteur de protection du logement le moins protégé de l'échantillon avec celui du logement le plus protégé est relativement faible.

Par contre, dans le Gard, il existe une forte opposition entre l'habitat ancien et l'habitat moderne. L'habitat ancien fortement mitoyen a des murs presque exclusivement en pierre, tandis que dans l'habitat moderne, qui regroupe 51 % du parc de logements, le matériau dominant - même dans le collectif - est le parpaing. De plus, la proportion de surface d'ouvertures de l'habitat individuel moderne est largement supérieure à la moyenne nationale. Par suite, l'écart entre le facteur de protection du logement le moins protégé avec celui du logement le plus protégé est grand. La moyenne retenue est le facteur de protection moyen de l'habitat moderne qui est - pour la plupart des radionucléides - voisin de celui du logement le moins protégé du Pas-de-Calais.


FACTEURS DE PROTECTION
.4
.35
.3
.25
.2
.15
.1
.05
0
SEINE MARITIME
GARD
PAS DE CALAIS
logement le moins protégé
facteur moyen
logement le plus protégé
XE133
KR85m
KR85
BA137m
CO60
KR88
NA24
GARD
PAS DE CALAIS
plus protégés que la moyenne
13.6 %
23.8 %
moins protégés que la moyenne
logements non représentés dans l'échantillon
collectif moderne
individuel moderne
collectif ancien
individuel ancien

Le département de Seine-Maritime, dont les facteurs de protection sont proches de ceux du département du Gard, a la particularité de présenter un habitat individuel défavorable : non seulement, dans le moderne où les murs de parpaing sont dominants, mais aussi dans l'ancien où une part importante des maisons ont des murs de bois (pans de bois et terre).

4 - Conclusion

Les principaux objectifs du projet ont été réalisés. Ce sont :

- l'établissement d'une base de données de dose par unité de concentration atmosphérique des différents radionucléides qui peuvent être rejetés dans l'atmosphère, au cours d'un accident dans une centrale nucléaire ;

- la réalisation d'une enquête sur l'habitat permettant de définir, pour chaque site nucléaire, un échantillon représentatif d'habitations ;

- la réalisation d'expériences de validation des codes de calcul ;

- l'établissement d'une base de données de facteurs de protection par nucléides, permettant d'évaluer l'efficacité d'une mesure de confinement de la population dans les habitations, autour de chaque site nucléaire.

Les caractéristiques de l'habitat qui ont le plus d'influence, en France, sur les facteurs de protection contre les rayonnements émis dans un nuage radioactif sont d'une part la prédominance de l'habitat individuel dans presque toutes les régions, et d'autre part l'utilisation de technologies légères, très souvent dominantes, dans la construction des murs de l'habitat moderne.

Cependant, il faut noter que ces facteurs de protection ont été déterminés pour un nuage semi-infini et que l'environnement urbain n'est qu'imparfaitement représenté. Celui-ci, qui est surtout important dans le cas des dépôts sur les surfaces en contact avec l'atmosphère, nécessiterait une étude sur les formes urbaines dans le but de définir une typologie des formes d'urbanisation mettant en évidence les différences régionales.

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

Centre de Recherche sur l'Habitat -
Ecole d'Artchitecture de Paris la Défense
41, allée Le Corbusier 92023 NANTERRE CEDEX.

J.P. PATAU - Faculté de Pharmacie
Service de Biophysique
UNIVERSITE Paul SABATIER
35 Chemin des Maraîchers
31062 - TOULOUSE CEDEX

V. Publications:

[1] J. LE GRAND, J.C. CROIZE, T. de DORLODOT et Y. ROUX.
Statistical survey of the housing characteristics and evaluation of shielding factors in the surroundings of Frendh nuclear sites.
Rad. Prot. Dosim. 21/(1/3) 87-95 (1987)

[2] J. LE GRAND, Y. ROUX
Les configurations physiques du parc de logements dans les départements français. Table Ronde Internationale - Actualité de la typologie architecturale. Paris 16-17 mars 1989 (to be published).

[3] J. LE GRAND, Y. ROUX et J.P. PATAU
Evaluation of doses and protection afforded by dwellings against atmospheric releases
Rad. Prot. Dosim. 11(1) 41-48 (1985).

[4] J. LE GRAND, Y. ROUX et J. PATAU
Dose reduction factors from a radioactive cloud for large buildings
Rad. Prot. Dosim. 15(4) 245-252 (1986).

[5] J. LE GRAND, Y. ROUX
Kerma rates and whole-body absorbed dose rates for immersion in a semi-infinite cloud.
Proc. IVth European Congress. XIIIth Regional Congress of IRPA, SALZBOURG, 1986.
Ed. by Austrian Association for Radiation Protection (1988).

[6] J. LE GRAND, Y. ROUX, G. KERLAU
Expériences de validation des codes de calcul utilisés pour déterminer les facteurs de protection apportés par les habitations.
Rapport CEA-R-5464 (1988).

[7] J. LE GRAND, Y. ROUX
Facteurs de dose pour l'irradiation externe : immersion dans un nuage et dépôt surfacique de radionucléides.
Rapport DPS 88/07/SEAPS.

Title of the project no.:

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.

III. Progress achieved:

1. INTRODUCTION

The purpose of this report is to describe the work which led to the development of a simulation model showing the transfer of pollution into the environment, capable of being adapted to various situations.

The first task necessary was therefore to carry out a vast amount of bibliographical research in the various methods of resolving the diffusion-advection equation. The methods analysed have been classed into four families:

- Analytical or semi-analytical resolutions of the diffusion-advection equation (DAE) which, although particularly interesting, are limited either to homogeneous n-dimensional infinite or semi-infinite media, or to non heterogeneous mono-dimensional finite media.
- Numerical resolutions effected by substituting discrete values into the DAE over a grid covering the medium being studied, using the finite differences method, the finite elements method or the nodal method. These methods, especially the first two, have inspired a large number of publications and many computer codes with varying performances. Despite the considerable interest of these methods, they unfortunately do not meet up to the objective at hand, since:
- they lead to the development of computer codes which are extremely greedy as far as data processing resources are concerned, whenever it is necessary to carry out three-dimensional calculations with a fairly high degree of heterogeneity,
- they lead to numerical diffusivity, due to the substituted spatial discrete values, which increase the diffusion term of the equation and require a fairly small time-scale steps in order to obtain a correct level of stability.
- Numerical resolutions using quasi-Lagrangian methods. These methods include the "Particle-in-cell" and "Puff-on-cell" methods.

  Here again, these methods have inspired many publications. These methods can be called quasi-Lagrangian because the diffusion-advection equation is resolved by "tracing" a group of particles (Puff) in the cells of a grid (on-cell). The particles are assumed to be spread out in a Gauss-type spatial distribution over the grid, at a given instant t. In the next instant, the mean of this Gauss-type distribution moves by advection, and its quadratic deviation increases by diffusion.

  The Gauss-type distribution then "spreads" over the grid cells around the calculation cell, and is broken down into several Gauss-type distribution systems with the same global mass.These resolution methods seem quite attractive, at first sight, except for the fact that:

- the breakdown into several Gauss-type distribution systems leads to a diffusion phenomenon, which needs to be counter-balanced by coefficients which control the quadratic deviation values.
- the stability condition of the method is very difficult to satisfy if the characteristics of the medium vary considerably between different points: it is necessary to adjust two control coefficients per cell of the medium.

  In reality, these methods only appears to be valid if the medium is quasi-homogeneous and if the advection element is preponderant over the diffusion element.
- Numerical resolutions using Lagrangian methods, that can be considered as "Monte-Carlo" methods, constitute the fourth family. These methods have been widely used in many fields, especially those involving the resolution of the diffusion equation and the Boltzmann equation. Bibliography concerning the stochastic aspect of the diffusion-advection equation resolution, has revealed the great interest in these methods shown by Soviet mathematicians (Khintchine, Guikman, Skorokhod, Kolmogorov...) and by the Japanese mathematician K. ITO. This report therefore contains a part of the work done by these mathematicians, and proposes a method for resolving the diffusion-advection equation that seems to be sufficiently rigourous from the mathematical point of view, and not very greedy as far as data processing facilities are concerned. The first advantage of this resolution method is that it provides interesting analytical results, i.e.
- a figure representing the probability of a particle, located at any given point in a medium, exiting that medium,
- figures showing a distribution of the departure time from the domain, and the moments, of any order, in this distribution,

This means that the trajectory of a particle can be simulated, and the equation resolved in any medium by using a number of simulations.

The most important part of our work is dedicated to establishing the formulae which will subsequently be put into program form. The demonstrations of the fundamental theorems used to generate these formula are not described - they are merely stated. Any reader wishing to look further into the subject will find details in the cross-referenced bibliography.

The flexibility of the method used therefore makes it possible to solve the dispersion-convection equation in highly heterogeneous media. In other words it is possible, in the case of atmospheric transfers, to take the effects of relief or urban agglomerations into account when working on a macro- or a meso-scale, or to take the effects specific to a hill, a building or a hedge of trees into account when working on a micro-scale.

In theory, this model has no limits. It is indeed capable of describing complex media at different scales (macro, meso and micro) with various conditions at the different limit (absorption - total or partial reflection with albedo, on one of the faces of any cell used to describe the medium). The only limits to the model are either program-induced and/or linked with the capacity of the computer in which the model is running: number of cells used to describe the medium, power of the computer used.

2. DESCRIPTION OF COMPUTER CODE

The computer code corresponding to the model discussed in Annexe 2 is written in FORTRAN ; it consists of one main program and thirteen sub-routines.

During a calculation, there is no Input/Output action, which means that the program code and tables are resident in the main memory during execution.

The required memory space is almost entirely taken up by the space reserved by three tables:

- A first table containing the data of the wind field at different time steps after emission of the particles. This table therefore contains as many elements as the number of grid-cells, multiplied by the number of wind fields, and multiplied by the number of wind vector components.
- A second table contains the description of the medium. It has as many elements as there are grid-cells.
- A third table contains the vector of the number of "mother" particles present in a cell at a given time step. It also has as many elements as the number of cells, multiplied by the number of given "steps".
  This table can be larger if:
  . the user requires results on a finer grid than the one used to describe the medium ;
  . the user requires an additional table showing the value of the number of "daughter" particles created by radioactive depletion, present in a cell at a given time step. The table created in this way will be of the same size as the third table.

If we use NX, NY, NZ to designate the number of steps in X, Y and Z of the grid describing the medium, NTL to designate the number of observation steps in the distribution of the particles in the domain, IC to designate the number of descendents from the mother particle, NV the number of wind fields used, the size of these tables will be equal to:

NX. NY. NZ. (3 NV + 1 +IC.NTL)

Example: If we take the case of a domain limited to a grid of 20 elements on the X-axis, by 20 elements on the Y-axis, by 20 elements on the Z-axis ; If we assume the use of 3 wind fields (NV = 1), and carry out the calculations for a radio-nuclide and its descendent (IC = 2) ; If we wish to set 5 observation steps (NTL = 5), then the required size will be: 21 200 bytes.

Execution time

The execution time cannot be specified, as it depends to an enormous extent on the machine used. Execution time depends on three factors:

- the maximum observation time: the greater this time is, the longer the particles will have to be followed ;
- the number of particles whose history needs to be followed: the greater the number of particles followed, the more accurate are the results. Indeed, if N is the number of particles emitted, the precision will be in $1/\sqrt{N}$.

The tracking of 10 000 particles induces a precision of 10^{-2} , which means that the third decimal in the calculation will be meaningless.

However, the calculation times for 10 000 particles and a few hours of simulation will, on most minicomputers, take only a few minutes.

Portability

The written program, without its graphics module, is entirely written in Fortran, and does not call upon any specific mathematical library : it is therefore totally portable.

a) Description of the grid system and the parameters associated with each cell

The medium studied is described by a regular three-dimensional or two-dimensional Cartesian grid. Eight parameters are then associated with each cell of the grid:

- An axial turbulence coefficient.
- A transverse turbulence coefficient.
- A vertical turbulence coefficient.
- An absorption coefficient which takes various processes into account, such as changes of phase or wash-out by rain. This coefficient is then translated into a probability term. Thus the speed of deposit of the particles on the ground, from the lower layers of the

atmosphere, becomes the probability of a particle being deposited ground when it arrives there, i.e. the probability of it being retained, especially if the ground is rough.

- Four coefficients showing the behaviour of the particle when it crosses one of the sides of the rectangular cell, or six coefficients showing the behaviour of the particle when it crosses one of the faces of the parallelipiped shaped cell in a three dimensional situation. Each coefficient indicates the albedo between 0 and 1. If it is equal to β, the particle has a probability β of passing through the face or side of the cell, and a probability $(1-\beta)$ of being reflected. One coefficient is defined per side or face of the cell.
- One field of vectors is associated with the medium. A two- or three-dimensional vector is associated with each cell. This vector is defined by its components in the fixed Cartesian coordinate system defined by the two- or three-dimensional grid. The origin of this vector is located at the centre of each of the cells.
  All these vectors together define a wind field.

b) Construction of a medium

The technique for defining a medium described above enables any one- two- or three-dimensional medium to be defined. It is therefore easy to define a medium using, for example, three types of cell:

- a "ground" type cell, whose coefficients of turbulence, absorption, and advection are defined for the lowest level of the atmosphere, and whose faces representing the ground are assigned with the total reflection limit condition (β=1).
  The other faces of the cell must be assigned with a total transmission limit condition.
- an "air" type cell, whose coefficients of turbulence, absorption and advection are defined for the corresponding layers of the atmosphere ; in this case, all the faces of the cells are assigned with a total transmission limit condition.
- an "inversion limit" type cell, whose coefficients of turbulence, absorption and advection are defined for the highest layer of the atmosphere, and whose topmost face is assigned with a β albedo transmission-reflection type limit condition. All other faces of this cell are assigned with total transmission limit conditions.

c) Characterisation of the particle

In order to characterise the particle, we define the particle's radioactive depletion coefficient. This coefficient is also translatable into a probability, i.e. the probability of a particle becoming disintegrated after a transfer time T.

d) Definition of the source

The emission point is defined by its coordinates in the Cartesian coordinate system in which the grid is defined.

4. DATA INPUT

The data necessary for the calculations is input in the form of a sequential file. The data to be input are as follows:

4.1. Definition of the reference grid of the medium

NRX PX Number of intervals (NRX) with a pitch PX on the X-axis.
NRY PY Number of intervals (NRY) with a pitch PY on the Y-axis.
NRZ PZ Number of intervals (NRZ) with a pitch PZ on the Z-axis.

4.2. Definition of the exit grid

NRLX PRX Number of intervals (NRLX) with a pitch of PRX on the X-axis.
NRLY PRY Number of intervals (NRLY) with a pitch of PRY on the Y-axis.
NRLZ PRZ Number of intervals (NRLZ) with a pitch of PRZ on the Z-axis.

4.3. Definition of the different media

NLIM Identification of the medium linked to a type of grid-cell.

J1 J2 I1 I2 K1 K2: Definition of a block of cells of the type identified by NLIM. This block consists of the cells between indices I1 and I2 on the X-axis, J1 and J2 on the Y-axis and K1 and K2 on the Z-axis. We define as many blocks as we need, up until the entire medium has been described. If a block overlaps one or several of the previously defined blocks, it is the overlapping block that takes precedence. When NLIM is equal to zero, this means that the description of the medium is finite. We will have defined the medium made up of NLIM different types of cells.

NLIM

J1 J2 I1 I2 K1 K2

. . . . . .

. . . . . .

NLIM

J1 J2 I1 I2 K1 K2

0

4.4. Definition of parameters connected with each type of cell

For each type of cell, we define ten parameters as described in previous chapters:

NLIM Identification of the first type of cell

PRM1 Coefficient of axial turbulence

PRM2 Coefficient of transverse turbulence

PRM3 Coefficient of vertical turbulence

PRM4 Coefficient of absorption

PRM5 Albedo with respect to face 1 (associated with X_{min} minimum abscissa)

PRM6 Albedo with respect to face 2 (associated with X_{max} maximum abscissa)

PRM7 Albedo with respect to face 3 (associated with Y_{min} minimum ordinate)

PRM8 Albedo with respect to face 4 (associated with Y_{max} maximum ordinate)

PRM9 Albedo with respect to face 5 (associated with Z_{min} minimum)

PRM10 Albedo with respect to face 6 (associated with Z_{max} minimum)

NLIM Identification of second type of cell:

. . . . . .

. . . . . .

5. OUTPUT OF RESULTS

The results are output in the form of particle concentrations in each of the cells of the output grid: ratio between the number of particles present in a cell, determined at an observation step T, and number of particles thrown out by the source. The output file has the following structure:

I1 J1 K1 D1 T1 VAL

in which I1 J1 K1 identifies the cell

D1 identifies whether it is a group of "mother" particles or "daughter" particles resulting from radioactive depletion.
(1 for "mother", 2 for "daughter")

TI identifies the observation step index.

There are as many recordings as there are cells in which there is at least one particle. Cells which are not identified in the output file do not contain any particles, and are associated with a zero concentration.

6. CONCLUSIONS

Our work has made it possible both to obtain interesting results on the diffusion-advection equation, considered in a stochastic differential form, and to write a computer code which solves the diffusion-convection equation in the most general cases.

The primary interest of the proposed methods is based on the modicity of the data processing resources required at the end. The resolution code for the DAE in a two-dimensional case, comprising 1 500 grid points, requires:

- less than 60 Ko of memory assigned to the program,
- no "input-output during the resolution of the equation.

The calculation codes produced using this type of method therefore seem particularly well adapted to a fairly wide range of mini- and microcomputers. They provide the possibility of processing complex cases, in terms of both the media description (relief, cell heterogeneity) and the limit conditions.

The conclusion of this report will consist in an enumeration of the developments necessary for this calculation code. The main difficulty resides in the generation of a coherent data set: it is not particularly easy make up the medium to be studied using a stack of cells of different types. A first development, which is currently in progress, consists in writing software that will carry out this task on the basis of a description of the relief identified by a range of points, each of which is associated with an altitude. A second development, also in progress, consists in enabling the operator to introduce a radioactive source that is more complex that a an instantaneous point source, i.e. a source that can be spread out over time and space. A third development, which should make the calculation code more interesting to use, consists in enabling particles of different types to be traced simultaneously (different radioactive depletion characteristics) ground sedimentation speed, etc, ...), and also enables different levels of "daughter" particles, produced by radioactive depletion, to be traced.

Even without resorting to new calculation code developments, many improvements can be made to the atmospheric transfer simulation:

It is recognised that the use of a simple wash-out coefficient is not a correct representation of the process of wash-out by rain, on radioactive or non-radioactive particles contained in the atmosphere. The model presented here enables atmosphere wash-out to be represented as an absorption, whose intensity can vary with altitude. This type of model representation, based on experimental observations, should provide a better estimation of humid radioactive particles on the ground.

It is also recognised that the use of a constant rate of deposit does not provide a correct micro-scale representation of the ground deposit of radioactive particles contained in the atmosphere. Here again, the model we have presented enables the particles deposited on the ground to be represented by a phenomenon of interaction with the ground surface, involving a roughness factor and therefore taking ground occupation into account.

In general terms, the superiority of the resolution in the DAE that we have put forward resides in its ability to solve, at each time interval and at the same time as the transfer equation is solved, one or several equations describing phenomena which are remotely associated with the transfer but which can have an effect on the result of the simulation. For example, it is fairly easy to incorporate an equation into the model to describe the elevation of the particles above the emission zone, if the emission involves the release of heat.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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und medizinische Forschung der
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Contract no.: BI6-F-130-D

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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, Dr. J. Kesten, Dipl.-Phys. G. Butterweck

I. Objectives of the project:

In all dosimetric models the aerosol particle size and the unattached fractions of the radon daughters are important parameters for the estimation of the radiation exposure to the human body. By means of different measuring techniques (high-volume impactors, low pressure impactors and screen diffusion batteries) the activity size 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 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 measurements of the properties of the shortlived radon decay products and of the mass will be continued in the outdoor atmosphere.
A thoron gas monitor must be calibrated, and it is planned to perform first measurements in rooms with higher thoron gas levels.
Properties of the thoron decay product ^{212}Pb (ThB) will be measured by the low pressure cascade impactor and by the screen diffusion battery technique indoor as well as outdoor.
The data evaluation of the European intercomparison measurements will be finished. The results will be published in the next reporting period as a European report.

III. Progress achieved:

1. Methodology:

Experimental methods and data evaluation

SIZE DISTRIBUTION MEASUREMENTS

In this study size distribution measurements were perforemd with a low pressure cascade impactor and high volume screen diffusion batteries. The low pressure cascade impactor consists of eight stages and a back-up filter and operates at a flow rate of 1.8 m^3h^{-1}. Efficiency curves and interstage losses were measured with monodisperse liquid, radioactive labelled aerosol particles in the diameter size range 70-6000 nm for two different pressures at the last stage. The measured 50% cut-off diameters range from 80 nm up to 6000 nm. The total interstage losses of aerosol particles are less than 2% of the total activity. Calibration measurements carried out with unattached ^{212}Pb show that about 75% of the unattached activity is lost in the entrance and on the walls of the first three impactor stages. To determine activity size distributions after air sampling the activities of ^{214}Pb, ^{214}Bi and ^{212}Pb on the eight impactor stages (deposited on thin aluminium foils) and on the back-up filter are measured by means of low level pure germanium, or by NaI detectors. For the determination of the sampled aerosol mass, the aluminium foils were weighed under controlled conditions before and after air sampling.

The particle diameters of the unattached Rn daughters (0.5-5 nm) and of aerosol-attached activities up to 400 nm were measured with screen diffusion batteries with different screen and mesh numbers at a volumetric flow rate of 2 m^3h^{-1}. In the diameter size range 4-200 nm the penetration of the different screens was measured with monodisperse test aerosols. Agreement was obtained between experimental results and theoretical predictions (Cheng et al. 1980) taking into account the individual efficiencies for diffusion, direct interception, and inertial impaction. The measurements of Rn daughter activity sizes consist of taking four parallel samples, three with screen diffusion batteries and one without as an absolute sample. This procedure was repeated twice with different screen numbers. The activities are collected on membrane filters and the emitted alpha particles are registered with surface barrier detectors during sampling and during a period after the end of air sampling. From the measured alpha counts the concentrations of the Rn daughters ^{218}Pb, ^{214}Pb and $^{214}Bi/^{214}Po$ and the corresponding penetration values are calculated.

Activity and mass size distributions are obtained from the size fractionated activities, measured with the low pressure impactor and the diffusion batteries by comparing the measured activities with simulated ones. The simulated values were calculated using the efficiency curves of the impactor and the wall losses of the unattached activities or the penetration curves of the diffusion batteries, respectively, whereby the true size distributions are approximated by a sum of log-normal distributions. In addition to this numerical evaluation, the impactor data were evaluated by a graphical method (cumulative method).

The median diameters of the accumulation mode of aerosol-attached activities of the shortlived Rn daughters range from 100 to 400 nm. This size range is the upper measuring limit of screen diffusion batteries or the lower one of impactors. Therefore comparison measurements were performed to check the accuracy and calibration curves of both methods. Measurements performed in a low ventilated room without additional aerosol

sources yield unimodal log-normal distributions for the Rn decay product ^{214}Bi with mean median diameters of 239 nm (screen diffusion batteries) and of 254 nm (low pressure impactor). The mean geometrical standard deviations of the calculated log-normal distributions are also in fairly good agreement for the different devices and range from 2.0 (screen diffusion batteries) up to 2.4 (low pressure impactor). From the screen diffusion battery data bimodal ^{218}Po - distributions were evaluated. The median diameters of the unattached cluster mode was about 1 nm with a geometric standard deviation of 2.0 and the median diameter of the accumulation mode was 210 nm with a geometric standard deviation of 1.9.

MEASUREMENT OF THE UNATTACHED FRACTIONS OF THE RADON PROGENY

For the separation of the unattached from the aerosol-associated Rn daughters and for the determination of the amount of the unattached fractions a single-screen with a 50% - penetration diameter of 4.0 nm was used. The measurement of the activity was performed by alpha spectroscopy (see above chapter). However, the rather flat penetration versus size dependence of single-screens and the ambiguity of the penetration curves - diameters in the coarse mode size range and in the ultrafine size range are associated with the same penetration value - have to be considered. These influence of the aerosol-attached activities deposited on the screens by impaction and interception was considered by a new developed data evaluation method. Our studies show that under realistic living conditions, indoors and outdoors, the unattached fraction of ^{214}Bi (^{214}Po) can be neglected (< 1%). Together with the experimental result that the size distributions of the aerosol-attached activities of all Rn progeny are identical the unattached fractions of ^{218}Po and ^{214}Pb were corrected for the screen collected aerosol-attached activities by means of the measured ^{214}Bi (^{214}Po) activities deposited on the screen. With this method it is only necessary to make sure that all unattached activities are collected, i.e. the 50% penetration value of the screen must be in the order of (4-6) nm. It is not necessary to know the exact shape of the penetration curve of the single-screen or the entire size distribution of the radioactive aerosol.

RADON GAS AND THORON GAS MEASUREMENTS

Radon gas in the air was measured continuously by electroprecipitation of the positively charged ^{218}Po ions in an electric field (10 kV) on a surface barrier detector. For this purpose the air was dried, filtered and sucked into an aluminium sphere (1.8 l) with a flow rate of 100 l h^{-1}. The measured alpha counts of the radioactive decay of ^{218}Po and ^{214}Po are proportional to the radon gas activity concentration in the air. This Rn gas monitor could detect down to 1 Bq m^{-3} with three hours counting time and 30% statistical uncertainty.

For the measurement of Tn gas under environmental conditions a similar device as for the Rn gas measurements was built. Due to the short half-life of Tn (56 s) an aluminium sphere of 14 l was used, a flow rate of 900 l h^{-1}, and the electric field was increased up to 18 kV. The Tn gas monitor was calibrated using a Tn gas source with a constant emanation rate of about 55%. This Tn gas monitor could detect down to 5 Bq m^{-3} with three hours counting time and 30% statistical uncertainty.

MEASUREMENT OF THE AEROSOL PARTICLE CONCENTRATION

Parallel to the size distribution measurements the particle concentration was registered by means of a calibrated condensation nuclei counter (General Electric). The counting efficiency and detection limit was measured with monodisperse Ag and NaCl particles in the diameter size range between 2 and 200 nm. For particle diameters of 3–4 nm a counting efficiency of some percent was obtained, whereas the 100% efficiency was reached for particle diameters of (10–20) nm (Scheibel and Porstendörfer 1986).

ROOM MODEL CALCULATIONS

The concentration of Rn decay products, the equilibrium factor, F, and the unattached fraction of the total potential alpha energy, f_p, are influenced by the basic processes of attachment, recoil and deposition (plateout) and by room specific parameters of Rn emanation rate e and ventilation rate v. The attachment rate to the atmospheric aerosol X is a linear function of the particle concentration Z: $X=\beta \cdot Z$ (β=attachment coefficient). The recoil factors r_i define the probability of an attached radioactive atom desorbing from the particle surface in consequence of an alpha decay. Surface deposition is the most important parameter in reduction of the Rn decay products in room air. The influence of the deposition rates q^f (unattached or "free" activity) and q^a (aerosol-attached activity) on the air activity concentrations can be described by room model calculations. Assuming a constant emanation rate, a spatial homogeneous activity distribution and 100% prefiltering of the unattached factions of the incoming air, the radon gas, the unattached and attached Rn daughter activity concentrations indoors (c_0^i, c_j^{if}, c_j^{ia}) and outdoors (c_0^o, c_j^{of}, c_j^{oa}) under steady state conditions are:

$$c_0^i = (e+vc_0^o)/(\lambda_0+v) \qquad (1)$$

$$c_j^{if} = (\lambda_j c_{j-1}^{if} + r_{j-1}\lambda_j c_{j-1}^{ia})/(v+\lambda_j+q^f+X) \qquad (2)$$

$$c_j^{ia} = (vc_j^{oa} + (1-r_{j-1})\lambda_j c_{j-1}^{ia} + Xc_j^{if})/(v+\lambda_j+q^a) \qquad (3)$$

with $c_0^{if} = c_0^i$, $c_0^{ia} = 0$, $c_j^{of} = 0$ and j=0: ^{222}Rn, j=1: ^{218}Po, j=2: ^{214}Pb, j=3: ^{214}Bi(^{214}Po).

Under the conditions $v < 1\ h^{-1}$ and $c_j^i >> c_j^o$ the attachment rate X could be calculated by means of the measured values c_1^{ia} and c_1^{if}:

$$X = c_1^{ia}(\lambda_1+v+q^a)/c_1^{if} \approx c_1^{ia}(\lambda_1+v)/c_1^{if} \qquad (4)$$

with $(\lambda_1+v) >> q^a$

Then the plateout rate q^f of the unattached Rn daughters can be determined from the measured activity concentration of the unattached ^{218}Po and the Rn gas ^{222}Rn:

$$q^f = \lambda_1 (c_0^i / c_1^{if}) - v - \lambda_1 - X \qquad (5)$$

In addition an equation of q^a can be evaluated by means of the measured values of c_2^{ia}, c_3^{ia} and c_2^{if}:

$$q^a = \lambda_3 (c_2^{i,a}/c_3^{i,a}) - \lambda_3 (c_2^{i,f}/c_3^{i,a}) X/(v+\lambda_3+q^f+X) - \lambda_3 - v \qquad (6)$$

JOINT MEASUREMENTS

Different experimental methodologies for the determination of the dynamics of Rn daughters in realistic indoor environments were compared by means of joint measurements performed in houses with elevated Rn concentrations in Belgium and in Northern Bavaria (FRG). Groups of the Gent State University (B), the National Radiological Protection Board (Chilton, GB) and the Isotopenlabor of the University of Göttingen (FRG) have been participating at this European Intercomparison of Rn daughters measuring equipment.

2. Results

Indoor and outdoor size distributions

INDOOR ACTIVITY SIZE DISTRIBUTIONS WITHOUT ADDITIONAL AEROSOL SOURCES.

Several acticity size distribution measurements were carried out in different rooms with Rn levels in the range 200–1700 Bqm^{-3}. All rooms were low or moderate ventilated ($v < 1h^{-1}$) and aerosol particle concentrations between 2000 and 16000 cm^{-3}.

The size distributions of ^{218}Po and $^{214}Bi/^{214}Po$ were calculated by means of the diffusion battery data. In some cases the ^{214}Pb distributions were also determined from the measured penetration values. It was found that the particle size of this aerosol–attached activities agree with those of ^{218}Po and $^{214}Bi/^{214}Po$.

The activity median diameters AMD (diffusion equivalent), the geometric standard deviation σ_{g1} and the fraction f_1 of the different parts of the derived log–normal distributions (mean values and extreme values) are listed in Table 1. All activity size distributions of ^{218}Po can be approximated by bimodal log–normal distributions, whereas the size distributions of $^{214}Bi/^{214}Po$ are nearly unimodal log–normal. Depending on the aerosol particle concentrations more than one third of the ^{218}Po activity was unattached and the diffusion equivalent diameters range from 0.5 to 2.0 nm with a mean diameter of 1.2 nm. The fairly high geometric standard deviations of this mode (σ_{g1}=1.3–2.6) show that before attachment the Rn daughters grow by cluster formation. Perhaps these clusters are stable because sometimes a small fraction (average 3%) of the $^{214}Bi/^{214}Po$ activities are measured in the size range up to 8.4 nm. However, it must be mentioned that this part of the $^{214}Bi/^{214}Po$ size distribution can also be explained by the ambiguity of the penetration curves (large particles would be misinterpreted as small particles), for statistical reasons or by the approximation of the true size distribution by a log–normal distribution.

The activities attached to aerosol particles (accumulation mode) are the dominant part of all activity size distributions and this mode of the ^{218}Po distributions agree with those of the $^{214}Bi/^{214}Po$ distributions. The AMDs range from 97 up to 313 nm, the geometric standard deviations σ_{g2} between 1.4 and 3.1 and the corresponding mean AMDs are 181 nm for ^{218}Po and 198 nm for the $^{214}Bi/^{214}Po$, where the mean geometric standard deviations are 2.0 and 2.1, respectively.

The size fractionated activities of the Rn decay product ^{214}Pb were determined using all impactor measurements. In some cases the activities of ^{214}Bi were also determined, and the same activity size distributions were

obtained for both Rn daughters. The activitiy median aerodynamic diameters (AMAD) of the accumulation mode range from 132 up to 285 nm with a mean value of 197 nm and with a mean geometric standard deviation of 2.5 (range 1.9–4.3, see Table 1). In addition to the activities resulting from the accumulation mode in the size range smaller than 100 nm (nuclei mode) about 3% of the total activty was measured. There is no evidence for another maximum of the coarse mode in the size range of some um.

In order to compare the results of the diffusion battery and of the impactor measurements, in a first approximation aerodynamic diameters (AMAD) can be calculated from diffusion equivalent diameters (AMD) by multiplying with the square root of the particle density. A density of 1.4 g cm^{-3} yieldes mean AMADs of the attached fraction of 214 nm (^{218}Po) and 234 nm ($^{214}Bi/^{214}Po$). These values are 8–19% higher than the AMAD measured with the impactor.

INDOOR ACTIVITY SIZE DISTRIBUTIONS IN CLOSED ROOMS WITH ADDITIONAL AEROSOL SOURCES

The influence of aerosol particles from additional aerosol sources on the activity size distribution was studied in three rooms with the diffusion battery technique and during the european intercomparison measurements in one other room with the low pressure impactor. About one hour before the sampling period the aerosol source was started and the production was carried on during the measurement. Besides the maxima of the unattached and aerosol attached activities of the accumulation mode, a third mode in the nuclei mode size range (<100 nm) was sometimes found. In the case of very small primary particles (candle light) from the screen diffusion battery measurements AMDs of several nm were obtained, whereas sooty particles yield median values of 150 nm. In the case of a burning candle the impactor measurements yield activity size distributions with a mean AMAD of 53 nm and a mean σ_g of 4.4. The aerosol particles of an electric motor producing aerosol particle concentrations of $(50–100)*10^3$ cm^{-3} led to additional nuclei modes with median values in the range of 37–160 nm. Cigarette smoke and aerosol particles from a tiled stove yielded AMDs of 300 and 270 nm, respectively (screen diffusion measurements). However, in the case of cigarette smoke impactor measurements yielded smaller median diameters of 130 nm.

OUTDOOR ACTIVITY AND MASS SIZE DISTRIBUTIONS

In the open atmosphere (vicinity of Göttingen) activity size distributions of the Rn decay product ^{214}Pb and the Tn decay product ^{212}Pb and mass size distributions were measured with the impactor. The parameters obtained for the measured activity size distributions are listed in Table 2. Since the measurements were performed at different days under variable meteorological conditions, a greater variation of the size distributions than that indoors was found.

The activity size distributions of the Rn and Tn daughters are similar. About 90% of the activity is associated to aerosol particles of the accumulation mode with median diameters of (300–400) nm. Due to outdoor aerosol sources (re–suspension and combustion processes) and meteorological conditions (turbulent exchange, precipitation) sometimes activities in the nuclei mode size range (<100 nm: 0–28%) and coarse mode size range (>2000 nm: 0–30%) were measured.

Table 1. Mean values of the median diameters AMD and AMAD, geometric standard deviations σ_{gi}, and activity fractions f_i of the log-normal size distributions, measured in closed rooms without additional aerosol sources with a low pressure impactor (IM) and the screen diffusion battery (DB) technique, The extreme values are listed in parenthesis.

Method No.of measur.	Nuclide	AMD/AMAD (nm)	σ_{g1}	fraction f_1/f_2	AMD/AMAD (nm)	σ_{g2}	fraction f_2
DB	^{218}Po	1.2	2.0	0.35	181	2.0	0.65
(18)		(0.5-2.0)	(1.3-2.6)	(0.20-0.54)	(97-265)	(1.5-2.7)	(0.46-0.80)
DB	^{214}Bi	2.7	2.3	0.03	198	2.1	0.97
(18)	^{214}Po	(0.7-8.4)	(1.6-3.6)	(0 -0.11)	(115-313)	(1.4-3.1)	(0.89-1.00)
IM	^{214}Pb	(10 -100)	?	0.03	197	2.6	0.97
(30)				(0 -0.17)	(132-285)	(1.9-4.3)	(0.83-1.00)

Table 2. Mean values of the median diameters AMAD and MMAD, geometric standard deviations σ_{gi}, and activity fractions f_i of the log-normal acticity and mass size distributions, measured in the outdoor atmosphere (vicinity of Göttingen) with the low pressure cascade impactor.

nuclide/mass No. of measurements	fraction f_1 (AMAD/MMAD<100 nm)	AMAD/MMAD (nm)	σ_{g3}	fraction f_3	AMAD/MMAD (nm)	σ_{g4}	fraction f_4
^{214}Pb	0.08	369	2.5	0.89	2820	1.5	0.03
(12)	(0 - 0.15)	(173-645)	(1.6-4.4)	(0.69-1.00)	(1660-4430)	(1.2-1.9)	(0 -0.25)
^{212}Pb	0.12	336	2.1	0.84	4540	1.9	0.04
(34)	(0 - 0.28)	(149-551)	(1.5-3.6)	(0.57-1.00)	(1350-7600)	(1.2-3.2)	(0 -0.30)
mass		460	2.2	0.59	4130	1.8	0.41
(25)		(173-737)	(1.4-3.3)	(0.37-0.87)	(2720-7000)	(1.2-2.9)	(0.13-0.63)

The mass median aerodynamic diameters (MMAD) of the accumulation mode is shifted to greater sizes (mean value: 460 nm) compared with the activity size distributions. About 60% of the total mass was found in this diameter size range and the remaining 40% of the mass was found in the coarse mode size range with median diameters of about 4000 nm (see Table 2).

The unattached fractions (f_1, f_p), the equilibrium factor F, plateout rates, and attachment rates

INDOOR MEASUREMENTS WITHOUT ADDITIONAL AEROSOL SOURCES

Measurements were performed in 10 furnished rooms of different houses with elevated Rn gas concentrations. Several measurements were carried out in each room at different times and under various outdoor conditions. The results of measurements in rooms without additional aerosol sources at low or moderate ventilation rates ($v < 0.5\ h^{-1}$) are summarized in Fig. 1 and 2. For each room the mean unattached fraction of ^{218}Po and ^{214}Pb, the corresponding f_p-values and the standard deviations are shown as a function of the aerosol particle concentration. In these figures the experimental results are compared with predictions of room model calculations (see eqn. 1-3). In closed rooms with an aged aerosol, the behavior of the Rn decay products can best be described by mean plateout rates $q^f = 54\ h^{-1}$, $q^a = 0.21\ h^{-1}$ and a mean attachment coefficient $\beta = 5.2 \cdot 10^{-3}\ cm^3 h^{-1}$. The decrease of the unattached fractions of the Rn progeny with increasing aerosol concentrations is evident. The f_p-values of the single measurements ranged from 0.016 and 0.246 with a mean value of 0.096 and the aerosol particle concentrations ranged between 1100-18000 (mean value 6100 cm^{-3}). The mean equilibrium factor, F, was determined to be 0.30 (range 0.15-0.49) and F increases with increasing aerosol particle concentrations. In rooms without additional sources, a mean attachment rate X of 28 h^{-1} (range 12-53 h^{-1}) was determined from the measured ^{218}Po concentrations (see eq. 4).

INDOOR MEASUREMENTS WITH ADDITIONAL AEROSOL SOURCES

The measurements in rooms with additional aerosol sources can roughly be devided into three groups. The first kind of aerosol sources (burning candle, electric motor, gas heating) produce small particles in the nuclei mode size range with diameters smaller than 100 nm. Although the particle concentrations ranged up to $10^6\ cm^{-3}$, the unattached fraction of the Rn daughters are only slightly smaller than in closed rooms with an aged aerosol (f_p~(0.03-0.10)). This result is due to the relatively small attachment rate X in the order of 100 h^{-1}.

Another class of aerosol sources mostly produce particles in the larger size ranges of the accumulation mode or coarse mode: cigarette smoke, burning wood in a tiled stove, burning candle (sooty particles). During measurements with these sources, the particle concentrations ranged up to $4 \cdot 10^5\ cm^{-3}$. Due to greater particle sizes the attachment rate increased up to about 1000 h^{-1}. The increasing attachment rates yield decreasing unattached fractions f_p below the detection limit of 0.005 and increasing F-values of 0.8.

Measurements in rooms with partly opened windows were performed to study the influence of the outdoor atmosphere. Because of different aerosol sources, the outdoor aerosol is characterized by a larger amount of aerosol particles in the nuclei and coarse mode size ranges compared to an aged

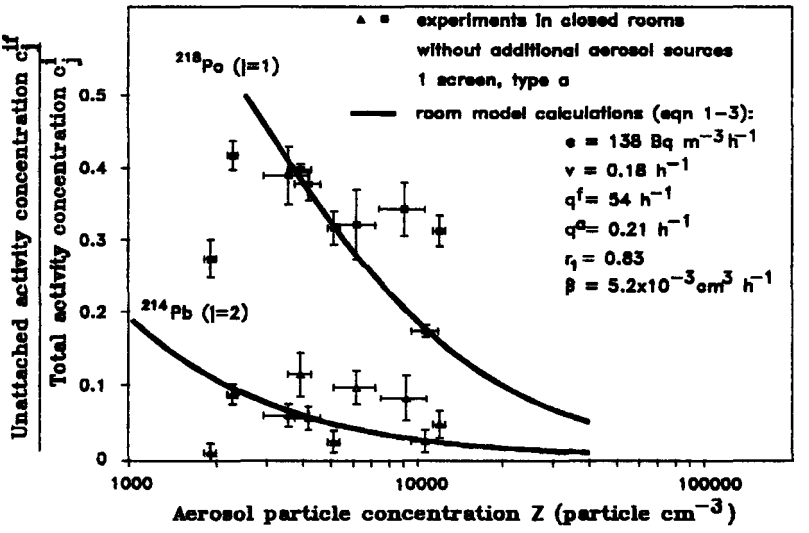

Figure 1. Unattached fractions of ^{218}Po and ^{214}Pb in closed rooms without additional aerosol sources versus particle concentraion Z, experiments and theory

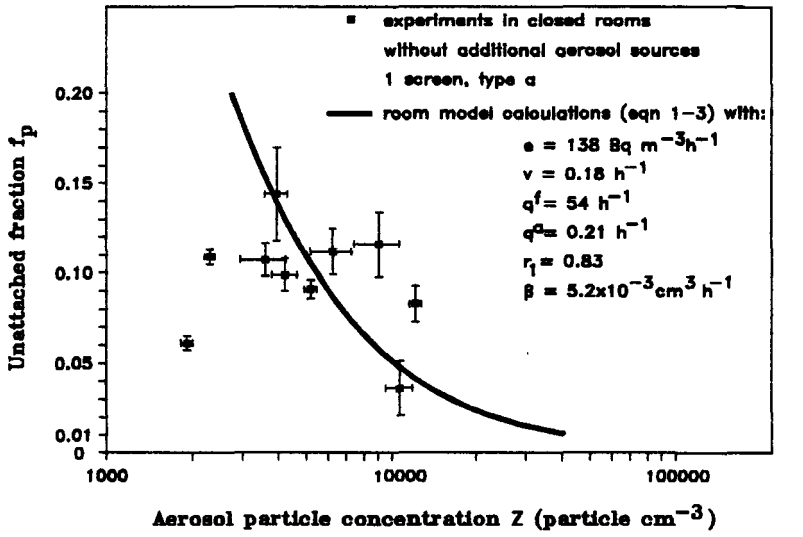

Figure 2. Unattached fraction f_p of the short-lived Rn decay products in closed rooms without additional aerosol sources versus aerosol particle concentration Z, experiments and theory

room aerosol. Therefore in rooms with partly opened windows rhe aerosol concentration increases and the unattached fraction f_p decrease slightly.

OUTDOOR MEASUREMENTS

In the outdoor atmosphere, the unattached activities were measured about 1 m above the ground (meadow) in the daytime. Different weather conditions influenced the turbulent exchange rate and exhalation rate and yielded rather large variations of the Rn gas concentrations (1-38 Bqm^{-3}) and of the concentrations of the Rn decay products. The aerosol particle concentrations ranged between $(7.5-93)*10^3$ cm^{-3} with a mean value of $3.4*10^4$ cm^{-3}. The unattached fraction f_p ranged between (0-0.22) with a mean value of 0.02, and for the F-factor, a mean value of 0.67 was measured (range: 0.23-1.19).

3. Discussion

Activity size distribution measurements of the Rn daughters yield the result that due to different plateout and coagulation rates the mean AMAD of the main activity mode (accumulation mode) of the indoor size distributions are significantly shifted to smaller diameters (AMAD=197 nm) compared with the outdoor results (AMAD=369 nm). Nearly the same results were obtained in former studies with a high volume impactor, where 170 and 390 nm were measured (Becker et al. 1984). These results also agree with measurements by Sinclair et al. (1978) where 150 nm was determined indoors and about 500 nm outdoors. Only Bondietti et al. (1987) published outdoor size distributions of ^{214}Pb with an averaged AMAD of 160 nm, which is smaller than the present values by a factor of two.

The results of measurements of the unattached fraction f_p show that most of the published calculations of the natural radiation exposure of the human public based on incorrect values of the unattached fraction of the short-lived Rn decay products (NEA 1983, ICRP 1987). Under normal conditions (closed room without aerosol sources, e.g. bedroom) the mean f_p-value of 0.095 is three times higher than proposed in the literature (f_p=0.03) (NEA 1983, ICRP 1987). Only in rooms with additional aerosol sources (cigarette smoke, aerosols from cooking) and in the outdoor environment, the aerosol particle concentrations are high enough to give an unattached fraction is equal or smaller than 0.03. Since in the dosimetric models, the dose conversion factor for the bronchial dose equivalent increases nearly lineary with f_p (James 1988), the natural radiation dose due to the short-lived Rn progeny is probably underestimated.

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Sinclair, D.; George, A.C.; Knutson, E.O. Reprint of an article published in the American Nuclear Society Proceedings entitled "Assessment of Airborne Radioactivity"; 1978

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V. Publications:

J. Porstendörfer, A. Reineking "Free Fractions, Attachment Rates, and Plateout Rates of Radon Daughters in Houses" in: Radon and its Decay Products. Occurence, Properties, and Health Effects (edited by P.K. Hopke), ACS Symposium Series 331 pp285-300, 1987

A. Reineking, J. Porstendörfer, K.H. Becker "Comparison Measurements of Radon Daughter Activity Size Distributions with Cascade Impactors and High-Volume Screen Diffusion Batteries" in: AEROSOLS, Formation and Reactivity, pp. 1158-1163. Proceedings of the Second International Aerosol Conference Berlin (West), September 1986

H. Vanmarcke, A. Reineking, J. Porstendörfer, F. Raes "Comparison of two Experimental Methods to determine the Unattached Fraction of Radon Daughters in Houses" Radiation Protection Dosimetry 24, 281-284, 1988

A. Reineking, K.H. Becker, J. Porstendörfer "Measurements of Activity Size Distributions of the Short-Lived Radon Daughters in the Indoor and Outdoor Environment" Radiation Protection Dosimetry 24, 245-250, 1988

A. Reineking, J. Porstendörfer "Activity Size Distributions of the Shortlived Radon Decay Products and their Influence on the Deposition Probability in the Human Lung" Journal of Aerosol Science 19, 1331-1337, 1988

A. Reineking, J. Porstendörfer "The Unattached Fraction of the Short-lived Rn decay Products in the Indoor and Outdoor Environment: an improved single-screen method and results", accepted for publication in Health Physics 1990

A. Reineking, J.C. Stong, H. Vanmarcke, R. Van Dingenen "Intercomparison of Methods for Investigating the Physical Characteristics of Radon Decay Products in the Environment" CEC report in preparation

RADIATION PROTECTION PROGRAMME
Progress Report

1989

Contractor: Contract no.: BI6-F-314-E

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Departamento de Ciencias Médicas y Quirurgicas
Facultad de Medicina de Santander
Catedra de Fisica Medica
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Telephone number: (942)337100

Title of the research contract:

Natural exposure from radon and radon progeny in Spanish houses.

List of projects:

1. Natural exposure from radon and radon progeny in Spanish houses

Title of the project no: 1

Head of the project:

Prof. Luis S. Quindos Poncela

Scientific staff:

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Prof. Fernandez Navarro
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Profa. Bonet Hortelano
Profa. Diaz Caneja Rodriguez
Profa. Gutierrez Velarde
Prof. Oyarzabal Amigo
Prof. Perez Muñoz
Prof. Peña Bernal
Prof. Calvo Aguilar
Prof. Vega Fernandez
Profa. Rossell Bueno
Prof. Galvez Delgado
Prof. Valverde Valverde
Prof. Arteche Garcia

I.- OBJECTIVES OF THE PROJECT

.- Determination of radon gas in spanish houses using a grab sampling method to calculate the national average of radon level in Spain.

.- Measurement of radioactivity in soil samples to correlate it with the presence of radon in houses.

.- Selection of areas in Spain with high radon levels in houses where develop a more defined studies concerning to the importance of different sources in the presence of radon in houses.

.- Evaluation of external dose from the radium, thorium and potasium content in soils and building materials.

.- Study of sources of radon in houses in selected areas with special emphasis in soils, building materials and water.

.- Measurement of the exhalation rate from soils and building materials.

.- Detection and study of radon in water supplies comparing the results with his natural radium content.

.- Study of the relationship between the external dose derived from the radioactivity of soils and the experimental resultsfrom a portable monitor.

.- Measurement of radon progeny using a grab sampling method, the WL and the equilibrium factor in a group of houses of selected areas in Spain.

.- Study of the relationship between the measurements of radon using passive dosimeters and the instantaneous method employing modified Lucas cells in order to evaluate the dose to the public.

.- Geographical distribution of lung cancer in Spain:Study of the incidence in ratio to the radon levels measured and smoking.

II.- Objectives for the reporting period (1st November 1988-1 st November 1989)

.- Determination of radon gas in spanish houses using a grab sampling method to calculate the national average of radon level in Spain.

.- Measurement of radioactivity in soil samples to correlate it with the presence of radon in houses.

.- Selection of areas in Spain with high radon levels in houses where develop a more defined studies concerning to the importance of different sources in the presence of radon in houses.

III. Progress achieved

During the period reported in this paper and according with the general objectives of the Project more than 2,300 measurements of radon levels in spanish houses have been finished succesfully. From them, 1,600 are related with the national survey and another 700 corresponding to the comunity of Cantabria located in the north of Spain where is located our University.

Figure I show the location of sampling points along the country, taking into account that each point mean an average of approximately 30 individual measurements of radon concentration in houses.

The measurements of radon levels in houses were made employing modified Lucas cells developed in our laboratory and calibrated in the NRPB at the third CEC intercomparison of active detectors in October, 1987 (Report EUR 11882 EN).The measurements were under standard conditions for the houses: a) close house (12 hours) with minimum level of ventilation b) winter season c) meteorological data recording and d) location of stable conditions for the room. In the same way, quality control in the development of the measurements was carefully take into account specially with the measurement of background and systematic control of the LLD that for our proposal was evaluated in 17 $Bq.m^{-3}$ for a counting time of 10 minutes.

The geometric mean for the total of measurements was 40.7 $Bq.m^{-3}$ with an standard deviation of 2.6.The values founded appear, as suspected, to follow a lognormal distribution showed in the Figure II.In the Figure III are indicated the percentages of homes for different intervals of radon levels for each of the 17 different communities of Spain.

This figure show that approximately a 13% of the houses in Spain are exceeding the recomended EPA value of 148 $Bq.m^{-3}$. Nevertheless this percentage is bassically corresponding to a few places or areas in Spain. In Figure IV is also shown the percentages of houses with high radon levels for two typical values: higher than 148 and 286 $Bq.m^{-3}$ respectively. Finaly, the Figure V include the areas taht focus our research interest for the next period.

The measurement of the radioactivity of soils have been acchieved and we are now proccesing data in the computer.More than 700 soils samples corresponding each one to a grid of 10x10 km^2 were collected along the total surface of Spain.A gamma spectrometry technique has been used in the identification and evaluation of the samples at the laboratory.

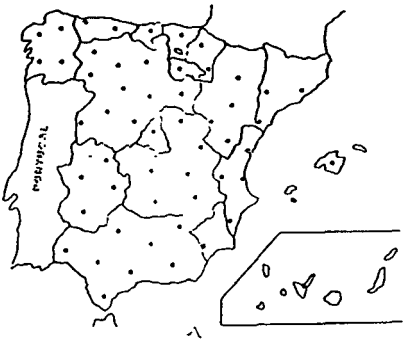
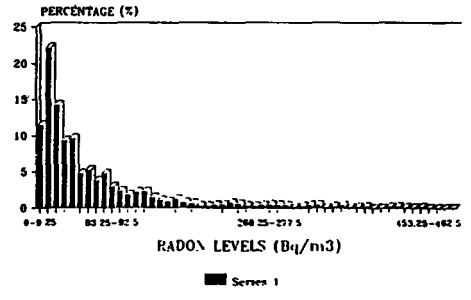


Figure I

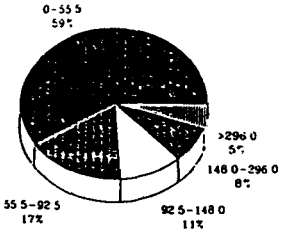
RADON LEVELS IN SPAIN
LOG–NORMAL DISTRIBUTION



(1988)

Figure II

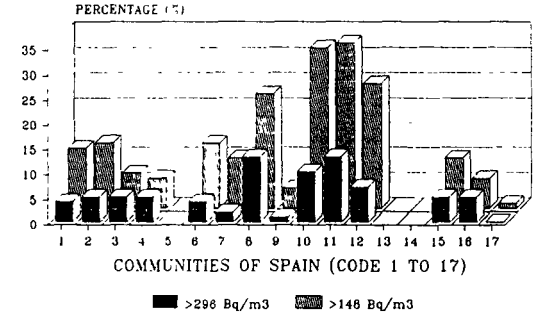
RADON LEVELS IN SPAIN
(In Bq per cubic meter)



(1988)

Figure III

SPANISH HOUSES
WITH HIGH RADON LEVELS



(1989)

1.- ANDALUCIA
2.- ARAGON
3.- ASTURIAS
4.- BALEARES
5.- CANARIAS
6.- CANTABRIA
7.- CASTILLA-LA MANCHA
8.- CASTILLA-LEON
9.- CATALUÑA
10.- EXTREMADURA
11.- GALICIA
12.- MADRID
13.- MURCIA
14.- NAVARRA
15.- LA RIOJA
16.- PAIS VASCO
17.- VALENCIA

Figure IV

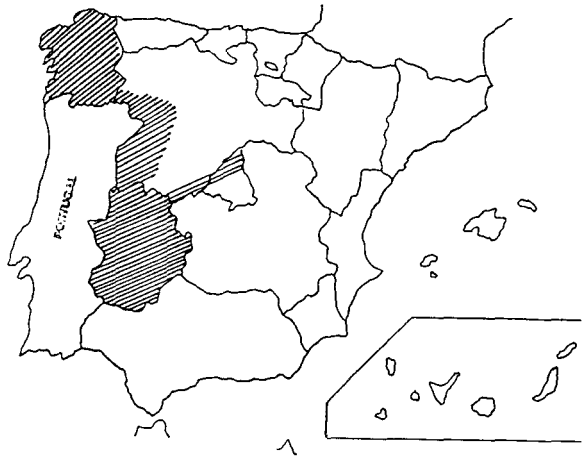


Figure V

IV.- Objectives for the next reporting period

.- Evaluation of external dose from the radium, thorium and potasium content in soils and building materials.

.- Study of sources of radon in houses in selected areas with special emphasis in soils,building materials and water.

.- Measurement of the exhalation rate from soils and building materials.

.- Detection and study of radon in water supplies comparing the results with his natural radium content.

.- Study of the relationship between the external dose derived from the radioactivity of soils and the experimental results from a portable monitor.

VI.- Publications

Radón, Principal fuente de radiación natural (in spanish)
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Alpha detection by zinc sulfide
Report LMF-121,35-38 Albuquerque, New Mexico, USA.

Niveles de radón en España (Primeros resultados) (in spanish)
Internal Report. Universidad de Cantabria.

Niveles de radón en la Comunidad de Cantabria (in spanish)
Internal Report. Universidad de Cantabria.

Survey of radon in Cantabria homes
XXXV Health Physics Society Annual Meeting, Albuquerque, New Mexico.

Dosis de radiación debida al radón en distintas localidades españolas
(in spanish)
VII Congreso Nacional de Física Médica. Oviedo.

Niveles de radón en casas de la Sierra de Guadarrama (Madrid)
(in spanish)
XXII Reunión Bienal de la Real Sociedad Española de Física. Palma de Mallorca.

RADIATION PROTECTION PROGRAMME
Final Report

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)]:

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Telephone number: 42 37 12 12

Title of the research contract:

Behaviour of accidentally released radionuclides in urban areas.

List of projects:

1. Experimental and modelling approach to assess indoor doses in urban agglomerations, and evaluation of the decontamination through run-off of deposited material.

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:

III. Progress achieved:

1. Introduction

The parameters dealt with in this study, namely run-off, weathering, dry deposition on external surfaces, indoor deposition, and indoor/outdoor air concentration, will be treated in sequence.

2. Run-off

2.1. Introduction to run-off
Run-off is the amount of rainfall that is not retained by the surface on which it falls. As run-off water from roofs and roads may retain some or all of the radioactive material and carry it into drainage and sewage treatment systems, the effect could have an important bearing on a consequence calculation for an urban area.

The following equation may be applied

$$Q = P - I_a$$

where Q is the direct run-off, P the total and I_a the initial accumulated rainfall.

2.2. Methodology for run-off measurement
To find the run-off from roofs and roads, constructions have been built that allows measurements to be made of the amount of water per unit horizontal projected area that runs off. This is done for roof constructions by collecting the run-off water in a vessel. The amount of rainwater per unit area is found by direct sampling of rainwater in a vessel. For the road construction a tent has been erected covering the road, and the contaminated rain has been simulated by a simple rain machine, which controls the total "rainfall". The run-off water is collected in a vessel.

2.3. Results for run-off
The result from a rainfall occurring on 8 May 1986 are shown in Table 2.1.

The table shows that for the silicon treated material Q = P, as there is the same amount of rainwater (sec. 0 column 2) as run-off water (sec. 4 column 2). This means that all the deposited rainwater runs off.

However, 20-30% of the deposited caesium, 40-65% of the deposited ruthenium, and 35-40% of the deposited barium was retained. For the same material with less slope (section 6), the retention is almost similar. Here, however, I_a has risen to 0.003 m.

In sections 3 and 5, both were covered with corrugated eternite but with different slopes: 80-90% of the caesium, 40-65% of the ruthenium and 20 to 35% of the barium were retained. I_a for the

45° slope was 0.0014 m and for the 30° slope 0.0035 m. This implies that the concentration of the radioactive isotopes mentioned is higher for the roof material with the steep slope than for the one with low slope.

Table 2.1. Run-off during the first shower following the Chernobyl accident; 8 May 1986.

Section No.	W* ($m^3.m^{-2}$)	^{137}Cs	^{134}Cs	^{131}I	^{103}Ru	^{106}Ru	^{140}La	^{140}Ba
		F**	F	F	F	F	F	F
0	0.0092							
1	0.0074	0.42	0.38	1	0.73	0.58	0.32	0.31
2	0.0050	0.32	0.29	0.57	0 47	0.40	0.31	0.32
3	0.0078	0.13	0.12	1	0.36	0.35	0.32	0.32
4	0.0092	0.78	0.71	1	0.59	0.37	0.67	0.68
5	0.0056	0.20	0.19	1	0.45	0.41	0.37	0.32
6	0.0063	0.82	0.75	1	0.53	0.51	0.78	0.78

* W = Volume of rainwater or run-off water per horizontally projected area.
** F = Material removed with run-off water/Material deposited.

0. Rain sampler
1. Cement tile 45° slope
2. Red tile 45° slope
3. Corrugated eternite 45° slope
4. Silicon-treated eternite 45° slope
5. Corrugated eternite 30° slope
6. Silicon-treated eternite 30° slope

The red tile (section 2) has the lowest run-off as it is very porous and it retained between 40 and 60% at the different deposited radioactive isotopes. The red tile was the only roof material that retained the iodine. On all other surface types the dry and wet deposited iodine were removed by the run-off water.

On the roads a 6 mm rainfall was simulated and the rainwater was contaminated with ^{132}Cs, ^{134}Cs, and ^{136}Cs.

On the asphalt road the initial accumulated rainfall, I_a was 0.0038 m and on the concrete road I_a was 0.0034 m.

Table 2.2 shows the result:

Table 2.2.

	W* m^3/m^2	^{132}Cs F	^{136}Cs F	^{134}Cs
Rain	0.0060			
Asphalt	0.0022	0.06	0.07	0.06
Concrete	0.0014	0.09	0.08	0.10

*W Volume of rainwater or run-off water per surface area
**F Material removed with run-off water/material deposited

Table 2.2 shows that only a small portion of the deposited caesium is removed with the run-off water for the asphalt road, as 93-94% of the caesium was retained. On the concrete road 90-92% was retained.

3. Weathering

3.1. Introduction to weathering

The fraction of the deposited radioactive material that is retained on the different hard surfaces is exposed to wind, rain, snow, and other processes that will tend to deplete retained material.

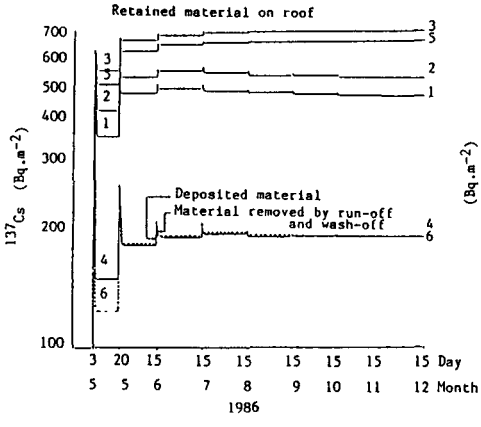
The depletion effect on the retained material is called weathering.

A very important depletion process when considering impervious urban surfaces is the wash off by rain.

3.2. Results for weathering

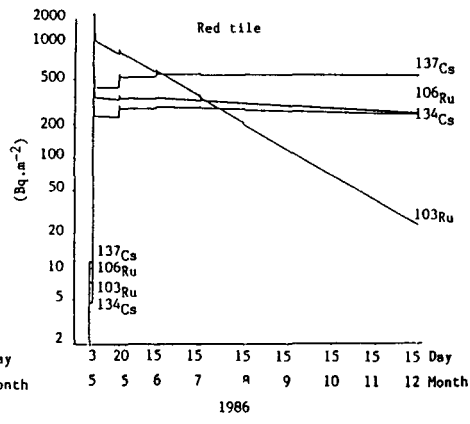
In order to determine the extent to which the weathering processes occur, measurements on roofs were performed during the last 8 months of 1986. The results are shown in Figures 3.1, 3.2, and 3.3.

Figure 3.1.



Caesium-137 retained on roof. Key to lines (top to bottom of figure):
3. corrugated eternite 45°slope
5. Corrugated eternite 30° slope
2. Red tile 45° slope
1. Cement tile 45° slope
4. Silicon-treated eternite 45° slope
6. Silicon-treated eternite 30° slope

Figure 3.2.

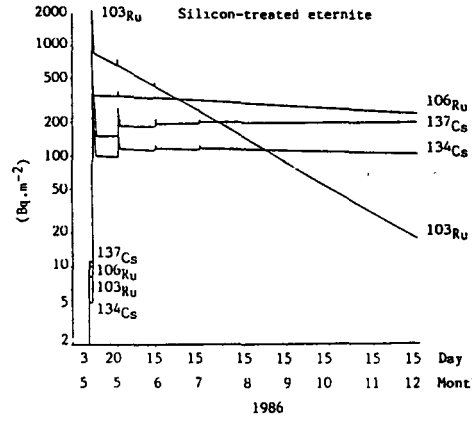


The retention of different isotopes on corrugated red tile.

The overall picture from these figures is that the wash-off process is very slow. More than six months after only a minor part of the material still retained after the first shower had been removed . Note that the slope of the curve for ^{103}Ru corresponds to its shorter half-life. To analyze this process

in greater detail, the wash-off effect of the rain that had fallen in August and December 1986 is calculated (see Table 3.1). The fraction removed by run-off had fallen from about 1% at the retained caesium in August to 0.7-0.8% in December.

Figure 3.3.



The retention of different isotopes on corrugated silicon treated eternite.

Table 3.1. Comparison of the wash-off from roofs in September and in December 1986. Figures expressed as % of retained material and as % per mm rainfall.

Isotope	Roof	August 1986				December 1986			
				Mean				Mean	
		(%)	(% mm⁻¹)	(%)	(%.mm⁻¹)	(%)	(%.mm⁻¹)	(%)	(%.mm⁻¹)
	1	1 4	0 044			2.1	0.051		
	2	1.8	0.056			0.9	0.020		
^{137}Cs	3	0	0	1.0	0.031	0.1	0.001	0.8	0.017
	4	1.5	0.048			0.8	0 020		
	5	0	0			0	0		
	6	1.3	0.040			0.6	0.015		
	1	1.3	0.040			2.0	0 046		
	2	1.6	0.050			0 7	0 018		
^{134}Cs	3	0	0	0.9	0.026	0.1	0 001	0.7	0.016
	4	1.2	0.037			0 7	0.016		
	5	0	0			0	0		
	6	1 0	0.031			0.5	0.013		
	1	3.6	0.112			4.4	0.100		
	2	2.3	0.071			0.6	0.014		
^{103}Ru	3	1.0	0.031	1.7	0.057	1 3	0.036	1.5	0.035
	4	0.9	0.027			0.8	0.018		
	5	1.4	0.042			1.1	0 025		
	6	0.9	0.029			0 6	0.015		
	1	1 9	0.059			2.1	0.049		
	2	1.8	0.057			0.5	0 013		
^{106}Ru	3	0 9	0 027	1.1	0.034	1.2	0.029	1 0	0.023
	4	0.5	0 015			0.4	0.010		
	5	1.1	0.034			0 9	0.021		
	6	0.4	0.012			0.6	0.013		

Key to roof type as Table 2.1.

The wash-off effect of the first "shower" of 3 mm simulated rain after the deposition of radioactive caesium on surfaces of asphalt and concrete was also considered. In this case the road was not dry when the "rain" began; the initially deposited rainfalls on the roads were therefore less than those reported in section 2.3. Table 3.2 shows the results of the wash-off experiment.

Table 3.2. Wash-off from roads

	$^{W*}_{m^3/m^2}$	^{132}Cs D	^{136}Cs *D	^{134}Cs D
Rain	0.0030			
Asphalt	0.0011	0.027	0.025	0.023
Concrete	0.0014	0.050	0.046	0.061

*W Volume of rainwater or run-off water per surface area

*D Material removed with run-off water/material retained before the rain.

As can be seen from Table 3.2. the 3-mm rainfall only 3 hours after the deposition removed 2-3% of the retained material from the asphalt and 5-6% of the retained material from the concrete.

4. Dry deposition

4.1. Introduction to dry deposition

The distribution of the dry deposited radioactive material on the different urban surfaces is important in the context of risk assessment.

4.2. Methodology for dry deposition measurements

The dry deposition velocity was measured taking samples from real urban surfaces such as plastered walls, roof material, street concrete flagstone, road asphalt, grass, trees, and bushes. The sampling was done immediately after the first cloud from Chernobyl had arrived at the city of Roskilde, Denmark.

4.3. Results for dry deposition

Tables 4.1., 4.2., 4.3., and 4.4. show the results for dry deposition on impervious surfaces.

Tables 4.1 and 4.2 shows deposition velocities on road material. There is no obvious indication of different deposition velocities in different areas. The deposition velocities obtained were clearly different for the various isotopes. The deposition velocities of the volatile group Cs and Ru are lower than those of the refractory group Ba, Ce, and Zr.

Table 4.1. Deposition velocities (units: 10^{-4}m.s.$^{-1}$) for asphalt surfaces.

	Rural area	Suburban areas		City areas	
			Sample No.		
Isotope	184	152	157	186	187
^{134}Cs	1.1			0.45	1 1
^{131}I	7.7	7.5	5.4	3 0	3.3
^{141}Ce	11	8 9	11	2.1	15
^{140}La	6 1		4.7	4.2	4.3
^{103}Ru	2 9	5.7	5 3	1.2	4.6
^{95}Zr	8.2	31	11	1.0	12
^{95}Nb	8 8		11	1.3	13

Table 4.2. Deposition velocities (unit: 10^{-4}m.s.$^{-1}$) for concrete flagstone.

	Suburban areas		City areas	
		Sample No		
Isotope	183	185	145	147
^{134}Cs	0 8	0.5	0.5	0 6
^{131}I	2.5	7.3	2.2	2.9
^{141}Ce	12	4.7	0.9	7.9
^{144}Ce	17	3.3	0.8	7.5
^{140}La	3 4	3.0	7.7	3.3
^{103}Ru	5.8	2.5	1.2	2.4
^{106}Ru	18	5.6		
^{95}Zr	12	4.1	1.0	8.2
^{95}Nb	13	5.1	0 8	6.2

Table 4.3.

Deposition velocities (units: 10^{-4} m.s^{-1}) for plastered house walls.

Sample No.	^{131}I	^{137}Cs
138	3.0	0.2
140	3.0	0.1
141	3.1	0.1
142	2.7	0.1

Table 4.4.

Deposition velocities (units: 10^{-4} m.s^{-1}) for roof material.

	Corrugated cement tile	Corrugated red clay tile	Corrugated eternite
^{137}Cs		3.5	2.8
^{134}Cs	2.9	2.7	
^{131}I	32	43	20
^{140}La	65	47	47
^{103}Ru	4.1	2.9	3.3
^{141}Ce		40	

As shown in the literature these two groups have different particle sizes. The first group has AMAD sizes of about 0.4 μm, the other of 1-4 μm. The lower deposition velocities for the particulates in the first group are in good agreement with the observations that 0,4 μm particulates have a lower deposition velocity than those of 1-4 μm.

The dry deposition velocities on green surfaces are shown in Tables 4.5. and 4.6.

Table 4.6 shows that the deposition velocities on bushes in the urban area are about 10 times higher than those on road surfaces. The deposition velocities on grass (Table 4.5) were found to be nearly proportional to the mass of grass per unit area, so that the deposition velocities cover a wide range. The values of the bulk deposition constant, however, seem to be much more stable for the different grass types than are those of the deposition velocity.

Table 4.5.

Deposition velocity V_d: 10^{-4} m.s^{-1}, bulk deposition B_d: 10^{-4} m^3.s^{-1}.kg^{-1}, for grass.

Sample No		^{137}Cs	^{134}Cs	^{131}I
1384	V_d	4.3	4 4	22
	B_d	21	21	110
1387	V_d	1.8	1.5	18
	B_d	10	8 7	100
1388	V_d	8.8	7.2	93
	B_d	10	8.5	110
1391	V_d	6.0	6 6	86
	B_d	7.9	8 7	110
1392	V_d	7 4	9 9	120
	B_d	9 1	12	140

Table 4.6.

Deposition velocities (units: 10^{-4} m.s^{-1}) for suburban trees and bushes.

Isotope	Yew trees		Juniper berry
	Height 2.5 m Sample No.155	Height 2.4 m Sample No.156	Height 2 m Sample No 153
^{134}Cs	13	5	3
^{131}I	110	100	32
^{141}Ce	63	30	23
^{144}Ce	69	23	28
^{140}La	33	30	14
^{103}Ru	36	28	13
^{106}Ru		47	28
^{95}Zr	78	32	26
^{95}Nb	84	31	26

5. Indoor deposition and indoor/outdoor concentration

5.1. Introduction to indoor deposition

In reactor safety studies it is important to estimate the indoor deposition. We combine this with filter-measurements and measurements of the air-exchange rate to find the indoor-to-outdoor concentration of an air pollutant of outdoor origin.

5.2. Methodology for indoor deposition measurement

An alternative methodology was used to find the different parameters. In some experiment the outside air was ducted directly into the test house and the air-concentration at the inlet and indoors were measured together with the air-exchange rate. In other houses the indoor and the outdoor air-concentrations were measured in a normal situation.

5.3. Result for indoor deposition and indoor/outdoor concentration

During the Chernobyl accident some measurements were made in a test house. Here the air was ducted directly into the house. The results are shown in Tables 5.1 and 5.2.

As can be seen in Table 5.2 the mean deposition velocities of the volatile group Cs, Ru, and I are lower than those of the

refractory group, Ce and Zr; the reason for this difference is the same as was given in section 4.3.

The transfer factor D = Indoor concentration/outdoor concentration is given in Table 5.3.

Table 5.1.

Indoor deposition. Air exchange rate λ_r = 1.6.

Isotope	Indoor/Inlet air concentration C_i/C_o	Rate coefficient of deposition λ_d (h^{-1})
^{137}Cs	0.80	0.39
^{134}Cs	0.81	0.38
^{131}I (particulate)	0.71	0.65
^{7}Be	0.78	0.44
^{103}Ru	0.56	1.26
^{106}Ru	0.61	1.02
^{141}Ce	0.46	1.89
^{144}Ce	0.40	2.44
^{95}Zr	0.31	3.56

Table 5.2.

Indoor deposition velocities.

Isotope	$\overline{U}_d$, mean deposition velocity ($m.s^{-1}$)
^{137}Cs	6.4×10^{-5}
^{134}Cs	6.2×10^{-5}
^{131}I (particulate)	1.1×10^{-4}
^{7}Be	7.1×10^{-5}
^{103}Ru	2.0×10^{-4}
^{106}Ru	1.7×10^{-4}
^{141}Ce	3.1×10^{-4}
^{144}Ce	3.9×10^{-4}
^{95}Zr	5.8×10^{-4}

Table 5.3. Transfer factor for exposure integral

Isotope	$D = C_i/C_o$
^{131}I (particulate)	0.39
^{137}Cs	0.27
^{7}Be	0.49
Air exchange rate λ_r = 0.4 h^{-1}	

Later some deposition velocity measurements in a test house were made using ^{137}Cs and ^{7}Be as tracers. The results are given in Table 5.4.

Table 5.4. Deposition parameters

	Isotope	Deposition velocity · m/s	Rate coefficient of deposition h^{-1}
partly furnished house	^{7}Be	0.000018	0.11 ± 0.03
	^{137}Cs	0.000150	0.91 ± 0.16
unfurnished room	^{7}Be	0.000010	0.06 ± 0.03
furnished room	^{7}Be	0.000033	0.21 ± 0.05

By comparing the deposition velocities in Table 5.2 with those given in Table 5.4 it can be seen that the deposition veloci-

ties given in Table 5.2 is generally higher than those in Table 5.4. The reason for this is primarily the higher air-exchange rate in Table 5.2 compared with that in Table 5.4. This tends to give a higher turbulence and thereby a higher deposition velocity. In Table 5.2 the deposition velocity of Cs is about the same as Be. This situation has changed markedly in Table 5.4, so that the deposition velocity for Cs in the latter case is 8 times higher than that of Be because in the in the latter case the Cs-marked particulates are mainly resuspended and resuspended particles are larger than those that come directly from the source.

In Table 5.5 some direct measured deposition velocities are given.

Table 5.5. Deposition velocity on internal surfaces · m/s
Air exchange rate λ_r = 0.5

		Particles		Particles and gas
	Sample no.	^{137}Cs	^{103}Ru	^{131}I
Wallpaper	148	0.000001	0.000001	0.000009
(vertical)	150	0.000002	0.000001	0.000008
Vinyl floor	149	0.000009	0.000010	0.000023
(horizontal)	154	0.000013	0.000010	0.000020
Wooden floor	207	0.000008	0.000008	
(horizontal)	208	0.000005	0.000007	

The deposition velocities measured directly on very smooth surfaces are much lower than the mean velocity given in Tables 5.2 and 5.4. This is because the rough surfaces in the house, e.g. carpets, are included in the mean deposition velocity.

6. Conclusion

The Chernobyl accident gave us a unique opportunity to collect data for use in the investigation of the different parameters important in reactor safety studies. For all the parameters new and better values are given; this will allow us to construct a much more reliable risk assessment model for the urban area. Producing this model is very important for the countries of the European Community as most of the people here live in cities.

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

V. Publications:

Roed, J. (1987). Dry deposition in rural and in urban areas in Denmark. Radiation Protection Dosimetry, Vol. 21 No. 1/3 pp 33-36, 1987.

Roed, J. (1987). Run-off from and weathering of roof material following the Chernobyl accident. Radiation Protection Dosimetry, Vol. 21. No. 1/3 pp 59-63 1987.

Roed, J. and R.J. Cannell (1987). Relationship between indoor and outdoor aerosol concentration following the Chernobyl accident. Radiation Protection Dosimetry, Vol. 21, No. 1/3 pp 107-110 1987.

Roed, J. (1988). Parameters used in consequence calculations for an urban area, NKA/AKTU 245(88)1. Presented at the Joint CEC/OECD (NEA) Workshop on Recent Advances in Reactor Accident Consequence Assessment, Rome, Italy 25th-29th January, 1988.

Roed, J. (1988). The distribution of dry-deposited material on trees from the Chernobyl accident. NKA/AKTU-245(88)2. Joint CEC/OECD (NEA) Workshop on Recent Advances in Reactor Accident Consequence Assessment, Rome, Italy 25th-29th January, 1988, Proceedings of the second part of the workshop, pp 165-178.

Roed, J. and R.J. Cannell (1988). The deposition of beryllium-7 marked particles on surfaces in unfurnished and furnished rooms, NKA/AKTU-245(88)3. Joint CEC/OECD (NEA) Workshop on Recent Advances in Reactor Accident Consequence Assessment, Rome, Italy, 25th-29th January, 1988, Proceedings of the second part of the workshop, pp 208-221.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no : BI6-F-120-NL

Kernfysisch Versneller Instituut
FOM
Van Vollenhovenlaan 661
NL-3527 JP Utrecht

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

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Zernikelaan 25
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Telephone number: (50) 63.36.00

Title of the research contract:

Investigation of the mechanisms leading to radon concentrations in dwellings.

List of projects.

1. Investigation of the mechanisms leading to radon concentrations in dwellings.

Title of the project no.:

Investigation of mechanisms leading to radon concentration in dwellings.

Head(s) of project:

R.J. de Meijer, L.W. Put

Scientific staff:

F.J. Aldenkamp
R.J. de Meijer
L.W. Put

I. Objectives of the project:

- Improve knowledge and understanding of mechanisms 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:

- Measurements of exhalation rates of walls, floors and soil in a number of test dwellings;
- Investigating the variability of radon exhalation by soil;
- Modelling "static" radon concentrations in terms of sources and average flows;
- Modelling radon concentrations in crawl spaces as function of ground water level, ventilation rate and soil properties;
- Conclusion of the activated charcoal canister investigation.

III. Progress achieved:

1. Introduction

In the period 1980-1984 a national survey in the Netherlands was conducted to obtain a scope on the distribution of radon concentrations in Dutch dwellings and to see if there were building parameters which could be correlated with elevated levels of radon. The project was part of the Dutch National Research Programme SAWORA and revealed:

- The median concentrations in the Netherlands are only slightly lower than in adjacent countries; the width of the distribution, however, is more narrow.
- Soil under the dwelling was suspected to be an important source of radon for the dwelling.
- Certain building materials and construction techniques seemed to be correlated to higher mean concentrations of radon.

To deduce measures which could reduce radon concentrations in dwellings it was felt necessary to investigate the mechanisms responsible for the infiltration of radon. Two proposals were made; one to investigate the static (diffusive) aspects, the other to study the role of pressure driven flow. Both proposals were carried out in a joint financial cooperation with the Dutch Ministry of Housing, Planning and Environmental Hygiene (VROM), as part of the National Research Programme "RENA" and the University of Groningen as part of their "Centrale Beleidsruimte".

This report deals with the static aspects of radon infiltration. As stated in the proposal the objectives of this investigation were:

- Development of instruments for measuring radon exhalation rates and time-averaged radon concentrations.
- Study of 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 physical phenomena.

These objectives were met by:

- Investigation of neutralization processes and their role on the collection efficiency of a device based on the electrostatic collection of charged radon-decay products. This investigation led to the final design and construction of radon-exhalation meters.
- Study on the possibility to use activated charcoal cannisters as short-time radon meters.
- Measurements of the influence of groundwater level on the radon concentration in soil and crawlspace.
- Investigation of the nature of the effective decay constant deduced from ingrowth curves obtained with the exhalation meter; relation between the measured quantities and the free-exhalation.
- Measurements of time-integrated radon concentrations in crawl space and living room of dwellings with an elevated radon concentration.
- Investigation of a possible relation between Ra-concentrations in soil and outdoor radon concentrations.
- Measurements of time integrated radon concentrations and exhalation rates of various surfaces in the crawl space and the living room in a test dwelling.

On various subtopics reports have been made or are in progress. In this final report only summaries of these investigations will be given. For details we refer to reports and/or papers.

2. Instrumentation

2.a) *Exhalation meter*

To determine the static source strength of radon in a room an instrument has to be available to measure the radon exhalation by the various surfaces *in situ*. At the start of the project a prototype exhalation meter was available from its designer Ackers[1]. This instrument consisted out of two coaxial perspex cylinders, on one side open, on the other side covered with a perspex lid. In the lid an ZnS(Ag) scintillator mounted to a photomultiplier tube (PMT) was placed close to a thin aluminized mylar foil at ground potential. Near the open end of the inner cylinder a grid was mounted, put at +110 V to create an electrostatic field (5 $V.cm^{-1}$) which causes charged radon decay products to move towards the foil.

A further development turned out to be necessary when measurements indicated that the count rate of this device was dependent on exposure to light and variations in temperature. These effects resulted in uncertainties of a factor two to three in the deduced exhalation rate. A new design was made in which the perspex cylinders and lid were replaced by stainless steel ones, the ZnS scintillator was replaced by a surface barrier detector and the grid was placed at ground potential and the foil in front of the detector at negative potential.

Measurements under controlled conditions of radon concentration, relative humidity and temperature and in an air-argon mixed atmosphere revealed information on removal processes. It was found that the dependence on relative humidity and temperature could be replaced by a dependence on absolute humidity and that the dependence on this parameter and on radon concentration decreased with increasing electrostatic field. The main influence on the electrostatic collection was found to be the neutralization of charged decay products due to small ion recombination, likely caused by OH radical formation due to radiolysis. A field strength of 125 $V.cm^{-1}$ was found to be the optimum between reduction of effects and introducing discharges. An extensive description of the measurements and their interpretation is given in a report[2].

2.b) *Activated charcoal cannisters*

Radon concentrations were measured by our group with time integrating cups of the Karlsruhe type[3]. The usual exposure time for measuring indoor radon concentrations is four months. In the beginning of the investigation it was felt necessary to also have available information over shorter periods. Experiences in the U.S.A. indicated that the activated charcoal cannister could be a good candidate.

For the investigations we developed a canister which was able to detect concentrations as low as 5 $Bq.m^{-3}$ for an integration time of one week[13]. As was experienced simultaneously by other investigators[4] the adsorption properties for radon in such canisters is strongly influenced by the amount of adsorbed water. This effect reduces the reliability of such results to an indicator of the radon concentration rather than a measurement.

Investigations[5] indicated that thin polyethylene foils would be a good water-vapour barrier but would be rather transparent to radon. Various types of polyethylene foil have been tried out; none of them seemed to be transparent enough to allow reliable radon concentration measurements in Dutch dwellings. The reduction factors observed are in the order of 20.

This large reduction factor and the results in contrast to previous experiences with track-etch detectors can be understood from the large effective volume of the charcoal canisters (600 l). For the uncovered canister the number of radon atoms will correspond to that in a 600 l volume; for the covered canister only radon atoms will be collected, which have diffused through the barrier.

The results indicate that for the Dutch situation only uncovered charcoal canisters may be used, with an intrinsic uncertainty in sensitivity of a factor of three.

3. Results of investigations

3.a) *Investigation of the relation between radium content of soil and time-averaged outdoor-radon concentrations.*

One of the results of the Dutch radon survey[6] was that at places in the country the time-averaged outdoor-radon concentration (TORC) appeared to vary over rather short distances. Variations of a factor of 3 over a distance of 40 kilometers were observed in the annual average. A number of regions with a definitely higher or lower TORC than in their surroundings were recognised. This result is unexpected since in this windy and flat part of the country wind is thought to wash out the local variations in the momentary radon concentration, due to differences in exhalation from the soil.

The soil composition in this part of the Netherlands may vary over short distances. South of the city of Groningen the soil consists mainly of silty fine clay often with intercalations of boulder clay and sometimes upon "pot" clay (region I). Towards the north this layer of sand dips under sea level and is covered by clay (region III). The east border of region I is formed by a sand ridge that was pushed up by glacial ice. Further east (region II) cover sands with layers of peat were originally present. Most peat has been removed and a new agricultural soil was obtained by mixing the top layer of sand with the upper layer of the former peat.

For the measurements passive radon dosemeters were used of the Karlsruhe type[3]. Two cups were placed in a type of nest box with openings covered with a mesh, allowing free exchange with ambient air. One cup remained for the full measuring period, the other was replaced after six

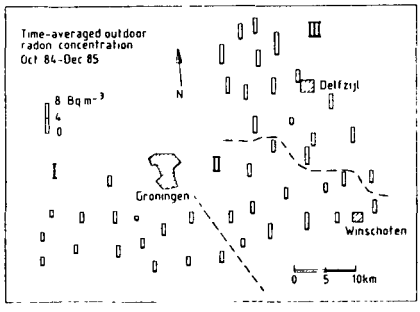

Figure 1. Outdoor radon concentrations (length of bars) at 43 locations averaged over a time interval of one year (TORC).

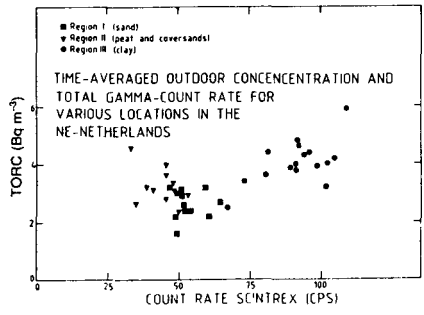

Figure 2. Two-dimensional representation of TORC values and total y ray intensity. The values have been indicated with symbols corresponding to regions indicated in fig.1.

months. The boxes were attached to trees at a standard height of 1.5 to 2 m above soil surface. The exposures started in November 1984 and ended December 1985.

The γ-ray intensities (GRI) were measured with a portable 4x4x5 cm^3 NaI crystal, mounted on a photomultiplier tube and connected to accesory electronics, including a single channel analyser and a scaler display.

In this report we restrict ourselves to a short description of the results. For more details we refer to ref.[7]. The TORC results are shown in Fig. 1; the values range from 1.6 to 5.9 $Bq.m^{-3}$. Going from the most western locations in an easterly direction and from Winschoten to the northwest one observed a trend of increasing concentration values.

Figure 2 shows a two-dimensional comparison between the TORC values and the GRI. The figure shows an almost perfect clustering of data points according to region, the clustering, however, occurs mainly via grouping in γ-ray count rate. Except for region III there is no indication for a correlation between GRI and TORC values.

Radon concentrations are a result of dynamic processes, which are determined physical parameters of the soil e.g. permeability and ground-water level. Such dynamic effects are not reflected in the (static) radium content of the soil and are not necessarily present in soil gas concentrations. The variations as observed indicate that TORC values may be a better indicator for exhalation rates by the soil than radium concentration.

3.b) *Dwellings with a high radon concentration*

For a period of one year time-averaged radon concentrations were measured in the living room, crawl space and some additional spaces of twenty dwellings. Fifteen dwellings are located in areas where relatively high indoor radon concentrations were measured in the Dutch national survey; five dwellings formed a block of "identical" houses. Of the latter five two crawl space floors were left bare, three of them were covered with foil and/or concrete during the construction.

In the beginning of this study it was becoming evident that the crawl space plays an important role for the radon concentration in the rest of the dwelling. It was therefore felt useful to investigate a number of dwellings with relative high radon concentrations and adjacent, if possible identical, dwellings. Such a study may give an answer to the question whether the high radon concentrations were caused by:

- the type of underlying soil (radium concentration, porosity);
- an important contribution of the crawl space to the ventilation of the dwelling;
- specific properties of the dwelling.

An important question is also whether covering the crawl space floor has a reducing effect on the radon concentration in crawl space and dwelling.

Radon concentrations were measured with the time-averaging dosemeters[3]. To investigate a possible correlation between radium concentration in soil and radon concentration in the crawl space soil samples of about 1 L were taken at about 10 cm depth. These samples were analysed in the laboratory with a low-background γ-ray spectrometer under the general assumption of equilibrium between radium and its decay products.

The procedures and results of this investigation have been reported in ref.[8]; in this report only a summary will be presented.

In Arnhem the investigated dwellings are located in the north western part of the city on the southern slope of glacial ridge. The soil consists

of coarse sand, with a humus layer of less than 30 cm and pebbles starting from 40 cm depth. The highest and lowest ground-water levels are >80 and >160 cm below the soil surface. The natural structure of the soil has not or hardly been destroyed during the construction of the dwellings.

The four dwellings have been built in the mid 1950's; the walls are made from brick and the dwellings have wooden floors. Almost all windows have double pane. In one of the houses the highest radon concentration of the national survey[7)] was found: averaged over one year 158 and 480 $Bq.m^{-3}$ for living room and crawl space, respectively.

In Maastricht two groups of houses were investigated; in the northwestern part a group of six, and a group of five in the southeastern part. The soil in both locations consists of loess, which is for 80-100% loam. The ground-water level is very deep below the surface. In the northwestern part the dwellings were built in 1970 as groups of five row houses and as twin houses. All six houses are within a few hundred meter of each other. As far as partitioning the six houses are identical to the other 99 dwellings of this type at this location. In the southeastern part the five houses are part of blocks of single-family row houses. Aside from mirror symmetry all houses are identical.

In Born the five investigated single-family houses form a block of row houses built in 1985. The soil is characterized as loamy fine sand with coarse sand or pebbles starting between 40 and 120 cm below the surface. The average high and low ground-water levels are >80 and >150 cm, respectively. These dwellings were selected due to the fact that at three of them a cover was placed at the crawl space floor. In two of these crawl space the cover consists of a 0.1 mm PVC foil plus a 15 cm thick aerated concrete layer. In the third crawl space only the aerated concrete is present. In all five dwellings the ceiling of the crawl space and the feed throughs in it were covered and sealed with a PUR-foam. This measure was sufficient to reduce the moisture problem in the dwellings. In one of the dwellings the crawl space was no longer accessible.

The uranium concentrations in the soil are lowest in Arnhem (~ 7 $Bq.kg^{-1}$) and highest in Maastricht (~ 25 $Bq.kg^{-1}$). For the outdoor radon concentration the values which are found in Arnhem (~ 3 $Bq.m^{-3}$) are considerably lower than in Maastricht (5-6 $Bq.m^{-3}$) and Born (~ 7 $Bq.m^{-3}$).

Two of the four houses in Arnhem have an indoor radon concentration below the average value found in the national survey, one has twice and the other four times the value. Only the latter house has also an enhanced high value in the crawl space. Since neither the outdoor concentration or the uranium concentration is obviously different for this house and since the house has been very tightly insulated it is assumed that the high values are the result of the low ventilation rate of the crawl space and the presence of the wooden floor between crawl space and living room. After the measurements the occupant installed a system for venting the crawl space. Preliminary results indicate a reduction of the concentration in crawl space as well as living room.

In all six dwellings investigated in NW-Maastricht the radon concentration in the living room is equal or higher than the average value in the national survey. In one of the dwellings an annual averaged value of 1060 $Bq.m^{-3}$ was measured in the crawl space; the highest value measured in a Dutch dwelling so far. The reason for this extreme value is unclear, the more since the uranium concentration of the soil is not drastically different from neighbouring crawl spaces and the radon concentration in the crawl space of the adjacent house is a factor of five lower.

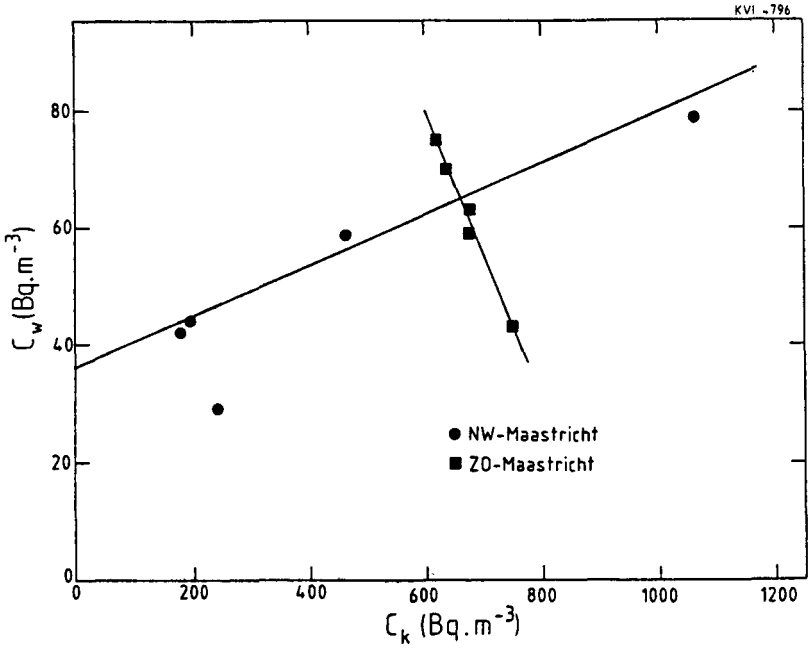

Figure 3. Two-dimensional representation of radon concentrations in living rooms (C_w) and crawl space (C_k) for two groups of dwellings, one in northwest (NW) and one in southeast (ZO) Maastricht. The statistical uncertainties are less than 10%. The lines are to guide the eye.

For all crawl spaces in the dwellings investigated in SE-Maastricht a similar high value (640-750 Bq.m^{-3}) was found; the concentrations in the living rooms are 1.5-2.5 times higher than the "Dutch average". The remarkable feature for this set of dwellings is that the concentration in the living room anticorrelates with the concentration in the crawl space.

This unexpected correlation shows up clearly in Fig. 3 where for the two groups of dwellings in Maastricht a two-dimensional correlation plot is given between radon concentrations in living room and crawl space. It is remarkable that for each of the groups the two concentrations are correlated. The correlation for the identical dwellings in NW-Maastricht indicate the expected linear relation based on a simple ventilation model and similar radon production. The slope of the dwelling suggests that about 4% of the air in the living room originates from the crawl space. The observed correlation between the two concentrations is then becoming remarkable because ventilation patterns of occupants may influence the concentration in the living room but in view of the small air exchange will not govern the concentration in the crawl space. Therefore the linear correlation itself and the large variation in the concentration in the crawl space are surprising. An estimate of the contribution from building materials and outside air is lower than the value obtained from the extrapolated value of C_{lr} at $C_{cs} = 0$.

For the dwellings in SE-Maastricht the almost constant radon concentration in the crawl space is to be expected for identical neighbouring dwellings. The anti-correlation can qualitatively be

understood if the total ventilation rate of all dwellings is approximately the same and the air current from crawl space to living room is the dominant air transport for the crawl space. In that case differences in the air transparency of the floor between crawl space and living room will change the air exchange for the crawl space. An increasing air exchange will probably decrease the crawl space concentration but will at the same time bring more radon to the living room.

The above data and conclusions are based on a small number of dwellings and on radon concentrations only. The observations remain remarkable and require further investigations before more solid conclusions can be drawn. In particular, to conclude whether the used model yields a satisfactory description it is necessary to have independent, quantitative information on the air exchange pattern.

In four crawl spaces and five living rooms in Born indoor radon concentrations were measured. The concentrations in the living rooms ranged between 30 and 40 $Bq.m^{-3}$; in the crawl space between 85 and 305 $Bq.m^{-3}$. In view of the experiences in NW-Maastricht it is uncertain what conclusions may be drawn from such a few number of cases. The two dwellings with as soil coverage foil + concrete have a lower concentration than the dwelling with a bare soil the value is higher than the one with concrete coverage only. This latter finding would, if real, be contrary to the first order expection that foil + concrete would be a better barrier than only concrete. To establish the effect of covering the soil in Dutch dwellings larger scale measurements are necessary, preferably with a measurement prior and after the installation of the soil coverage.

Further investigations including simultaneous ventilation measurements are necessary to better understand the observations. Both for Maastricht as for Born it can be concluded that such investigations should be carried out over at least 15-20 identical dwellings to eliminate uncertainties due to the normal spread in the value of "identical "parameters". Extension and further development of the radon concentration and radon transport models and comparison of their results with data are essential for further development of knowledge.

3.c) *Relation between ground-water level and radon concentrations in soil*

The diffusion length of radon in water is about one to two orders of magnitude smaller than in non water-saturated sand. This means that for situations in which the ground-water level is less than a few diffusion lengths in sand below the surface, the radon concentration in the soil gas near the surface, and hence the exhalation rate from the soil depends on the height of the ground-water level. Diffusion model calculations show an increase by about a factor of five for the radon concentration in a crawl space if the ground-water level is lowered from 10 cm below the surface to 50 cm.

Near a test house where many radon related investigations have been and will be carried out, ground-water levels and rainfall were recorded daily for about two years. Since September 1989 the frequency has been lowered to once a week.

Radon concentrations were measured in a pipe which was capped at the top and placed into a hole in the soil with a depth of 40 cm. Time-averaged radon dosemeters were exposed in the pipe for about one week.

The experimental results confirm model calculations which indicate that high radon concentrations (~ 10 $Bq.m^{-3}$) may occur in soil gas even in

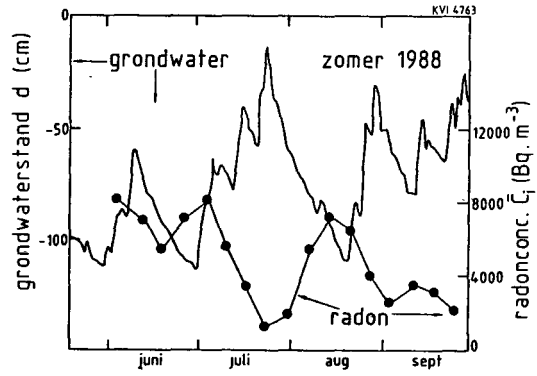

Figure 4. Daily variations in the ground-water level and the weekly averaged radon concentration in a capped tube, placed 40 cm in to the soil. Measurements were carried out in the summer of 1988.

soils with a low radium content (~ 10 $Bq.kg^{-1}$). The results indicate that there is a correlation between averaged radon concentration and the average ground-water level in the preceeding week. Fig. 4 shows the data collected in the period May to October. The data show a good correlation, especially if one realises that due to build up and decay of radon different radon concentrations are to be expected for situations with the same average ground-water level but with the ground-water level either increasing or decreasing.

Presently a report[9] is in preparation in which the correlation between ground-water levels at various locations around the building are compared and in which the correlation with radon concentration in soil and in the dwelling is measured. In this report also the effect of lowering the ground-water by installing a drainage system will be discussed.

3.d) *Radon exhalation measurements in and around a test dwelling*
3.d.1) *Results of measurements*

In and around a test dwelling radon exhalation was measured from surfaces in the crawl space, living room and yard. The measurements were carried out *in situ* with the device described in section 2.a. Each meausurement consisted of placing the instrument to the surface, if necessary sealing its outer contact and flushing it with dry nytrogen. Every half hour the average values of temperature and relative humidity inside the device and the pressure difference between in and outside of the measuring volume were registered together with a number of counts in the gates corresponding to α-particles emitted by decaying ^{218}Po and ^{214}Po on the foil in front of a 600 mm^2 solid state detector. Foil and detector housing were placed at -2.5 kV with respect to the grounded cylinders of the device.

Measurements were carried out for periods of a few days to a few weeks until the number of counts had reached a constant value. Radon exhalation rates were deduced from the measured ingrowth curve after converting the number of counts to radon concentration by fitting the growth curve by the function

$$C(t) = \frac{E \cdot A}{\lambda_e} (1 - e^{-\lambda_e t}). \qquad (3.1)$$

In this expression E is the exhalation rate, A the area of the surface covered by the device, λ_e an effective decay constant and C the radon concentration. In this expression E and λ_e are fitting parameters; the values have been, in first order, interpreted as the (bound) exhalation, and the sum of a leak constant (λ_v) and the decay constant of ^{222}Rn (λ_{Rn}), respectively. The leak constant represents the leakage of radon due to insufficient sealing and/or diffusion of radon to the surrounding due to the porosity of the material. As indicated by Samuelson[10] the value of the exhalation rate is influenced by the presence of the device. The rate of influencing depends on the porosity of the material, the diffusion length and the geometry. A start has been made to try to deduce free-exhalation rates, preliminary results indicate that for the present setup the difference between free and bound exhalation is not too large. The complexity of this problem needs further attention. In this report we present the bound exhalation rates only and make estimates on radon concentrations in crawl space and living room based on these values. It should be reminded that the estimates may change in future if the exhalation rates are corrected for the presence of the instrument.

TABLE 1. EXHALATION RATES (E), EFFECTIVE DECAY CONSTANT (λ_{eff}), AND SURFACE AREA (A), FOR WALLS, FLOOR AND SOIL OF THE CRAWL SPACE AND LIVING ROOM IN THE SOUTHERN PART OF THE TEST DWELLING.

	E ($Bq.m^{-2}.h^{-1}$)	λ_e (h^{-1})	A (m^2)	E.A ($Bq.h^{-1}$)
crawl space (V=26 m^3)				
soil d = 58 [1]	1.78 ± 0.08	0.015 ± 0.001	48	85
" d = 48	0.88 ± 0.06	0.011 ± 0.001		42
" d = 46	1.02 ± 0.06	0.011 ± 0.001		49
inner wall	2.44 ± 0.06	0.111 ± 0.003	10	24
outer wall	1.96 ± 0.04	0.043 ± 0.002	15	29
ceiling[2]	3.40 ± 0.05	0.054 ± 0.001	48	173
living room (V=208 m^3)				
floor	1.34 ± 0.04	0.032 ± 0.001	48	65
wall	0.26 ± 0.05	0.074 ± 0.008	97	25

1) water level relative to the soil surface, averaged over the measuring period
2) taken identical to the value of the floor in the study

Table 1 lists exhalation rates, effective decay constants and surface areas for the surfaces in the crawl space and living room. The exhalation

rate for the ceiling is taken equal to the value obtained for the floor in the study because the value for the ceiling could not be measured directly due to its curved surface. In the study the prefab concrete bars are covered by a few centimeters of cement. In the table three measurements are reported taken at different levels of the ground-water in the crawl space. Here one notes a decreasing exhalation rate with a decreasing thickness of the sand layer between the exhalation meter and the ground-water. In the crawl space the exhalation rate of the soil is a factor of two to three smaller than of the walls and the ceiling. It is clear from the table that the ceiling yields the largest contribution to the diffusive source strength in the crawl space. From the values of λ_e one notices the large value for the inner wall. This value indicates the roughness of the surface which made it difficult to mount the device leak-tight.

The exhalation rates listed in table 1 are obtained by converting the measured count rate by a calibration factor determined per measurement from a sample taken with a Lucas cell. The relative accuracy is thought to be in the order of 15%; the absolute value are known within about 40%. Another experimental uncertainty, especially for the measurement on the soil, is the change in E with time due to varying circumstances as ground-water level and water content of the soil. Some of these effects were noticed in the analysis of the soil measurements where the data were not too well described by eq. 3.1. As mentioned before it is unclear to what extent the values of the exhalation rate are influenced by the presence of the device[10].

3.d.2) *Model calculations*

In the extremely simplified model for the radon concentration in a room which is ventilated with radon-free air one considers constant static sources and a constant ventilation rate. For the equilibrium situation one may write for the radon concentration C in a room with ventilation rate λ ($\lambda \gg \lambda_{Rn}$) and volume V:

$$C = \frac{1}{\lambda V} \sum_i E_i A_i, \qquad (3.2)$$

where the summation is over all radon exhaling surfaces indicated by i with exhalation rate E_i and surface area A_i. Here it is assumed that the radon concentration in the incoming ventilation air may be neglected.

For the crawl space, a ventilation rate λ=0.5 h^{-1} (based on the average value in fig.2e) and V, E_i and A_i taken from table 1 one obtains a value of C=21 and 17 $Bq.m^{-3}$ for a dry soil (d=46 cm) and a barely inundated (d=0 cm) crawl space, respectively. In the latter case the contribution from the soil has been neglected. In the period 1980-1987 time averaged radon concentrations were measured[11]; the average value was 50±20 $Bq.m^{-3}$. The indicated uncertainty is the standard deviation. It is large, presumably due to variations in the ground-water level[11].

From the numbers one may conclude that based on the exhalation measurements the radon concentration in the crawl space is two to three times higher than expected on diffusion from the materials. This discrepancy could partly be attributed to the uncertainties in the measured values but it may also indicate another source of radon which, for this crawl space, is equally or even more important.

A similar simplified model yields for the living room with constant source terms and a constant ventilation with a constant fraction, α, of air

from the crawl space:

$$C_{lr} = \alpha C_{cs} + (1-\alpha) C_0 + \frac{1}{\lambda V} \sum_i E_i A_i .$$

Substituting C_{lr}=22 Bq.m^{-3} (ref. 11), C_{cs}=50 Bq.m^{-3}, C_0=3 Bq.m^{-3}, λ_{lr}=0.5 and E_i and A_i from table 1 one obtains α=0.4. This value would correspond to a crawl space ventilation rate of at least 1.6 h^{-1} and hence a discrepancy between the radon contribution calculated from static sources and the observed value for the crawl space of a factor of 8. Such a discrepancy cannot be accounted for by the uncertainties in the measured values.

The values of the radon exhalation rates of the soil at different ground-water levels can be compared with a one dimensional diffusion model calculation. For this purpose the radium concentration of the upper layer of the soil was determined: 6.9±0.2 Bq.kg^{-1}. Assuming the following values for porosity: ε=0.3, diffusion length in soil: l=1.0 m, and emanation factor: η=0.2, one obtains values of 12, 13 and 15 Bq.m^{-2}.h^{-1} for d=46, 48 and 58 cm, respectively. From the comparison with the experimental values listed in table 1 one may conclude that the calculated exhalation rates are an order of magnitude larger than measured. This might be due to too high values for diffusion length, porosity and/or emanation factor. It should be pointed out that the soil is well compacted due to the fluctuating ground-water level, which before the installation of the drainage system regularly caused inundation of the crawl space. From the discrepancy in magnitude it is clear that we have presently insufficient knowledge on the value of the parameters. From the relative values it is clear that the drastic change in exhalation rate occuring for ground-water levels between 48 and 58 cm below the surface can not be explained with a simple diffusion model for a homogeneous soil. It therefore seems likely that this change is due to a change in radium concentration in the soil at a depth between 48 and 58 cm and/or that dynamic processes have influenced the values. An argument for the first two possibilities is that the building pit was partly refilled with sand of a different kind than the excavated soil. Analysis of the dynamic aspects of radon[12] indicates that the value at d=58 cm may be influenced by a short period of heavy rain after a long dry period. It has been hypothesized that the percolating water acted as a slowly moving piston, partly blocking the radon flux to the surface in the yard and thereby increasing the concentration in the deeper layers of the soil. As the overall conclusion one may draw that our knowledge on radon transport in soil is presently insufficient to properly model the radon entry in the crawl space.

4. Conclusions and recommendations

It can be stated that the objectives of this part of the research programme have been met. The development of a radon exhalation meter has succesfully been concluded with a device that operates with a collection voltage of -2.5 kV on a foil in front of a surface barrier detector whilst the surrounding stainless steel cannister is at ground potential. The instrument works satisfactorily in atmosphere with 100% relative humidity as crawl space or soil in the yard. Although the instrument provides reliable information the interpretation of the data in terms of free-exhalation rates needs further investigation.

Activated charcoal cannisters were found to have a humidity dependent radon absorption efficiency. Attempts to circumvent this problem by

applying a radon-transparent vapour barrier have so far not been too successful; application of those barriers strongly reduced the sensitivity of the instrument. It is recommended to use activated charcoal cannisters only as first indicators in cases where an accuracy of a factor of three is sufficient. The use in humid environments (e.g. crawl spaces) is not recommended. For more accurate measurements we suggest the use of passive dosemeters[3] with an integration time of one to several months or quasi-continuous monitors[12] with sampling times of about 30 minutes. The first method is recommended for large scale surveys; the latter one for more detailed investigations of the dynamic transport of radon.

The investigations in houses with a relative high concentration have shown that in one house the high radon concentrations could be related to an extreme reduction of the ventilation of the crawl space. In Maastricht a set of identical houses was found with a large spread in the radon concentration in the crawl space and a concentration in the living room proportional to the crawl space concentration. In a set of identical dwellings at the opposite side of Maastricht the crawl space radon concentrations were almost the same and the living room radon concentration is inversely proportional to the radon concentration in the crawl space. It is suggested that the ventilation pattern for these sets of houses is different. Presently it is impossible to indicate whether the observations are in agreement or not with radon transport models since no ventilation patterns have been made. Since we have made a proposal for simultaneous measurement of ventilation patterns with PFT's and radon concentrations it is clear that we recommend to make funds available to obtain the necessary additional information.

Investigations of a set of identical dwellings with various types of crawl space floor covers have not resulted in a clear statement that such covers reduce the radon entry. Part of the reason for this is that the number of dwellings was too small; another reason was that no measurements could be made prior to the installation of the covers.

The role of ground-water level on the radon concentration in soil could clearly be established qualitatively. It can be stated that due to the much smaller diffusion length in water compared to dry soil (2.5 cm to about 1 m) the ground-water acts as an impenetrable barrier for radon to reach the surface. Only the non water-saturated soil layer above the ground-water level was found to increase the concentration and exhalation. Again it should be stated that the results so far agree only qualitatively and no quantitative description could be made yet. A further investigation of this problem is foreseen in the near future.

In and near a test dwelling measurements were performed of exhalation rates by various surfaces and the influence of the ground-water level was investigated. In this test dwelling time-average radon concentrations were measured over a period of several years for crawl space and living room. If one ignores the uncertainty in the interpretation of the exhalation rates and if one assumes certain values for the average ventilation rate a radon concentration in the crawl space is estimated which is a factor of two to three lower than the measured value. For the living room one estimates that 40% of the ventilated air enters the room via the crawl space. Moreover, one notices that the largest contribution to the radon concentrations in the crawl space is coming from building materials.

There are several arguments which indicate that a description of the radon concentration from static sources only is insufficient and that dynamic aspects in the transport of radon in soil under certain circumstances play an important role. Also in this part of the investigation it is found that the measured exhalation rate of the soil in

the crawl space is not well described by a diffusion model with a homogeneous soil. In view of the fact that the crawl space or in general the subfloor space is one of the most important entry routes for radon we have suggested a study under laboratory conditions of radon transport in soil. Such an investigation will be possible in the coming years in a joint effort of the CEC Radiation Protection Programme, the Dutch Ministry of Housing, Planning and Environmental Hygiene and the University of Groningen.

From the recent results in Maastricht and Roden we conclude a discrepancy between the calculations based on simple models and the data. In our opinion this discrepancy is likely due to assumptions made on ventilation patterns. It is therefore strongly recommended to repeat some of the measurements with simultaneous measurements of ventilation patterns.

We like to acknowledge the financial support of the CEC Radiation Protection Programme, the Dutch Ministry of Housing, Planning and Environmental Hygiene (VROM) via the National Research Programme RENA and the University of Groningen. The development of the project was influenced by the constructive interaction with the "Stuurgroep RENA". The technical and administrative assistance by the KVI is gratefully acknowledged.

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

Drs. J.G. Ackers, RD-TNO, Arnhem, The Netherlands

V. Publications:

P. Stoop, F.J. Aldenkamp, E.J.T. Loos, R.J. de Meijer and L.W. Put Measurements and modelling of radon infiltration into a dwelling. Paper selected for oral presentation at the 1990 International Symposium on radon and radon reduction technology, Atlanta, Ga, Febr. 19-23, 1990.

E.J.T. Loos, Modelling and measurments of dynamics of radon concentrations in a crawl space. KVI Internal Report 156i.

L.W. Put en R.J. de Meijer, Metingen van luchtdrukverschillen in en rond een woning. KVI Internal Report R-003.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

College van Bestuur der
Rijksuniversiteit Groningen
Postbus 72
NL-9700 AB Groningen

Contract no.: BI6-F-210-NL

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

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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.:

Measurements on, and control of infiltration of radon into dwellings.

Head(s) of project:

R.J. de Meijer, L.W. Put

Scientific staff:

R.J. de Meijer
L.W. Put
P. Stoop

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:

- Final tests of the continuous radon monitor;
- Correlation measurements of radon concentrations, pressure, temperature differences and rainfall etc. as function of time in two test dwellings;
- Evaluation of data with model calculations;
- Suggestions for countermeasures.

III. Progress achieved:

1. Introduction

In the period 1980-1984 a national survey in the Netherlands was conducted to obtain a scope on the distribution of radon concentrations in Dutch dwellings and to see if there were building parameters which could be correlated with elevated levels of radon. The project was part of Dutch National Research Programme SAWORA and revealed:

- The median concentrations in the Netherlands are only slightly lower than in adjacent countries; the width of the distribution, however, is more narrow.
- Soil under the dwelling was suspected to be an important source of radon for the dwelling.
- Certain building materials and construction techniques seemed to be correlated to higher mean concentrations of radon.

To deduce measures which could reduce radon concentrations in dwellings it was felt necessary to investigate the mechanisms responsible for the infiltration of radon. Two proposals were made; one to investigate the static (diffusive) aspects, the other to study the role of pressure driven flow. Both proposals were carried out in a joint financial cooperation with the Dutch Ministry of Housing, Planning and Environmental Hygiene (VROM), as part of the National Research Programme "RENA" and the University of Groningen as part of their "Centrale Beleidsruimte".

This report focusses on the dynamic aspects of radon infiltration. As stated in the proposal the objectives of this investigation were:

- Improvement of knowledge and understanding of mechanisms leading to infiltration of radon into dwellings.
- Investigation of the role of vective transport in soil and dwellings by studying short-term radon concentrations in correlation with relevant physical parameter.
- Deduce input for model studies from (auto)correlations.
- Deduce countermeasures for reducing indoor-radon concentrations.

These objectives were met by:

- Design, construction and testing of a continuous radon monitor, suitable for measuring (low) radon concentrations in the average dwelling.
- Setup of equipment in a test dwelling to measure (quasi-) continuously radon concentrations and possible relevant parameters like pressure differences, temperatures, relative humidities, barometric pressure, precipitation rate and ground-water level.
- Construction of a multi-room model to describe radon concentrations based on static and dynamic sources and advective flow.
- Installation of a ventilation duct plus fan to connect the crawl space with the outside air to introduce forced ventilation either by creating underpressure or overpressure.

2. Instrumentation

2.1. Radonmeter

Measurements of radon concentration often proceed via the detection of α-particles emitted by its decay products collected electrostatically onto or nearby a detector. Such a detector can be a surface barrier detector or a scintillator in combination with a photomultiplier tube (PMT), either directly connected or via a light guide. The advantage of such a system is that a high sensitivity can be achieved by collecting the decay products from a large volume, the almost 2π geometry for the α-detection and the fact that two short-living decay products of radon emit an α-particle in their decay. The disadvantages are the fact that small amounts of trace gases like NO_2 already strongly influence the collection efficiency by neutralization of the charged decay products[1] and that a deduction of radon concentrations from count rates is reliably possible after equilibrium between radon and its short-lived decay products is established (> 3 h).

Since in the domestic environment traces of NO_2 are likely to be present and our goal is to measure short-term correlations (~ 0.5 h) we opted for a different approach in which the detection of radon itself is more prominent. In this approach we started with a large Lucas cell as previously described by Cohen *et al.*[2] and decided to further develop this type of detector to make it suitable for our purposes.

In the initial stage we investigated the optimal length of the cell and the pro's and con's of the use of a light guide. It was found that the optimal length of the cell was about 30 cm; at greater length the pulse height of the light, produced at the opposite end of the cell from the PMT and emitted by the scintillator in the interaction with α-particles, becomes comparable to the background. It was found that the high energetic part of the background is due to Cerenkov radiation produced in the photocathode and/or the quartz window of the PMT. The use of a light guide enhances the pulseheight of the background signal.

The final design of the instrument is a cylindrical, 30 cm long, Lucas cell with a diameter of 13 cm, segmented into eight longitudinal sections. The cell and segment walls are covered by ZnS(Ag) except for the side where the cell is mounted onto a 13 cm diameter, low-background PMT. Due to this geometry a high efficiency for radon is obtained: about 40%. The efficiency for each of the two short-lived, α-emitting decay products is about 50%. The background, mainly due to Cerenkov radiation in the PMT, is about 1 cpm. Compared to a 1.2 L cell described in ref.[3] with efficiencies for radon and decay products of 30 and 24%, respectively, our 3 L instrument has a three to four times larger sensitivity. Concentrations of 10 $Bq.m^{-3}$ can be measured with this setup with a statistical uncertainty of 50%.

With an algorithm based on the count rates for the decay series[3] the count rate of the detector is converted to radon concentration. Figure 1 shows an example in which the instrument is exposed to a "block-pulse" of radon. The solid line presents the data points, the dashed curve corresponds to the deduced radon concentration. From the figure one notices that indeed

the algorithm deduces from a block pulse radon concentration as input, a curve with prominent ingrowth and decay features of radon and its decay products. This curve reproduces the data.

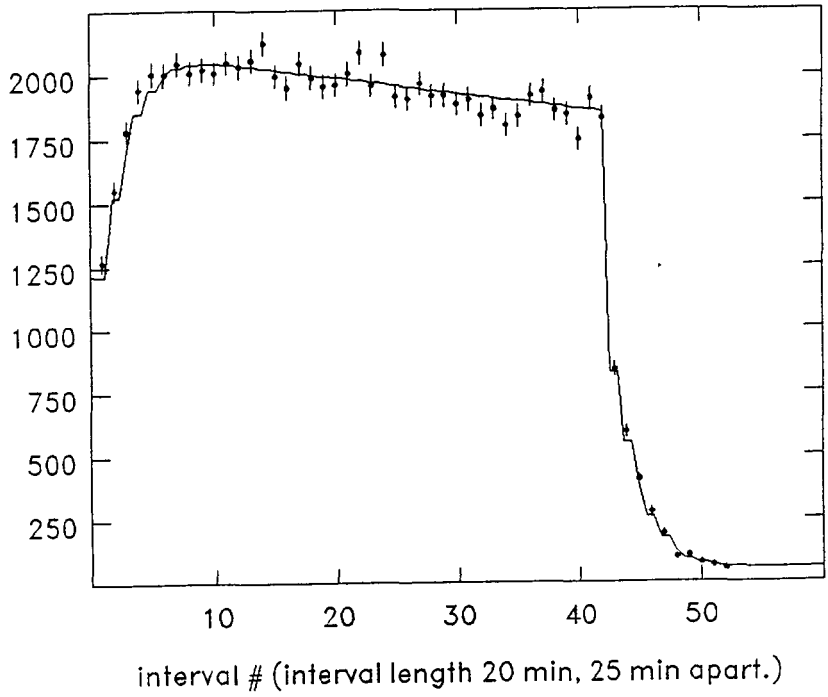

Figure 1. Count rate (counts per 25 min) measured with our continuous radon meter exposed to a block puls with an initial concentration of C_{Rn} = 338 $Bq.m^{-3}$.

2.2. Setup in the test dwelling

Measurements were made with two of the described radonmeters; sampling the air of the crawl space and the air of the living room respectively. The setup was operated in a quasi-continuous mode: a sample is taken by flushing the cell over a filter and a drying column for five minutes with a rate of 3 $L.min^{-1}$; subsequently the sample is counted for 25 minutes and the number of counts is stored in the memory of a data logger. Simultaneously the values of the following parameters, averaged over 25 minutes, are stored in the memory: pressure differences between outside air at the sides of the dwelling at the crawl space ventilation openings, the pressure difference between living room and crawl space, temperature and relative humidity of

outside air, air in the living room and air in the crawl space, temperature of the soil near the house and in the yard and barometric pressure. In addition the amount of precipitation in the period is registrated.

3. Dynamic ventilation model

A dwelling is considered with N rooms, each having a radon concentration $C_i(t)$ and a volume V_i; outdoor parameters are indicated by the index N+1. The radon concentration in room i is determined by the total strength of radon sources in the room, S_i, and the transport of radon by air currents. If q_{ik} denotes the air current from room k into room i the following mass-balance equation for room i can be written:

$$V_i \frac{dC_i(t)}{dt} = - \lambda_{Rn} V_i C_i(t) + \sum_{k=1}^{N+1} (q_{ik} C_k(t) - q_{ki} C_i(t)) + S_i. \qquad (3.1)$$

Defining $\lambda_i = \frac{1}{V_i} \sum_{k=1}^{N+1} q_{ki}$ as the ventilation rate of room i and $\Psi_{ik} = \frac{q_{ik}}{V_i}$,

eq. (3.1) transforms into:

$$\frac{dC_i(t)}{dt} = - (\lambda_{Rn}+\lambda_i) C_i(t) + \sum_{k=1}^{N+1} \Psi_{ik} C_k(t) + S_i/V_i \qquad (3.2)$$

Eq. (3.2) can be written for each of the N rooms, giving a system of N coupled linear non-homogeneous differential equations. The set of equations can be described more comprehensively using vector and matrix notation. In principle the equations can be solved analytically, in practice, however, instabilities occur and a numerical solution is preferred. The algorithms have been implemented in a computer programme (CARACO).

Essential for the calculation are the input parameters: the currents q_{ik} and the source strengths S_i. The currents q_{ik} were deduced from the pressure difference between room i and k, $\Delta p_{ik}=p_k-p_i$, and the air transparency of a barrier between the compartments i and k, T_{ik}.

$$q_{ik} = T_{ik} \left(\frac{\Delta p_{ik}}{1 \text{ Pa}} \right)^n \qquad (3.3)$$

The values of T_{ik} and n are determined by the air leaks in the dwelling, these were partly measured by ourselves[4], partly taken from ref.[5]. For the source term in the crawl space a pressure driven flow term (S_{pdf}) was added to the static sources (S_{st}) as measured with the radon exhalation meter. This pressure driven flow stems from the pressure difference between the

outside and the crawl space and causes a small, Darcy-type flow, through the soil (and the walls of the crawl space). This flow becomes important due to the relatively high concentration of radon in soil. The intensity of this term depends on the permeability of the soil (and walls), the radon concentration in the soil and the length of the crawl space perimeter. This model has been applied to a number of measurement series. Two examples will be discussed in section 4.3.

4. Measurements in a test dwelling; results

4.1. Test dwelling

The test house is located in Roden, southwest of the city of Groningen in the northern part of the Netherlands. It is located on the edge of the "Drents Plateau"; the local soil consists of silty fine sand with intercalations of boulder clay upon "pot clay". The impermeability of the pot clay causes large and rapid variations in the ground-water level. Prior to the installation of a water drainage system in February 1989 the ground-water levels often almost reached the soil surface. The crawl space thus contained 10 cm or more water during a major fraction of the year. Such situations are not uncommon for recently built houses in various parts of the Netherlands.

The dwelling is a single family house, built in 1973. It has a rectangular floor shape with a wall extending from the crawl space to the roof, which divides the house into a northern and southern part which can be regarded as reasonably independent. In the present investigation only the southern part is investigated. This part consists of a crawl space and a high loft living room, with an "open kitchen", directly covered by the roof. The ceiling of the crawl space consists of prefab hollow concrete bars which are covered by a layer of a few centimeters cement; in the living room this layer is covered with ceramic tiles. The outside walls are cavity walls consisting of masonry (about 10 cm thick); the cavity was filled in 1976 with a polyurethane foam for thermal insulation. The floor of the crawl space consists of uncovered sand and is situated at about 40 cm below the surrounding soil of the yard. Around the house, at a distance of 1 to 1.5 m, a ring type drain was installed in February 1989 at a depth of 70-90 cm below the surface. The dividing wall in the crawl space has two crawl openings in addition to feed throughs for central heating (water) and utilities. In June 1989 these openings were closed by 5 cm thick foam board. All remaining openings in the wall were sealed with caulk. In October 1989 a 20 cm diameter ventilation duct from crawl space to roof plus fan was installed in order to (de)pressurize the southern part of the crawl space. Natural ventilation of the crawl space occurs via ventilation shafts to the outside air; in principle these shafts should have no connection with the cavity wall.

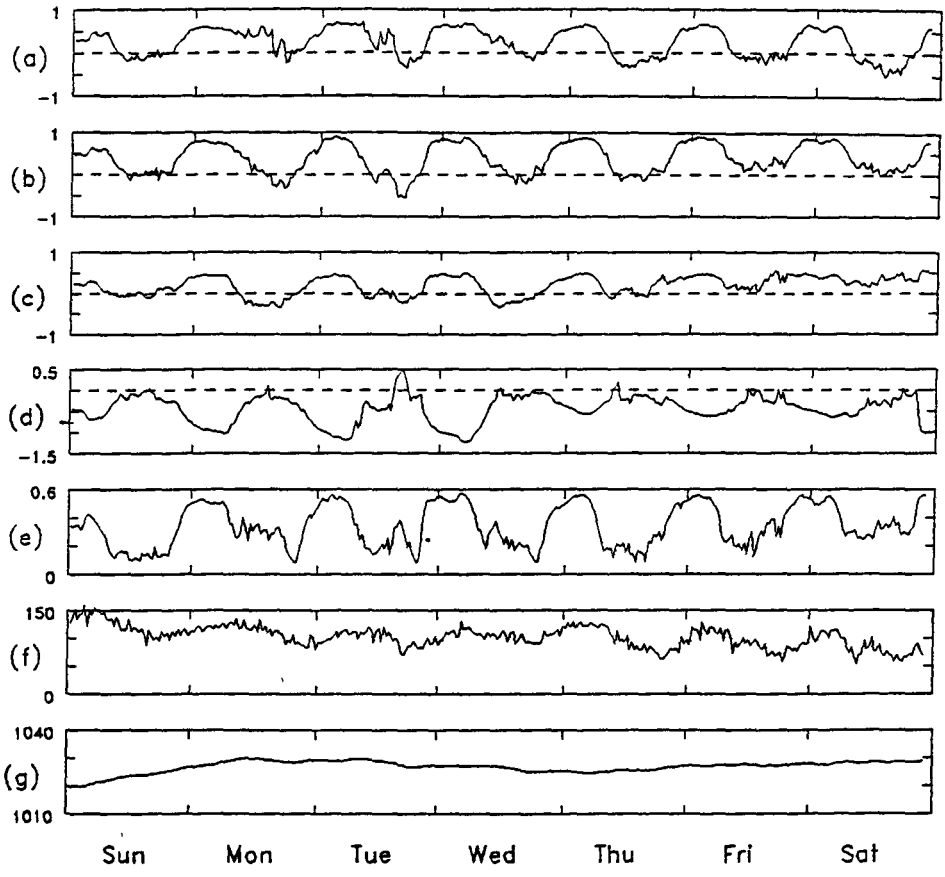

Fig. 2. Pressure differences $P_i - P_{cs}$ (Pa) with P_i being the pressure at the west (a), south (b) and east (c) outside wall and in the living room (d) and with P_{cs} being the pressure in the crawl space. The calculated ventilation rate (h^{-1}) of the crawl space and the measured radon concentration ($Bq.m^{-3}$) and the barometric pressure (hPa) are presented in parts (e), (f) and (g), respectively. All data were collected in week 24, 1989 (June 11-17).

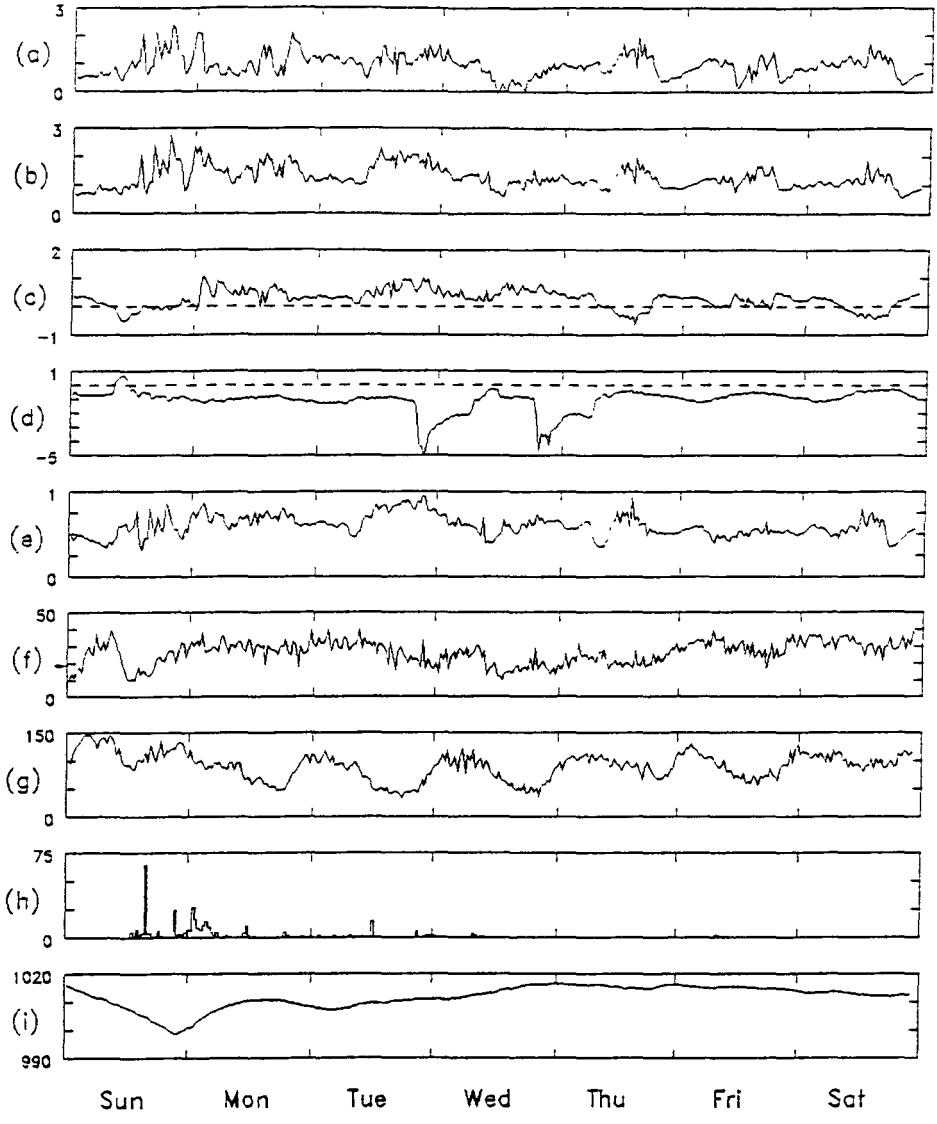

Fig. 3. Pressure differences $P_i - P_{cs}$ (Pa) with P_i being the pressure at the west (a), south (b) and east (c) outside wall and in the living room (d), the calculated ventilation rate (h^{-1}) of the crawl space, (e) the measured radon concentrations ($Bq.m^{-3}$) in the living room (f) and crawl space (g), precipitation rate ($mm.h^{-1}$) in part (h), and barometric pressure (hPa) in the bottom part. All data were collected in week 31, 1989 (July 30-August 5).

4.2. Results

Measurements were started in June 1989 and have, except for starting problems, been continued until the end of the year. As examples we present the results of two weeks. The analysis of the results of other weeks is still in progress. Fig. 2 shows from top to bottom four pressure differences $P_i - P_{cs}$ with i=west, south, east and living room, respectively, and P_{cs} the pressure in the crawl space, the deduced ventilation rate, the radon concentration in the crawl space, and the barometric pressure in week 24 (June 11^{th} - 17^{th}) of 1989. This week was characterized by absence of wind, high temperatures during day time and cool nights. In the figure one notices that during the night time the living room is at a lower pressure than the crawl space and the crawl space is at lower pressure than the outside air. This is attributed to the temperature difference between living room and outside air. In the absence of wind or forced ventilation this stack effect is considered the driving force for the pressure difference between crawl space and living room. The ventilation rate is calculated from conservation of air mass and the flow calculated from the pressure differences and the measured leak of the crawl space ventilation shafts. From the figure one notices that both ventilation rate *and* radon concentration in the crawl space are in phase with each other and the pressure differences over the wall.

Fig. 3 shows the time dependence of the radon concentration in crawl space and living room a number of parameters during week 31, 1989. In this week there was almost continuously wind from the southwest, resulting in overpressure at the south and west walls and underpressure in the living room, all with respect to the crawl space. Heavy rain fall occurred on Sunday afternoon and evening as showers. No obvious effect on the radon concentration is observed. On Tuesday and Wednesday evening the open fire was lit causing a sharp increase in the pressure difference between living room and crawl space. Surprisingly no effect of this increased underpressure is observed on either radon concentrations and/or pressure difference of the crawl space with the outside world. The radon concentration in the crawl space shows the diurnal cycle similar to the one in fig. 2. Also in the pressure differences such a cycle is noticeable. The difference with fig. 2, however, is that the maxima in the crawl space radon concentration are more pronounced and that for Tuesday, Wednesday and Thursday they coincide with the minima in the ventilation rate. However, they are more pronounced than the variations in the ventilation rate. The overall trend of the crawl space radon concentration is a decrease towards the middle of the week and an increase afterwards. For the radon concentration in the living room no diurnal cycle is observed; the concentration is somewhat lower on Wednesday and Thursday.

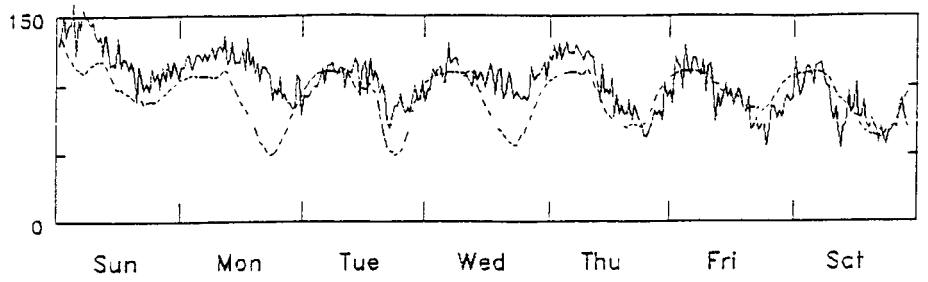

Fig. 4. Radon concentration in the crawl space of the test dwelling in Roden during week 24, 1989 (June 11-17). The solid curve represents the measurement, the dashed curve the concentration calculated with a dynamic model. Multiplication factors for the measured S_{st} and estimated S_{pdf} are 1 and 40, respectively.

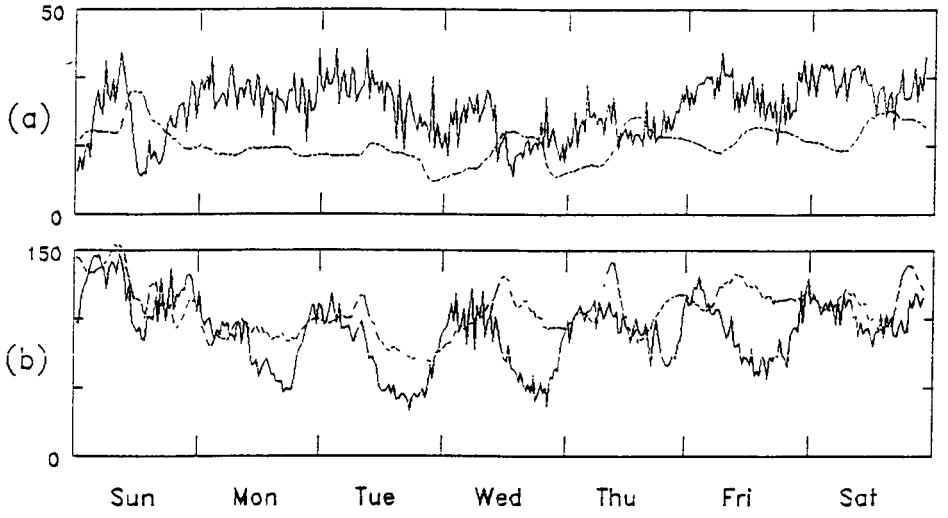

Fig. 5. Radon concentration in the crawl space and living room of the test dwelling in Roden during week 31, 1989 (July 30 - August 5). (See fig. 4). Multiplication factors for the measured S_{st} and estimated S_{pdf} are 7 and 1, respectively.

4.3. Analysis and interpretation

In the analysis of the data the calculations were carried out with measured leakage parameters for the ventilation shafts and estimated values for floor, windows, roof and doors[5]. The static source strengths S_{st} for the crawl space and living room, based on exhalation measurements[4] amount to 275 and 90 $Bq.h^{-1}$, respectively. The value of the pressure driven flow (PDF) depends on the pressure difference between the wall and the crawl space, the permeability of the soil (and walls), the radon concentration in the soil and the length of the crawl space perimeter. As starting values were taken: for the permeability 5.10^{-12} m^2, the value for fine sand, and for the radon concentration in the soil gas 10 $kBq.m^{-3}$.

Fig. 4 shows the radon concentration in the crawl space measured during week 24, 1989. The dashed curve is the calculated concentration based on measured leakage parameter, measured S_{st} and a pressure driven flow (PDF) term with a strength 40 times the estimated value of S_{pdf} based on the starting parameters. Without the adjustment of S_{pdf} the magnitude of the concentration is a factor of three too low and the oscillations are out of phase. This latter observation leads to the conclusion that even a larger static term, which cannot be excluded in view of intrinsic uncertainties in the determination of the exhalation rate, cannot explain the data; a static term alone results in a decreasing radon concentration with increasing ventilation rate. The results indicate that the missing radon concentration in the crawl space may be accounted for by a value of the S_{pdf} which is a factor of 40 larger than estimated from rather arbitrary starting parameters.

Fig. 5 shows radon concentrations in the living room (top) and crawl space (bottom) measured during week 31, 1989. The dashed curves represent the concentrations calculated with a two-room model. First the crawl space concentration was optimized by starting from values for parameters as found for week 24, 1989. The result in the bottom part of fig. 5 is obtained with a seven times larger value than the measured value of S_{st} and a value for S_{pdf} equal to the value estimated from the original starting parameters. With these values of S_{st} and S_{pdf} the average concentration in the crawl space is reproduced; in the living room the concentration is a factor of two too low. The larger static source term is necessary to reproduce the dips in the crawl space radon concentration at times of increased ventilation; the smaller S_{pdf} to obtain the correct average values.

At first sight it seems rather strange that the size of the terms changes in time and one may argue that the infiltration of radon is still a large puzzle. Without trying to reduce such a conclusion one could think of a possible explanation. During week 31 the ground-water level reached one of its lowest values of the year (d=58 cm). The exhalation of the soil in this period, measured at one location increases sharply (d=58 cm in table 1 of

ref. (6)). The increase of the exhalation rate is larger than expected on basis of difference of water level at d=48 and 46 cm.

As a hypothesis we propose a role to rainfall to qualitatively account for both the increase in exhalation rate and the reduction of the PDF-term. The concentrated precipitation did hardly change the ground-water level until the latter part of the week. This means that the percolating rain has been forming a type of piston on the soil closing of pores in air with water thereby reducing the porosity and permeability of the soil. The slowly moving piston blocks the diffusion of radon to the outdoor soil and thus causes an increase of the radon in the soil gas below the piston. This will enhance the exhalation of the soil in the crawl space, especially near the perimeters (the measurement of the exhalation was made close to the centre). Moreover the reduction of permeability will reduce the PDF-term. It should be stressed again that this is only a hypothesis, which qualitatively accounts for these rather large changes.

4.4. Countermeasures

Based on a simplified ventilation model with only static sources one would expect a diminishing concentration with increasing ventilation with outside air. Based on this expectation a ventilation duct and fan were installed in the test dwelling. The duct connects the southern part of the crawl space with the roof; the fan speed can be regulated.

After calibration of pressure difference over part of the duct to air speed using an anemometer, measurements started in November 1989. For the present series of measurements the fan sucks air from the crawl space. Measurements of about five days with fan on and five days with fan off are made with after each step an increasing fan speed. During this period of measurements the ventilation shaft to the outside air was closed off in an attempt to increase the ventilation from the living room to the crawl space. This increase was expected to reduce the radon concentration in the living room.

Figure 6 shows the daily averaged radon concentration in the crawl space and living room of the test dwelling; the solid line indicates the residing time (inverse of the ventilation rate) of the air in the crawl space deduced from the pressure difference over part of the duct and the volume of the crawl space. From the figure one notices that the concentration in the crawl space is in the fan-off periods a factor of two lower than in the weeks 24 and 31 despite the closure of the ventilation shafts. This seems to be correlated with a higher ground-water level in December compared to the summer. Ventilation of the crawl space has clearly an effect on the radon concentration in the crawl space; the concentration is proportional to the residing time.

In the periods with fan-on the pressure in the living room is always higher than in the crawl space: on the average 1, 2 and 4 Pa for the settings 0.5, 1.0 and 1.5, respectively. In the periods with fan-off the pressure in the living room is on the average about 1 Pa lower than crawl space. Despite the fact that the flow reverses sign during the fan-on period and the influx of "radon-rich" air is blocked the radon concentration in the

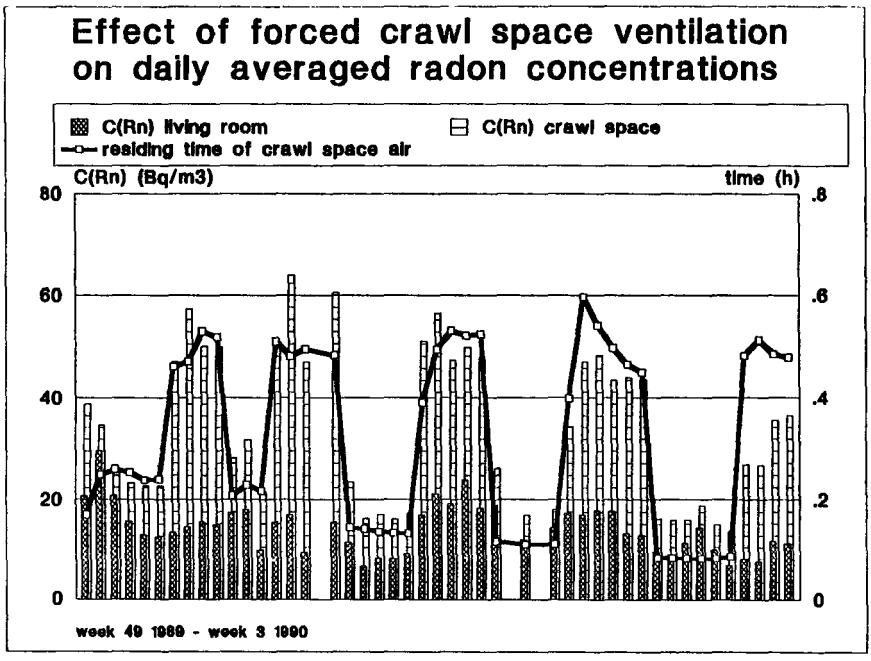

Figure 6. Daily averaged radon concentrations in living room and crawl space in December 1989 and January 1990 for various settings of the fan in the ventilation duct between crawl space and roof, corresponding to various values of the residing time.

living room remains practically unchanged. Moreover the radon concentration in the living room remains higher than expected on basis of the value of the outside air concentration and the exhalation of the walls and floor. Since also the northern part of the house is likely to be at underpressure the observation indicates an unaccounted entry route of radon to the living room. A possible way is via the cavity wall, which is connected to the soil and only via leaks connected to the crawl space. It is intended to propose a continuation of this part of the project in which ventilation patterns and leak areas will be measured simultaneously with radon concentration.

5. Conclusion

This research period has resulted in a continuous radon meter, suitable for measurements in dwellings with concentrations as low as 10 $Bq.m^{-3}$. Measurements in a test dwelling yield radon concentrations in crawl spaces and living rooms which are larger than calculated from measured static source strengths. Time evolution of radon concentrations measured simultaneously with pressure differences indicates that pressure driven flow through the soil may account for the missing contribution to the concentration as well in the crawl space as in the living room. This is the result of the preliminary analysis of two weeks in the summer of 1989 with different weather characteristics.

From this analysis we conclude that the effective strengths of diffusive and pressure driven terms are not constant in time. It is proposed as a hypothesis that changes in water content of the soil due to precipitation may temporarily increase the radon concentration below the wetted layer and that the increased water content may reduce the pressure driven flow.

Although we are still at the beginning of the analysis of the data and our hypothesis may be replaced by others we would be tempted to state that understanding radon infiltration into dwellings is not so much a problem of transport in the dwelling than it is a problem of radon transport in soil. In our present opinion the way to better understanding is a combination of analysing measurements, as described in this paper, over a longer period of time to identify possible important parameters and investigations of these parameters under controlled conditions in the laboratory.

Measurements on the effect of countermeasures indicate that bringing the crawl space at underpressure with a fan in a ventilation duct from crawl space to outside air has a clear reducing effect on the radon concentration in the crawl space but has no significant influence on the radon concentration in the living room. This latter result is surprising since during fan-on periods the direct current between living room and crawl space reverses compared to fan-off situations. Since the concentration remains higher than expected on basis of the concentration in the outside air and the exhalation by surfaces it is suspected that an unaccounted entry route of radon is present. A more detailed analysis and further investigations are imperative to understand these phenomena.

The analysis of the dynamic aspects of radon entry indicated that our knowledge is still insufficient to fully comprehend the question how radon enters a dwelling. The processes of radon transport are much more complicated than anticipated before. To our opinion the largest uncertainty is the transport in soil. Changing ground-water levels and precipitation vary source strength, porosity and permability for radon. For an actual dwelling inhomogenities in the soil, and uncontrollable variations in soil parameters make an assessment of the radon transport difficult. It seems therefore a logical step to investigate the transport in the laboratory with a setup having dimensions larger than the diffusion length of radon in soil. The results of such an investigation may eliminate some of the uncertainties in the present investigations and reveal presently hidden properties in

radon transport in a dwelling.

The present investigation is financially supported by the CEC Radiation Protection Programme, the Dutch Ministry of Housing, Planning and Environmental Hygiene and the University of Groningen. The work, therefore, is also part of the Dutch National Research Programme "RENA" and of the Environmental Radioactivity Research at the KVI.

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3. Busigin, A., van der Vooren, A.W. and Phillips, C.R., Interpretation of the response of continuous radon monitors to transient radon concentrations, Health Phys. 37, 653-667, 1979.
4. Loos, E.J.T., Modelling and measurements of the dynamics of radon concentrations in a crawl space, KVI internal report 156i, 1989.
5. Phaff, J.C., de Gids, W.F. and Knoll, B., Ventilatie van gebouwen. Metingen van de luchtlekken en voorspelling van de ventilatie van een woning in Roden, IMG-TNO Technical Report C535, Sept. 1983, TNO-Delft.
6. Aldenkamp, F.J., de Meijer, R.J. and Put, L.W., Investigation of mechanisms leading to radon concentrations, Report to the CEC Radiation Protection Programme, 1990. Contract # BI6-F-120-NL.

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

Drs. J.G. Ackers, RD-TNO, Arnhem, the Netherlands

V. Publications:

P. Stoop, F.J. Aldenkamp, E.J.T. Loos, R.J. de Meijer and L.W. Put, Measurements and modelling of radon infiltration into a dwelling, paper selected for oral presentation at the 1990 International Symposium on radon and radon reduction technology, Atlanta, Ga, Febr. 19-23, 1990.

L.W. Put, Radon concentraties in een twintigtal woningen. Studie naar voorkomen van relatief hoge radonconcentraties in woningen, en naar het effect van bodemafsluiting op de radonconcentratie, KVI Internal Report R-004.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no : BI6-F-116-UK

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

Dr. J.W. Stather
Biomedical Effects Department
NRPB
Chilton, Didcot
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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:

J C H Miles

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 modify the multichannel diffusion battery so that the size range of the instrument is extended to cover particle diameters down to 0.5 nm allowing better estimates to be made of the unattached activity particle size distribution. To make further measurements in a representative range of dwellings and domestic conditions and in a selection of mines.

III. Progress achieved:

During this reporting period, the NRPB multichannel diffusion battery was modified to allow measurements of the activity size distribution down to 0.5 nm. The channels were loaded with screens as follows:

Channel	Mesh No.	Wire diameter (μm)	Solid fraction (%)	Number of screens
0	-	-	-	None
1	200	35	29	1
2	400	24	30	1
3	400	24	30	4
4	400	24	30	14
5	400	24	30	45

The larger wire diameter for channel 1, with correspondingly larger gaps between wires, allows a larger fraction of the aerosols in the range 0.5-5 μm to penetrate. The computer routine used to unfold the size distribution was modified accordingly.

The instrument was used in the living room of a representative house. Activity median diameters in the range 150 to 200 nm were found for the attached component. In the case of the unattached component, AMDs in the range 0.8 to 2.9 nm were found with the unattached fractions, from 37 to 8 per cent, varying inversely with condensation nucleus concentration. Although activity measurements were not made during the night, the condensation nucleus concentration was found to fall to a minimum of 200 particles cm^{-3} around 0200 hours, about an order of magnitude lower than the daytime value, so unattached fractions are likely to be even higher at night. Tri-modal size distributions were observed when cooking was taking place in the adjacent kitchen. The additional mode, at about 6 nm, is assumed to be due to the growth of aerosols from vapours generated during cooking. These distributions were similar to those observed in earlier measurements in kitchens.

The instrument was also used, during the period, in an intercomparison held in a realistic mining environment at Limoges, France, which was sponsored by the CEC. Typical results of measurements using the diffusion battery are shown in Figure 1. The equilibrium factor found in the mine was around 0.4, similar to that in homes, but the unattached fraction of radon daughters was lower, at 1-3.5%. This implies that for this mine, at least, lung doses for a given potential alpha energy concentration will be lower than in homes. Further measurements in homes and mines will be carried out to determine how typical are these conditions.

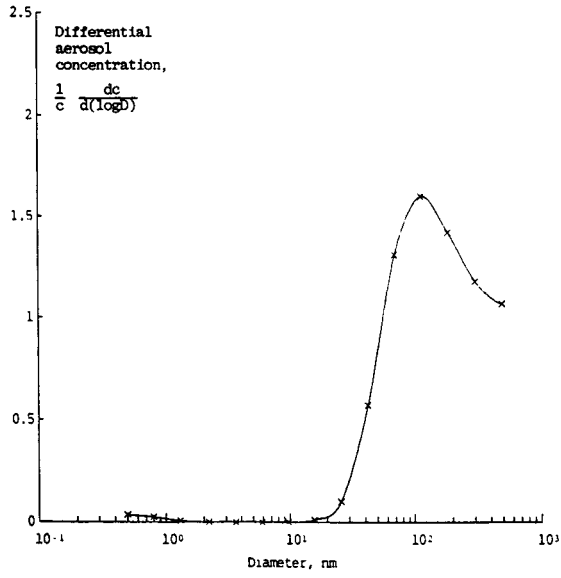

Figure 1: Typical distribution of aerosol sizes for the radioactive aerosol in the Limoges mine intercomparison

IV. Objectives for the next reporting period:

To make further measurements in a range of mines and dwellings, and to use the results to determine the relationship between radon concentration and lung dose in these conditions.

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

Dr A Reineking, Isotopenlaboratorium der Georg-August-Universität, Burckhardtweg 2, D-3400 Göttingen, F.R.G.

Dr H Vanmarcke, Nuclear Physics Laboratory, Gent State University, Proeftuinstraat 86, B-9000 Gent, Belgium

VI. Publications:

Strong, J C. The size of attached and unattached radon daughters in room air. Journal of Aerosol Science 19, 1327-1330, 1988.

Title of the project no.: 2

Deposition of aerosols in the upper respiratory tract.

Head(s) of project:

J C H Miles

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 investigate the spatial deposition of particles in the nasal cast within the size range 50 to 250 nm diameter and with unattached lead-212. To measure the penetration through the nasal cast of radon daughters in the size range 1 to 5 nm diameter.

III. Progress achieved:

The efficiency of the human nose as a filter of unattached Po-218, the first decay product of radon, was investigated using hollow casts. The apparatus used is shown schematically in Figure 1.

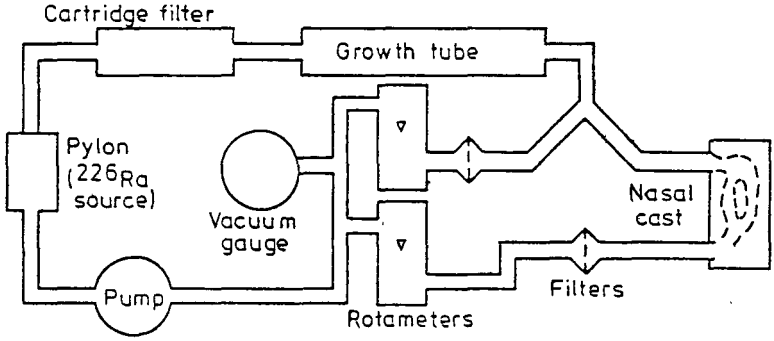

Figure 1: Schematic diagram of the apparatus used to measure penetration off unattached Po-218 through nasal cast.

Three airway models were used, two of which were nasal casts obtained from cadavers. The third was made by carrying out nuclear magnetic resonance imaging of a live subject. Sheets of polymethyl methacrylate were cut according to the slice images from NMR and assembled to make an airway model which included an oral cavity.

The three models were then used to determine the penetration of unattached Po-218 through the airways. The penetration through the oral cavity of the sectioned cast was similar to that through the nasal casts. implying that the lung dose from unattached radon daughters is not strongly dependent on the manner of breathing either by nose or mouth.

IV. Objectives for the next reporting period:

To analyse the data collected in the previous reporting periods in order to provide input to a model for calculating lung doses from radon decay products as a function of unattached fraction of radon decay products with account being taken of subject age.

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

Dr D L Swift, Associate Professor. Department of Environmental Medicine, Johns Hopkins School of Hygiene and Public Health. 615 N. Wolfe Street, Baltimore. MD 21205, USA.

Dr J E Agnew, Department of Medical Physics, The Royal Free Hospital. Pond Street. Hampstead. London NW3 2QG.

VI. Publications:

Strong J C and Agnew J E. The particle size distribution of Technegas and its influence on regional lung deposition. Nuclear Medicine Communications 10, 425-430, 1989.

Title of the project no.: 3

Application of CR-39 etched track detectors to low background counting and particle sizing of air samples.

Head(s) of project:

J C H Miles

Scientific staff:

S Naismith

I. Objectives of the project:

To develop the technique of alpha-particle registration of CR-39 to the stage where it becomes suitable for routine assay of long-lived alpha activity collected on personal air sampler filters.

II. Objectives for the reporting period:

To test software for automatic scanning and assessment of etched-track autoradiographs of personal air sampler filters.

III. Progress achieved:

In the last reporting period, revised software was developed by the Medical Research Council, Population Cytogenetics Unit, Edinburgh, to enable the NRPB Cytoscan 110 optical image analyser to identify automatically alpha tracks in chemically-etched CR-39 detectors. In this period, the capabilities of this software were explored to determine the feasibility of using etched-track autoradiography to assess long-lived alpha activity on personal air sampler filters.

The ability of the Cytoscan running the lastest software to recognise tracks and to record parameters such as x and y coordinates, area and perimeter has been tested. Figure 1 shows a map of the tracks on an etched track detector, clustered in the centre. This occurs because of the design of the sampling head of the personal air sampler (PAS), which deposits larger particles in the centre of the filter paper. The variation in track density across such an autoradiograph enables the aerodynamic size of the sampled aerosol to be determined.

Figure 2 shows a histogram of track areas. This is an essential check on the routine performance of the Cytoscan, as any errors in track recognition result in a change in the shape of the histogram. If etching conditions vary slightly from batch to batch, a shift is caused in the whole histogram. This can be corrected to allow all autoradiographs to be compared on a common basis.

The work carried out so far has demonstrated that the Cytoscan, though it can be used for development of this technique, is not entirely suitable for routine application. The current version of the software is simpler to use than previous versions, but is still time-consuming and requires a considerable understanding of the mode of operation of the Cytoscan. In order to scan autoradiographs without overflowing the memory available, it is necessary to postpone analysis of the data: the data are stored on a floppy disk, then a data analysis programme is run on the Cytoscan. This reduces daily throughput of autoradiographs to a level which would be unacceptable in a routine service. However, the throughput is sufficient to allow an assessment of the feasibility of the technique in routine operation on a faster image analyser.

0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	162	939	36	0	0	0
0	0	0	709	6837	627	0	0	0
0	0	0	143	2025	330	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

Figure 1: Map of etched tracks accepted by the Cytoscan

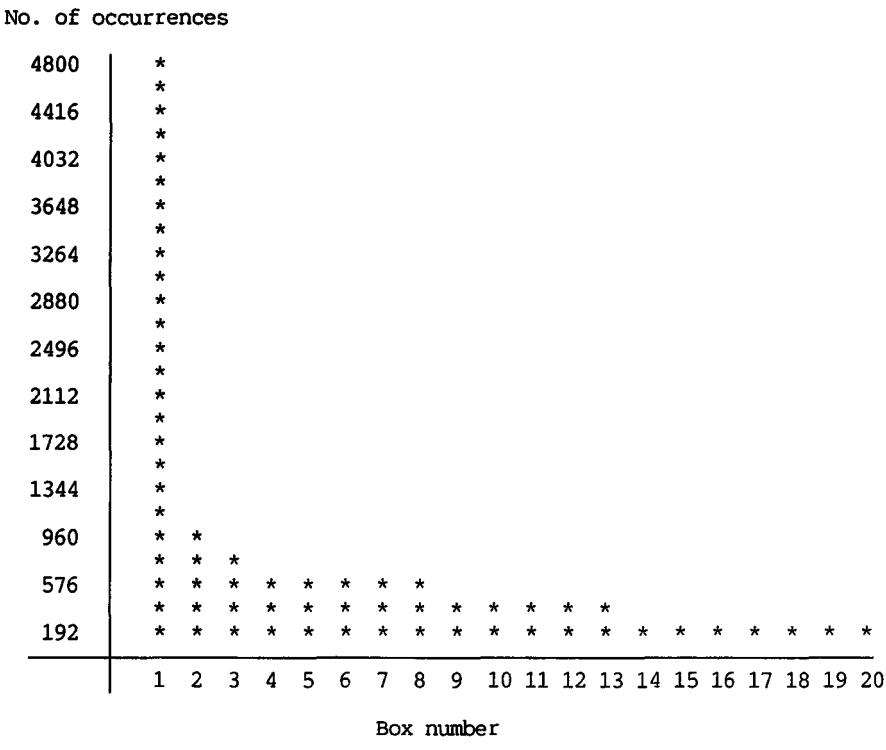

Figure 2: Histogram of areas of accepted tracks

IV Objectives for the next reporting period:

An evaluation of the potential of the technique for the routine assessment of PAS filters will be undertaken. The advantages and disadvantages of the technique will be examined with regard to cost, speed of reporting results, sensitivity, and extra dosimetric information obtainable. These characteristics will be compared with those of the existing evaluation technique using alpha drawer counters.

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

Population Cytogenetics Unit
Medical Research Council
Edinburgh

(Dr D Rutovitz)

VI. Publications:

None.

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
Miss B.K. Butland

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 develop methods for distinguishing relative (multiplicative) and absolute (additive) risk models.

To analyse data on cancer mortality among the U.K. ankylosing spondylitis patients and the Japanese atomic bomb survivors.

To develop cancer risk estimates applicable to a U.K. population.

III. Progress achieved:

Distinguishing Relative and Absolute Risk Models for Radiation-Induced Cancers

In projecting cancer risks observed in high dose studies over time, two approaches have generally been used. The relative (or multiplicative) risk projection is based on the assumption that the relative cancer risk observed in the period of follow-up for the study will remain constant into the future. The absolute (or additive) risk projection assumes that the annual absolute excess cancer risk observed in the study will remain constant over time. Since for most solid cancers the underlying risk increases sharply with age, the annual absolute excess risk also increases with attained age under the relative risk model, whereas under the absolute risk model it remains constant. Consequently these two methods can yield quite different estimates of lifetime risk, particularly if the relative or absolute risks also depend on age at exposure. UNSCEAR (1988) have presented results based on both projection models, using data from the Japanese atomic bomb survivors; the lifetime radiation-induced risk for fatal cancers of all types at high doses and high dose rates varied from 4% Gy^{-1} (low LET) based on an age-dependent absolute risk model to 11% Gy^{-1} (low LET) based on an age-dependent relative risk model. (Risk estimates applicable to a UK population are described below).

A formal statistical approach to distinguishing how well the relative and absolute risk models fit to data from a follow-up study has been developed (Muirhead and Darby, 1987). This involves the use of a wider class of risk models, of which the relative and absolute models are special cases. The goodness-of-fit of the best-fitting model within this wider class can then be compared with that of each of the relative and absolute models separately, in order to test whether either the relative or the absolute risk model (or both) is consistent with the data. These methods were initially applied to data from earlier follow-ups of the U.K. ankylosing spondylitis patients given x-ray therapy and the Japanese atomic bomb survivors in Hiroshima and Nagasaki (Muirhead and Darby, 1987, 1988); results based on more recent data are described below. Generally, the data on solid cancers are consistent with models under which the relative risk either remains constant over time or ultimately tails off, rather than with the model under which the absolute risk is constant over time.

It should be emphasised that even in those cases where the relative risk appears to be constant over the period of follow-up, there can still be great uncertainty in the estimation of lifetime risks. As mentioned later in relation to the development of risk estimates from the 1988 UNSCEAR report, lifetime risks based either on a relative risk projection or simply on the follow-up to date of the Japanese atomic bomb survivors can differ by more than a factor of 3.

Ankylosing Spondylitis Patients

Analysis of age and time trends in radiation-induced cancer risks has been undertaken (Muirhead and Darby, 1989) using data from the latest follow-up of U.K. ankylosing spondylitis patients who received x-ray therapy (Darby et al, Br. J. Cancer, 1987). For the grouping of all cancers other than leukaemia and colon cancer, the relative risk at a given time since exposure was found to decrease with increasing age at exposure, in line with results from the Japanese atomic bomb survivors (Shimizu et al, RERF TR5-88, 1988). However, the trends in risk with time since

exposure were such that both the relative and absolute excess risk began to tail off about 20 years following exposure. Thus different methods for projecting risks beyond the current period of follow-up yielded similar lifetime risks on the basis of these data. The above result contrasts with the most recent follow-up of the Japanese atomic bomb survivors (Shimizu et al, 1988), where the absolute excess risk of solid cancers is continuing to increase with increasing time since exposure and the relative risk is approximately constant up to 40 years post exposure. However, a tailing-off in the radiation-induced risk as in the spondylitics has also been seen, for example, in lung cancer among uranium miners and bone cancer in U.S. radium luminisers (BEIR IV, 1988).

Data on the Japanese Atomic Bomb Survivors, based on the DS86 Dosimetry System

The full data set on cancer mortality up to 1985 among the Japanese atomic bomb survivors, as based on the new DS86 dosimetry, is awaited from the Radiation Effects Research Foundation (RERF) in Japan. However, some analyses have been carried out using data in RERF Technical Report 9-87 (Preston and Pierce, 1987) and augmented by additional data supplied by the authors of this report to the Central Electricity Generating Board (Charles and Little, CEGB RD/B/6112/R89). These data were used to examine a claim by J.W. Gofman (Health Physics, 56, 117 (1989)) that the cancer risk per unit dose in the Japanese survivors increases to a statistically significant extent with decreasing dose. The methods that he used, however, were inappropriate. It was found when the mortality rates were calculated correctly and adjusted for sex, age and time since exposure that the data for both leukaemia and the grouping of all other cancers were consistent with linear dose-response relationships. These points were made in a letter published in Health Physics (Muirhead and Butland, 1989).

The Preston and Pierce data have also been used to study some of the results published by Little and Charles (J. Radiol. Prot. 9, 9 (1989)) based on these data. For mortality from cancers other than leukaemia the relative risk appears to be approximately constant over the period of follow-up, although, as pointed out by Shimizu et al (RERF TR5-88, 1988), there is an indication that the relative risk is tailing off amongst those aged less than 10 years at exposure. For leukaemia mortality Little and Charles suggested that, after allowing for effects of age at exposure and time since exposure, a model which is close to a relative (multiplicative) risk model provided the best fit to the A-bomb data. However, further analysis has indicated that there may be one or two data points exerting a disproportionate influence on the choice of risk model. After appropriate adjustment the best fit appears to be closer to an absolute (additive) risk model. Examination of these data is continuing.

Other Studies

Tapes have been received from the U.S. National Institute of Occupational Safety and Health (NIOSH), containing data on lung cancer mortality and radon exposure (plus information on smoking) among Colorado Plateau uranium miners. However, it has not yet been possible to perform analyses due to errors in some of the data on these tapes. A corrected data tape is due to arrive shortly. It is intended to study the joint effect of radon exposure and smoking on lung cancer mortality. Previous analyses, such as that by the BEIR IV Committee, suggest that the data are consistent with a multiplicative effect. The topic of risk projection will also be examined using these data, in conjunction with data from other high dose studies such as the ankylosing spondylitis and Japanese A-bomb data.

Risk Estimates Developed from the 1988 UNSCEAR Report

In the wake of recent publications by the Radiation Effects Research Foundation (RERF) in Japan on cancer risks among the A-bomb survivors based on the new DS86 dosimetry system and of the 1988 UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) review of risk estimates, health effects models applicable to a UK population have been developed (Stather et al, NRPB-R226, 1988). For cancer, risk estimates were derived mainly on the basis of the Japanese data, but using other studies where these were more informative for particular cancers (eg. using data on radium luminisers in the case of bone cancer, and on patients given x-ray treatment for enlarged thymus in the case of thyroid cancer). Owing to the uncertainty in the prediction of lifetime risks as described above, two sets of risk estimates were calculated for solid tumours; one set based on the follow-up of the A-bomb survivors to date (40 years post exposure) and the other set based on the assumption that relative cancer risks will remain constant until the end of life. Based on an analysis of radiation-induced cancer rates and baseline rates for individual cancer types in the Japanese survivors and UK ankylosing spondylitis patients, it was decided for solid tumours to transfer risk coefficients derived from the former population to a UK population on the basis of relative risk, since this parameter was more stable across populations than absolute risk. In order to extrapolate to low doses and low dose rates, a dose rate effectiveness factor (DREF) of 3 was used in most cases, as suggested by a review of animal studies; however, the DREF was taken equal to 2 for breast cancer.

Table 1 shows risk estimates applicable to a UK population of all ages and both sexes. The total fatal cancer risk at low doses and dose rates was estimated as 1.4-4.5% Gy^{-1} (low LET). The range of uncertainty due to lack of information from current cohort studies on risks over a complete lifetime is greatest for those exposed in childhood. For a working population aged 20-64 years at exposure, the range of uncertainty is less than for the whole population and estimates of 1.8-3.4% Gy^{-1} (low LET) were obtained for all cancers; see Table 2.

Table 1

Estimated excess cancer risks in a UK population (all ages, both sexes) associated with exposure to low LET radiation

Cancer type	Fatal cancer risks, 10^{-2} Gy^{-1} (low LET)				ICRP 1977 (adults)
	Lifetime projection		Risk observed to date[i]		
	HDR[g]	LDR[h]	HDR	LDR	
Leukaemia[a]	.84	.28	.84	.28	.2
Breast[b]	1.1	.55	.42	.21	.25
Lung[a]	3.5	1.2	1.15	.38	.2
Thyroid[c]	.075	.025	.055	.02	.05
Bone[d]	.15	.05	.15	.05	.05
Liver[e]	.45	.15	.23	.08	.1
LLI/colon[a]	1.1	.37	.38	.13	.1
Stomach[a]	.73	.24	.14	.05	.1
Remainder[a,f]	5.0	1.63	.565	.20	.2
Total[a]	12.9[j]	4.5	3.93	1.4	1.25

Notes:

(a) based on Japanese A-bomb survivors

(b) based on studies in western women

(c) from NCRP Report No 80 (1985)

(d) based on radium luminisers

(e) based on Thorotrast cases (BEIR IV, 1988)

(f) by difference

(g) high dose rate (> 100 mGy d^{-1})

(h) low dose rate (< 100 mGy d^{-1})
DREF: 2 for breast, 3 for other cancers

(i) based on period of follow-up of Japanese population to date (40 years)

(j) corresponds to 12.9% Gy^{-1}

(k) from Stather et al (1988)

Table 2

Estimated excess cancer risks in a UK working population (both sexes) exposed to low LET radiation at ages 20-64 years

Cancer type	Fatal cancer risks, 10^{-2} Gy^{-1} (low LET)				ICRP 1977
	Lifetime projection		Risk observed to date[i]		
	HDR[g]	LDR[h]	HDR	LDR	
Leukaemia[a]	1.01	.34	1.01	.34	.2
Breast[b]	.79	.40	.51	.26	.25
Lung[a]	2.09	.70	1.64	.55	.2
Thyroid[c]	.05	.017	.03	.01	.05
Bone[d]	.15	.05	.15	.05	.05
Liver[e]	.45	.15	.23	.08	.1
LLI/colon[a]	1.21	.40	.56	.19	.1
Stomach[a]	.59	.20	.19	.06	.1
Remainder[a,f]	3.33	1.10	.88	.28	.2
Total[a]	9.67[j]	3.4	5.20	1.82	1.25

Notes:

(a) based on Japanese A-bomb survivors

(b) based on studies in western women

(c) from NCRP Report No 80 (1985)

(d) based on radium luminisers

(e) based on Thorotrast cases (BEIR IV, 1988)

(f) by difference

(g) high dose rate (> 100 mGy d^{-1})

(h) low dose rate (< 100 mGy d^{-1})
DREF: 2 for breast, 3 for other cancers

(i) based on period of follow-up of Japanese population to date (40 years)

(j) corresponds to 9.67% Gy^{-1}

(k) from Stather et al (1988)

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

Imperial Cancer Reseach Fund Cancer Epidemiology Unit,
University of Oxford, Radcliffe Infirmary, Oxford, OX2 6HE, UK.
(Collaborating research worker - Dr. S.C. Darby).

V. Publications:

Muirhead, C.R. (1989). Transfer of risk estimates between populations. Summary of contribution to Workshop on Risk Estimates for Radiation Carcinogenesis, Bad Münstereifel, Federal Republic of Germany, 28-29 September 1989.

Muirhead, C.R. and Butland, B.K. (1989). Letter to the editors: Dose-response analyses for the Japanese A-bomb survivors. Health Phys., 57, 1035-6.

Muirhead, C.R. and Darby, S.C. (1987). Modelling the relative and absolute risks of radiation-induced cancers (with discussion). Journal of the Royal Statistical Society, A150, 83-118.

Muirhead, C.R. and Darby, S.C. (1988). Distinguishing relative and absolute risk models for radiation-induced cancers. IN Health Effects of Low Dose Ionising Radiation - Recent Advances and their Implications, 45-50. London, British Nuclear Energy Society.

Muirhead, C.R. and Darby, S.C. (1989). Relative and absolute risk models for cancer mortality in ankylosing spondylitis patients. In Low Dose Radiation: Biological Bases of Risk Assessment (eds. K.F. Baverstock and J.W. Stather), 162-170. London, Taylor and Francis.

Stather, J.W., Muirhead, C.R., Edwards, A.A., Harrison, J.D., Lloyd, D.C. and Wood, N.R. (1988). Health effects models developed from the 1988 UNSCEAR report. Chilton, NRPB-R226 (London, HMSO).

Stather, J.W. and Muirhead, C.R. (1989). Radiation risks and the 1988 UNSCEAR report. Radiological Protection Bulletin, 98, 15-19.

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
Miss B.K. Butland

I. Objectives of the project:

To develop and refine techniques for analysing epidemiological data on occupational and other populations exposed to low levels of ionising radiation. To develop and validate statistical software for use in the study of populations exposed occupationally to radiation.

II. Objectives for the reporting period:

To refine software for analysing data from cohort studies of those exopsed occupationally to radiation. To study methodological issues in the assessment of studies of childhood cancer around nuclear installations. To examine epidemiological information on the risks of cancer following irradiation in utero.

III. Progress achieved:

Software for Analysing Occupational Mortality Data

The computer program ARFAR (At Risk For Any Reason) has been developed at NRPB to assist in the analysis of epidemiological data for those exposed occupationally to ionising radiation (Barry, 1986). 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 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. It is intended that the program be used in the analysis of the UK's National Registry for Radiation Workers (NRRW).

While the stratification of person-years by variables such as age that vary linearly with time is relatively straightforward, the additional stratification by a variable such as cumulative radiation dose that generally changes in a non-linear manner over time is more complicated. It is in its ability to stratify person-years by dose that ARFAR differs from most other programs that perform cohort analyses. The calculations are based on histories of annual doses received by the workers. By assuming that dose is received at a constant rate over any year, a cumulative dose history can be constructed for each worker that is a piecewise linear (rather than linear) function of time. This allows person-years to be split according to various levels of cumulative dose.

As part of the validation of the program, some of its output was compared with that from other programs designed to analyse occupational mortality data, such as PERSON-YEARS (Coleman et al, Int. J. Epidem., 15, 134-137 (1986)). However, as pointed out above, ARFAR has the advantage over most such programs in the way that it can stratify person-years by cumulative dose. ARFAR has also been used in conjunction with a program that simulates occupational mortality data based on individual dose-histories from studies of the Hanford workforce in Washington State, USA. The latter program, named SIRIS, was developed by GSF Neuherberg, FRG (see section IV); its function was to simulate a date and cause of death for each worker in the Hanford cohort, based on sets of baseline mortality rates and various models for radiation-induced cancer risks. On applying ARFAR and its ancillary programs to the output from SIRIS, it was shown that under the assumption of ICRP (1977) risk estimates, it would not be possible to identify a radiation-induced excess cancer risk, even with the simulated life-long follow-up of the cohort. However, with recent changes in the models favoured for the projection of risks over time, namely from an absolute to a relative risk approach (UNSCEAR, 1988), the detection of an excess risk based on life-long follow-up of a larger cohort may be feasible if such a relative risk model holds for solid cancers.

Certain enhancements have been made to ARFAR and the ancillary programs. One refinement allows more general latency distributions for risk following exposure to be accommodated. In particular, rather than assuming that a radiation-induced cancer risk suddenly commences at a specific time following exposure, ARFAR can now stratify person-years on the basis of a model for which the risk increases linearly with time until it reaches a plateau. While the former model is routinely used in analyses of occupational studies and so will be used in the first analysis of the NRRW, the latter model is more flexible and allows the sensitivity of the latency assumptions to be assessed.

Another modification to ARFAR has been made that will reduce considerably the computing time arising from multiple runs of the program. This consideration is particularly important in relation to the NRRW, where large numbers of runs will be required. The saving in computing time has been achieved by producing a separate version of ARFAR in which only tables of numbers of deaths from a given cause are produced. Thus only one run of the original version of ARFAR is required to obtain tables of person-years, while the modified version can be run repeatedly for different causes of death. A program has also been written to calculate median doses within dose groups, such that trends in mortality can be examined in relation to these median doses.

Among more recent enhancements, ARFAR can now perform analyses on sub-groups of workers, such as all those monitored for internal emitters. ARFAR has also been modified to allow workers to enter the study some time after commencing radiation work. This is particularly important in the case of the NRRW, which commenced at the beginning of 1976 and for which, in the case of certain employers, follow-up of the workers did not start until this time.

ARFAR has been run satisfactorily on trial databases prior to the first analysis of the NRRW, which will commence shortly (this analysis is supported by the CEC Radiation Protection Programme under contract no. BI6-F-213-UK(H)). Documentation for ARFAR has been written, and copies of the program have been provided to other research workers in this field. ARFAR and its associated programs are available on request to NRPB.

The Geographical Distribution of Childhood Cancer in Relation to Nuclear Installations

During the last few years there has been considerable interest in reports claiming to show excesses of childhood cancer - and in particular, leukeamia - in the vicinity of British nuclear installations. Some of these reports, namely those concerning the reprocessing plants at Sellafield and Dounreay, and at AWE Aldermaston and ROF Burghfield, have been investigated by the UK Committee on Medical Aspects of Radiation in the Environment (COMARE). The interpretation of such epidemiological studies is generally not straightforward, particularly if the number of cancer cases is small. The choice of boundaries for the relevant study area, time period, ages and disease grouping, as well as the choice of control group, can often affect the statistical significance of the results. The sensitivity of the results with respect to these choices has therefore been assessed when examining reports concerning various installations, such as those described above. A review has been conducted of the different methodologies - particularly concerning the choice of geographical units - employed in several recent studies (Ennis, 1987). Also, a computer program has been written for demonstration and teaching purposes which allows a distribution of cases of a rare disease to be generated across a geographical grid. In this way it is possible to show how apparent 'clusters' can occur by chance.

Examination of data on the geographical variation in cancer rates throughout the country would be of great help in trying to interpret the results around nuclear installations. An important issue is whether the number of disease cases in any specified area follows a Poisson distribution (as is usually assumed in studies around nuclear installations) or whether it follows a distribution with a larger variance. A search of the statistical literature indicated that a test derived by Potthoff and Whittinghill (Biometrika, 53, 167 (1966)) should have good

properties at detecting such 'over-dispersion' in the numbers of cases. However, this test does not appear to have been used in an epidemiological setting until now. Some computer simulations were performed that confirmed the good statistical power of the techique; these results were presented at a Royal Statistical Meeting on Cancer near Nuclear Installations, held in London in May 1989 (Muirhead and Ball, 1989). The above test will be applied to a database on the geographical distribution of diagnoses of childhood leukaemia and lymphoma throughout Great Britain. This database, which has been constructed by the Childhood Cancer Research Group in Oxford (with support from NRPB), will be ready at the beginning of 1990.

Cancer Risks following Irradiation In Utero

An examination was made of a paper written by members of the Department of Social Medicine at the University of Birmingham (Knox et al, J. Soc. Radiol. Prot., 7, 177-189 (1987)), which contained a re-analysis of data on pre-natal x-rays and childhood cancer obtained from the Oxford Survey of Childhood Cancers (OSCC). In particular, it was suggested in the paper that adjusting in the analysis for the effects of both drugs administered to the mother and maternal illnesses during pregnancy increased the excess relative cancer risk associated with pre-natal x-rays by more than a factor of 2.

As a result of discussions with the authors and further analyses of the data it was found that an error had been made in their calculations; in particular, the cohort-specific cancer risks and the relevant years of birth had been mis-matched. When these were aligned correctly it was found that the relative risks were similar to those obtained in earlier analyses of this study, i.e. a relative risk of about 1.4. A joint letter with one of the authors pointing out the error and providing revised risk estimates (Table) was published in the above journal (Muirhead and Kneale, 1989).

Estimated Absolute Risk Coefficients for Incidence of All Cancers over Ages 0-14 Years associated with Irradiation In Utero

Source of estimates of dose per film	Absolute cancer risk/10^4/Gy,[a] based on: Deaths during 1964-79 with adjustment for pregnancy illnesses and drugs[b]	Deaths during 1953-79 with no adjustment for pregnancy illnesses and drugs[c]
Stewart and Kneale (1970)	1270[d] (840-1920)[e]	1360 (1000-1840)[e]
UNSCEAR (1972)	600[d] (440-810)[e]	640 (410-1000)[e]

[a] Geometric mean of the calendar period-specific ratios of risk per examination to mean dose per examination.
[b] Based on Table 7a of Knox et al (1987).
[c] See Muirhead and Kneale (1989).
[d] These values involve an extrapolation to risks arising for those born before 1950, i.e. prior to the period of observation in this instance. Restricting attention to those born since 1950 yields risk coefficients of 1030/10^4/Gy based on Stewart and Kneale's dose estimates and 600/10^4/Gy based on UNSCEAR's dose estimates.
[e] Approximate 95% confidence interval.

References

(1) Knox, E.G., Stewart, A.M., Kneale, G.W. and Gilman, E.A. (1987). Prenatal irradiation and childhood cancer. J. Soc. Radiol. Prot. 7, 177-189.

(2) Stewart, A.M. and Kneale, G.W. (1970). Radiation dose effects in relation to obstetric x-rays and childhood cancer. Lancet i, 1185-8.

(3) United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (1972). Ionising Radiation: Levels and Effects. 1972 Report to the General Assembly, with annexes. Vol. II: Effects (New York: United Nations).

As part of the development of health effects models in the light of the 1988 UNSCEAR report, data on cancer risks following irradiation *in utero* were reviewed (Stather *et al*, NRPB-R226, 1988). These consisted of data from the OSCC, from other studies of pre-natal x-rays and childhood cancer (for example, that by MacMahon in the N.E. United States), and from the Japanese atomic bomb survivors. Based on the OSCC and estimates of doses from pre-natal x-rays given by UNSCEAR (1970), a risk of cancer incidence following *in utero* exposure of 6% Gy^{-1} was adopted (low LET), with values of 2.5% Gy^{-1} for leukaemia and 3.5% Gy^{-1} for other cancers. Half of these cancers were assumed to be fatal.

IV. 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.

V. Publications:

Barry, S.F. (1986). ARFAR - A person years at risk program. British Journal of Industrial Medicine, 43, 572-3.

Ennis, J.R. (1986). Report review: "Epidemiological Studies of Occupational Exposure to Radiation" by J.M. Davies for OECD/NEA. Radiological Protection Bulletin, 72, 21-23.

Ennis, J.R. (1987). How should the health of communities near nuclear installations be monitored? Journal of the Society for Occupational Medicine, 37, 19-23.

Ennis, J.R., Ball, A.M. and Barry, S.F. (1987). 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.

Muirhead, C.R. and Ball, A.M. (1989). Contribution to the discussion of the Royal Statistical Society Meeting on Cancer near Nuclear Installations. Journal of the Royal Statistical Society, A152, 376.

Muirhead, C.R. and Darby, S.C. (1989). Introduction to the proceedings of the Royal Statistical Society Meeting on Cancer near Nuclear Installations. Journal of the Royal Statistical Society, A152, 305.

Muirhead, C.R. and Kneale, G.W. (1989). Letter to the editor : Prenatal irradiation and childhood cancer. Journal of Radiological Protection, 9, 209-212.

Stather, J.W., Muirhead, C.R., Edwards, A.A., Harrison, J.D., Lloyd, D.C. and Wood, N.R. (1988). Health effects models developed from the 1988 UNSCEAR report. Chilton, NRPB-R226 (London, HMSO).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-213-UK

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

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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.:
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
Mr. A.M. Ball
Miss B.K. Butland
Mr. A.J. Conquest
Mr. A.A. Goodill

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 national mortality rates.

II. Objectives for the reporting period:

To prepare both the registry database and software that will be used to analyse it.

III. Progress achieved:

The National Registry for Radiation Workers (NRRW) is a long-term epidemiological study of those exposed to ionising radiation in the course of their work and for whom radiation dose records are kept. The study is primarily one of mortality, though information on cancer incidence will also be examined. Since the expected number of radiation-induced cancers is small it is important that the cohort studied should be as large as possible.

Over the period of this contract work has continued on validating the last major contributions to the NRRW and on adding them to the database. So far as possible the task of bringing together different records for the same individual has proceeded in parallel with this work as have the overall checks on data validity. Great improvements have been made in the scope of the database and in data quality. Nevertheless, more work is needed before the NRRW will be ready for analysis. Tables 1 and 2 summarise some of the more important aspects of the database. Tables 3 and 4 give more detailed information.

One long standing 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; this has risen to 74% in the second supplement[2] and to 88% in the third[3] (see table). At the end of the contract period the percentage flagged had reached 94%, very close to the minimum acceptable figure of 95%. Nevertheless, there are sub-groups within study population for whom the situation is not yet satisfactory.

Table 3 describes the personal information supplied to the NRRW and Table 4 gives a histogram of dates of birth. The latter also gives provisional data for members of the study population who refused to participate in the NRRW. One important parameter for allowing for the Healthy Worker Effect is the date of employment or of starting radiation work. Ideally both these dates would be known and analyses using both would be compared. However, it can be seen that one or other date is missing in a substantial number of cases. Investigations have shown that at least one of the dates is available in almost all cases and it will be necessary to use one as a surrogate for the other. In many practical cases (probably in most) these dates will in fact be the same.

Plans for the analysis of the database have been formulated and produced as a report. This was circulated to a group of eminent epidemiologists for comment. After revisions the document was published in its final form as memorandum NRPB-M149[4] in 1989.

The analysis will be in two parts, as described in NRPB-M149. The external analysis will involve comparing mortality rates among the radiation workers with rates for the general population of England and Wales. While it is reasonably straightforward to calculate these national rates for the period from 1950 onwards, rates are also required for the earlier period of the late 1940s. To this end codes are being constructed to 'bridge' between the 5th and 9th revisions of the International Classification of Diseases. The resultant national mortality rates will be used in conjunction with a standard computer program (PERSON-YEARS) to perform cohort analyses.

The second, and more important part of the analysis will involve testing for any trend in mortality rates with the dose received by the workers. This internal analysis will be conducted using the computer program ARFAR (At Risk For Any Reason), which has been developed at NRPB under CEC contract B16-F-116-UK. During the reporting period further enhancements have been made to the program. These allow for analyses of sub-groups of the workers, such as those monitored for internal emitters, and for the possibility that workers may be entered in the study some time after commencing radiation work. A user guide for the program has also been prepared.

The above two computer programs have been run satisfactorily on several trial data sets and checks for the consistency of the results have been performed.

Some study has also been made of the likely effect of dose record keeping practices on tests for trend in disease rates with dose. In particular, systematic errors in cumulative recorded doses arise through the subtraction of values from annual doses as a consequence of recording levels and as a means of allowing for threshold doses. It appears that allowing for length of time monitored in the analysis may reduce the effect of such dose measurement errors. A possible alternative, if data on time monitored are not available, is to allow for length of employment.

Table 1: Status of Study Population, as given in NRPB-R219

Study population	Number enrolled	Positive refusals	Individuals of uncertain status
97,437	94,845 (97%)	1752 (1.8%)	840 (0.9%)

Number of individuals enrolled on NRRW	Number flagged	Number not flaggable
94845	83454 (88%)	11391 (12%)

Table 2: Distribution of Doses, as given in NRPB-R219

	Lifetime dose (mSv) <10	10–50	>50	Total
No. of employments:				
Participants	52347 (55%)	26529 (28%)	15969 (17%)	94845 (100%)
Non-participants	(49%)	(34%)	(17%)	(100%)
Collective dose for participants (man Sv)	133 (4%)	577 (19%)	2266 (76%)	2976 (100%)

Table 3

Personal data supplied to the NRRW[1]

Site	Number on NRRW	Date of employment[3]	Date of ceasing employment[4]	Year of commencing radiation work[3]	Date of entry to NRRW[2,3]	Industrial classification[3]	Update status[4]	Sex	
								M	F
Amersham Int	1707	1662	YES	1237	1705	1636	YES	1104	603
BNFL									
Capenhurst	1457	1255	YES	1457	667	1284	YES	1427	30
Chapelcross	897	814	YES	819	822	895	YES	847	50
Risley	740	739	YES	740	740	739	YES	720	20
Sellafield	9697	5682	YES	5014	3698	5519	YES	9042	655
Springfields	5150	3943	YES	4267	5141	5031	YES	5044	106
BNFL TOTAL	17941	12403		12297	11068	13438		17080	861
CEGB									
Berkeley Labs	567	0	YES	516	270	0		552	15
Berkeley power stn	489	0	YES	381	317	0		486	3
Bradwell	548	0	YES	547	218	0		546	2
Dungeness A	510	0	YES	510	202	0		506	4
Dungeness B	511	0	YES	507	352	0		509	2
Hartlepool	1209	0	YES	1072	1165	0		1205	4
Hinkley Point	1337	0	YES	1194	544	0		333	4
Oldbury	582	0	YES	481	332	0		78	4
Sizewell	358	0	YES	358	0	0		355	3
Trawsfynydd	577	0	YES	476	172	0		574	3
Wylfa	660	0	YES	539	370	0		657	3
CEGB TOTAL	7348	0		6881	3942	0		7301	47
MOD									
Civilians	15860	403	YES	15854	15860	13766	YES	13903	1957
Army	3003	1595	YES	3003	3003	2786		2538	468
Royal Air Force	5147	3718	YES	5147	5147	4980		4581	566
Royal Navy	9383	3494	YES	9383	9382	6776		8773	610
MOD TOTAL	33393	9210		33387	33392	28308		29795	3598
MRC - RBU	153	152	YES	22	125	146		99	54
NRPB	187	166	YES	187	177	167	YES	146	41
Other organisations	5134	2		5106	4643	0		4263	871
Rolls Royce & Assoc	703	699	YES	701	703	701	YES	670	33
SERC									
Daresbury	614	606	YES	612	614	611	YES	605	9
Rutherford	565	564	YES	565	565	563	YES	551	14
SERC TOTAL	1179	1170		1177	1179	1174		1156	23
SSEB Hunterston	1550	989	YES	1531	804	1519	YES	1541	9
UKAEA									
Dounreay	3229	2266	YES	2264	2905	3229	YES	3019	210
Harwell and Culham	3297	2554	YES	3284	3202	3294	YES	3049	248
AEA Risley	593	504	YES	464	593	507	YES	582	11
Winfrith	1895	1895	YES	1895	1895	1895	YES	1838	57
Ex-UKAEA	16536	16534	YES	16401	16534	16536	YES	15010	1526
UKAEA TOTAL	25550	23753		24308	25129	25461		23498	2052
GRAND TOTAL	94845	50206		86534	82867	58592		86653	8192

Notes

1. The need for some items in this table was not identified when the NRRW was first set up. This accounts for the delay which some sites have experienced in providing these data

2 A conservative estimate of date of entry to the NRRW can be made by the NRRW if the site is unable to supply this information. However it will usually be possible for the site to provide more accurate information.

3. The number in each column indicates the number of individuals for whom the data are supplied at each site

4 An entry in this column indicates that the information is supplied where applicable.

Table 4

Distribution of dates of birth for participants

Site	Years of birth															
	pre-1900	1900-1904	1905-1909	1910-1914	1915-1919	1920-1924	1925-1929	1930-1934	1935-1939	1940-1944	1945-1949	1950-1954	1955-1959	1960-1964	1965-1969	1970-
Amersham Int.[2]	0	0	0	1	23	65	125	133	149	147	165	235	301	257	99	0
Percentage	0%	0%	0%	0%	1%	4%	7%	8%	9%	9%	10%	14%	18%	15%	6%	0%
BNFL																
Capenhurst	0	0	0	50	106	163	137	129	104	116	164	211	169	105	3	0
Chapelcross	0	0	1	24	62	82	76	101	69	64	88	89	127	80	34	0
Risley	0	0	1	0	4	35	62	65	67	103	121	138	103	37	4	0
Sellafield	0	0	0	92	300	579	606	622	621	655	924	1123	1435	1207	483	0
Springfields	0	0	0	143	263	512	490	463	382	350	568	624	775	436	144	0
BNFL TOTAL	0	0	2	309	735	1371	1371	1380	1243	1288	1865	2185	2609	1865	668	0
Percentage	0%	0%	0%	2%	4%	8%	8%	8%	7%	8%	11%	13%	15%	11%	4%	0%
REFUSALS Percentage	0%	0%	0%	4%	10%	11%	11%	7%	8%	7%	11%	13%	16%	3%	0%	0%
CEGB[2]																
Berkeley Labs	0	0	0	1	16	31	55	72	71	116	104	58	25	14	0	1
Berkeley power stn	0	0	0	5	11	67	66	61	69	50	61	53	33	11	0	0
Bradwell	0	0	0	16	37	58	61	56	60	69	65	49	46	30	1	0
Dungeness A	0	0	0	5	26	41	54	51	59	57	58	81	59	19	0	0
Dungeness B	0	0	0	0	7	22	31	56	60	85	89	79	62	20	0	0
Hartlepool	0	1	0	0	7	32	55	93	127	187	254	165	159	118	0	0
Hinkley Point	0	0	0	9	32	89	166	171	172	205	217	153	87	26	0	0
Oldbury	0	0	0	8	16	43	61	65	69	82	88	59	42	35	0	0
Sizewell	0	0	0	5	34	57	61	55	46	41	23	21	13	2	0	0
Trawsfynydd	0	0	0	8	29	51	53	80	74	69	81	50	57	23	0	0
Wylfa	0	0	0	6	17	57	64	62	88	90	113	82	48	28	0	0
CEGB TOTAL	0	1	0	63	232	548	727	822	895	1051	1153	850	631	326	1	1
Percentage	0%	0%	0%	1%	3%	8%	10%	11%	12%	14%	16%	12%	9%	4%	0%	0%
MOD																
Civilians	0	1	7	122	518	1239	1396	1350	1390	1584	2008	1955	2271	1675	342	2
Army	0	2	0	6	5	17	37	71	184	364	486	580	704	443	104	0
Royal Air Force	0	1	0	3	4	14	52	157	360	568	863	1060	1126	829	110	0
Royal Navy	0	0	0	3	9	21	49	126	319	695	1610	1732	3016	1633	170	0
MOD TOTAL	0	4	7	134	536	1291	1534	1704	2253	3211	4967	5327	7117	4580	726	2
Percentage	0%	0%	0%	0%	2%	4%	5%	5%	7%	10%	15%	16%	21%	14%	2%	0%
REFUSALS Percentage	0%	0%	0%	0%	3%	12%	8%	6%	15%	8%	19%	10%	15%	2%	1%	0%
MRC - RBU	0	0	0	1	1	13	8	11	10	10	17	22	26	29	5	0
Percentage	0%	0%	0%	1%	1%	8%	5%	7%	7%	7%	11%	14%	17%	19%	3%	0%
REFUSALS Percentage	0%	0%	0%	0%	0%	0%	14%	43%	14%	0%	0%	14%	0%	14%	0%	0%

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 (London: HMSO) (1985).

(3) Kendall, G.M., O'Hagan, J.A., Rees, S., Walker, S.M. and Muirhead, C.R., Summary of data held by the NRRW. Chilton: NRPB-R219 (London: HMSO) (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. Chilton: NRPB-M149 (London: HMSO) (1989).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Commissariat à l'Energie
Atomique, CEA
IPSN
B.P. n° 6
F-92265 Fontenay-aux-Roses

Contract no.: BI6-F-122-F

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

M. G. Uzzan
IPSN-DPS-
CEA-CEN de Fontenay-aux-Roses
B.P. n° 6
F-92265 Fontenay-aux-Roses

Telephone number: (1) 654.71.39

Title of the research contract:

Consequences of irradiation of population and workers.

List of projects:

1. Assessment of industrial irradiation.
2. Assessment of the objective detriment and of its 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

ASSESSMENT OF INDUSTRIAL IRRADIATION

Head(s) of project:

A. DESPRES

Scientific staff:

A. DESPRES - S. BONNEFOUS

I. Objectives of the project:

Objective : Elaboration of a data base allowing the evaluation of sanitary consequences and of economical impacts of an accidental pollutant release.
This data base covers all the countries of the European Community, on a grid with 10000 km^2 meshes.
The parameters included concern population, land use, agricultural productions and employment.

II. Objectives for the reporting period:

Tasks : Four tasks were defined for the reporting period 1.1.89 - 31.12.89 :

- transfer of data files in floppy disks, in order to be workable on micro computers ;
- completion of the data base, namely by including milk production and all relevant parameters needed to evaluate transfer of radionuclides to man by water ;
- development of graphical and cartographic softwares in order to make easy the reading of the data files ;
- for particularly critical meshes (meshes including potentially polluting installations), or for very non-homogeneous meshes (costal areas for instance), evaluation of the same parameters on a 100 km^2 grid.

III. Progress achieved:

Results :

1 - The transfer of the data files on floppy disks is achieved. Two softwares have been written : The first one, devoted to internal use, introduces new data in the base and the second one assists the consultation of the data base. This software is entirely conversational, and allows to select

- a given data file
- a country
- one or several meshes
- the meshes for which the value of the parameter is in a given range.

All of these conditions can be mixed together: As an exemple, it is possible to obtain the list of the meshes for RDA, for which the population is in the range 10 000 - 30 000, and for which the annual wheat production is in the range 1 000 - 5 000 t.

2 - Data concerning the quantities of collected milk have been included in the data base, in the 10 000 km^2 grid. Data concerning water transfer are actually collected in the frame of the european CORINE Project. This data concern water abstraction (place, quantities) and water distribution (covered area, consumption for industrial, domestic and agricultural uses), both for surface and ground water, in all the european countries. When this data will be available (that is planned for the first half of 1990), they will be included in the data base.

3 - Graphical software and visualization

The map of Europe and the grid, have been computerized. Discussions are in progress to obtain a software able to assign the value of a parameter to a given mesh.

Two others works are developped in the frame of Project 1:

1 - An expert system is developped to provide a decision aiding system in case of contamination of foodstuffs after an accidental release of radionuclides. The objective of this system is to establish a classification of the countermeasures that can be envisaged, taken into account feasibility, dose reduction and economical consequences. This first step is achieved.

The second step consists to the representation of parameters that are not easy to

quantify:

the influence of the quantity of contaminated products;

the relations between public reactions and countermeasures.

Contacts are established with other european laboratories working in the same area (especially the RADE-AID Project).

2 - A bibliographic synthesis concerning the internal contamination measurements performed in the european countries after the Chernobyl accident had been written.

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

European laboratories working in the RAD-AID Project

V. Publications:

S. BONNEFOUS, A. DESPRES

Evolution of the European Data Base

Communication to be presented at the Seminar on Codes for Assessing the Off-Site Consequences of Nuclear Accidents (Athens, 7-11 May 1990)

A. DIAZ

A demonstration of the expert system is planned at the same meeting (Athens, 1990)

A. DESPRES

Internal Contamination Measurements Conducted in Europe after the Chernobyl Accident; Bibliographical Revue (in press).

Title of the project no.: 2

Evaluation of objective detriment in relation to economical considerations - Researchs on human beings.

Head(s) of project: R. MAXIMILIEN

Scientific staff: 1 person

I. Objectives of the project:

The aim of the project is to compare methods of evaluation of chemical and radiological detriment in order to propose an harmonization of criteria used in risk management.

II. Objectives for the reporting period:

Comparative analysis of methods of carcinogenic risk evaluation for non ferrous metals and ionizing radiations by examining animal carcinogenesis data.

III. Progress achieved:

1. Method :

In contrast to ionizing radiation, chemical carcinogenic risk cannot be assessed with unifying assumptions on the underlying carcinogenic process: considering the diversity of the mechanisms of action, progress in comparison of detriments may be achieved using a case by case approach focusing, at first, on directly acting chemical compounds (i.e. not requiring metabolic activation) for which carcinogenicity is highly suspected in humans. The aim of a study on non ferrous metals was thus to discuss procedures for making extrapolation to humans based on a detailed analysis of experimental results. Toxicological profiles were established for nickel and cadmium inorganic derivatives (speciation) from updated litterature. Animal data were standardized by making reference to the main recommendations of good laboratory practice (E.C. guidelines). Evaluation was performed using a scheme based on 3 critical parameters (Figure) : study duration which should extent to the life expectancy of the species under study since most of malignancies occur naturally in the last third of the life span ; sample size, the statistical significance of negative experiments being ascertained depending on sizes of exposed and control groups and on spontaneous tumour incidence ; survival rates of groups, since sufficient numbers of animals should be at risk during most of their life span (comparison of survival rates of exposed over controls and controls over historical series).

2. Results :

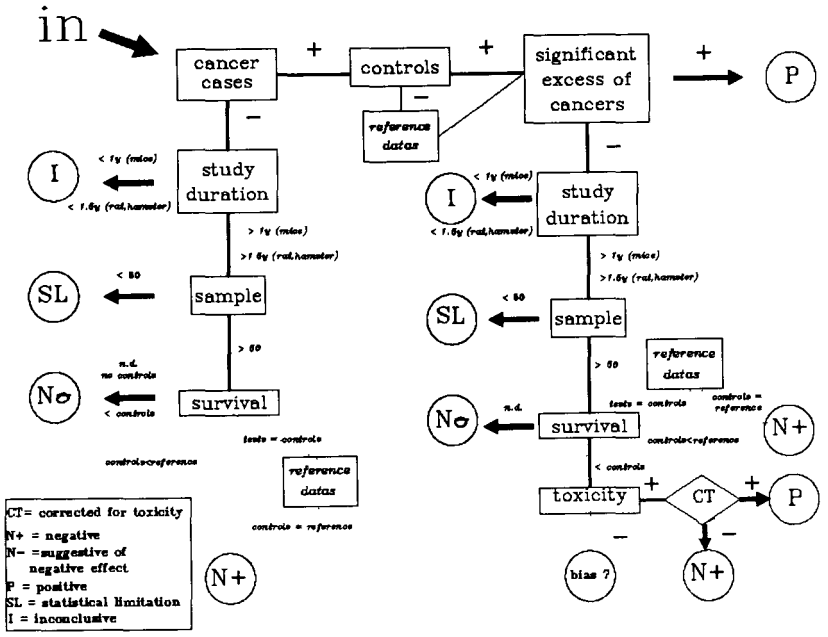
Experimental results were classified within 5 classes : Insufficient for interpretation (I = insufficient study duration), Statistically Limited (SL = negative experiment but numbers limited the power of the study), Presumably Negative (No = negative experiment but survival rate significantly reduced), Negative (N+ = negative experiment, survival rates reaching "acceptable levels"), Positive (P = significant excess of malignancies whatever the follow up duration, the sample sizes and survival rates).
For risk assessment purpose, extrapolation of these classified animal data was considered by adding scores taking into account the relevance of the administration routes : A = relevant human exposure routes, B = routes of unclear significance and C = non relevant routes.

Animal carcinogenic biassays relative to 9 nickel and 6 cadmium inorganic substances were reviewed : experiments on inhalation demonstrate lung carcinogenicity of nickel subsulfide and of cadmium oxide, cadmium sulfide, cadmium sulfate and cadmium chloride in rats ; for cadmium compounds, results were negative in mice and hamsters ; no carcinogenic effects were observed following oral administration. For routes of less or no relevance for human risk assessment, some results were clearly positive (subcutaneous, intramuscular injection ...) demonstrating cancers in situ but not in remote sites for nickel substances ; with respect to cadmium substances, systemic carcinogenic effects were noticed in testis and prostatic glands for soluble compounds. For both metals, numerous studies fail to demonstrate cadmium carcinogenicity, but methodologically acceptable negative ones are very limited in number. Accordingly strain dependent effects and dose effect relationship could not be thoroughly assessed.

3. Discussion :
Detailed analysis of animal experiments is then not confirming the unifying ion hypothesis proposed for assessing carcinogenic risk of metal inorganic compounds. For nickel, more results on relevant routes are needed ; for cadmium, additionnal studies on interspecies differences seem of great interest for a better understanding of extrapolation procedures.

Further work is undertaken for validation of the critical evaluation scheme : application to animal carcinogenesis by plutonium compounds and extension of the chemical data base to incomplete metal carcinogens (arsenical compounds).

Evaluation scheme of animal carcinogenesis



IV. Publications

R. MAXIMILIEN
Critical review of animal carcinogenesis by nickel and its inorganic compounds
Report EUR 12456

R. MAXIMILIEN, B. DERO
Critical review of animal carcinogenesis by cadmium and its inorganic compounds
Rapport CEA R-5516 (E)

TITLE OF THE PROJECT N°3

Assessment of the subjective dimension of the radiological detriment, in relation to sociological considerations.

HEAD OF PROJECT
J.BRENOT
IPSN/DPS, B.P. n°6,
92265 FONTENAY-AUX-ROSES, Cedex, FRANCE

SCIENTIFIC STAFF
J.BRENOT, M.H.BARNY

1. OBJECTIVES OF THE PROJECT

Analysis of public attitudes toward nuclear energy shows the considerable importance of subjective components. As far as risk is concerned, fear and anxiety are not the only explicative factors, and both ideology and culture must be taken into account. Within a comparative approach, are analyzed :

- the subjective dimensions of risk perception for activities, the nuclear one obviously but also others which are common in the everyday life,
- the difference between risk perceptions of specialists and of lay people which can explain the difficulty to establish a fruitful communication.

The final goal is to propose methods or at least recommendations for integrating this subjectivity in risk management and more precisely in risk communication.

2. OBJECTIVES OF THE REPORTING PERIOD : 1989

2.1 Study of risk perception among specialists and of lay people

A questionnaire has been prepared which addresses the following issues : the opinion on major hazards, the level of risk and its acceptability (as a willingness to do more for risk reduction) for various activities, and elicitation of criterias used by people when they manage the risk. The questionnaire has been proposed twice, firstly to 150 safety experts from various technical domains and secondly to a representative sample of lay people in the Bordeaux area (705 individuals). A first comparison has been made [1]. Search for more experts (specially in nuclear and chemical domains) and in-depth analysis will be pursued during 1990.

Results of the IFOP February 1987 survey about risk perception in the general public have been exposed in [2].

2.2 Synthesis of concepts and approaches used in risk perception studies.

A review of the risk perception discipline has been written. It details the definitions of risk, the place of risk perception in risk analysis, the various approaches and dimensions used to describe the individual and social phenomenon of risk perception, and the tentative modelizations of attitudes toward risk [3]. This review will serve as a basis of discussion for the next 39th UNSCEAR Session in Vienna 14-18 May 1990.

2.3 Chernobyl impacts on public opinion and governmental policy
A paper dealing with the french situation has been presented at the IIASA Conference "Policy responses to large accidents" Vienna 16-17 January 1989. It has been published in the Conference proceedings [4]. Perception of the information following a major accident is also emphasized in [3].

2.4 Realization of non directive interviews in the public to see the importance of risks in people minds
This task has been delayed for two reasons : a lack of time and up to now the suspended decision of COGEMA to realize with us risk perception studies around nuclear sites as it was planned. Nevertheless a compilation of survey data regarding to subjects of worry in the public has been performed.

3. OBJECTIVES FOR THE NEXT REPORTING PERIOD : 1990

3.1 Study of risk perception among specialists : achievement.
3.2 A comparative analysis of public opinion on risks and nuclear activities in EC countries is planned. Various european research teams will be involved during a workshop in Paris.
3.3 Radioactive wastes perception was studied some years ago. The seriousness of the problem for the public leads us to re-analyze the data obtained during the last years.

4. OTHER RESEARCH GROUP COLLABORATING ACTIVELY ON THIS PROJECT

Groupe de Recherche Energie, Technologie et Sureté (GRETS)
Direction des Etudes et Recherches - EDF
30, rue de Condé, 75006 PARIS

5. PUBLICATIONS

[1] **BARNY M.H., BRENOT J., PAGES J.P.** Perception des risques majeurs dans la population bordelaise et chez les experts. Note SEGP/LSEES, 90/02, Janvier 1990.
[2] **BASTIDE S., MOATTI J.P., PAGES J.P., FAGNANI F.** Risk perception and social acceptability of technologies : the french case. Risk Analysis, Vol. 9, N° 2, p.215-223 (1989)
[3] **BRENOT J.** The perception of risks, Note SEGP/LSEES 89/26, Oct.1989.
[4] **BARNY M.H., BONNEFOUS S., BRENOT J., PAGES J.P.** The evolution of french opinions about nuclear matters before and after Chernobyl. p.177-184 in : IIASA Conference "Policy Responses to Large Accidents", Laxenburg, 16-17 Jan.1989.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor.

Université Paul Sabatier
Division des Affaires Scientifiques
118, Route de Narbonne
F-31062 Toulouse Cedex

Contract no.: BI6-F-122-F
Sub Contract SC-003-F

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

Dr. M. Delpoux
Lab. de Botanique et Biogéographie
Université Paul Sabatier
39 Allée Jules Guesde
F-31062 Toulouse Cedex

Telephone number:

Title of the research contract:

Comparative genotoxicity of the principal environmental agents.

List of projects:

1. Comparative genotoxicity of the principal environmental agents.

Title of the project no.: 2

EVALUATION DU DETRIMENT OBJECTIF GLOBAL ET DE SON COUT EN RELATION AVEC LES CONSIDERATIONS ECONOMIQUES - RECHERCHES SUR LE VEGETAL

Head(s) of project:

M. DELPOUX

Scientific staff:

M. DELPOUX, H. DULIEU, M. CHAMEAUD, H. METIVIER, S. PONCY, L. TORRES, M. ING, P. MAGNES; S. MURATET, O. VERNET

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:

- étude de la forme de la réponse dose-effet génétique autour des sources de cobalt 60 successivement installées à la Section de Toxicologie expériemtale et de Cancérologie expérimentale de l'IPSN-DPS-SPE, CEA, de Bruyères le Chatel.
- étude des effets génétiques du radon avec des atmosphères diversment enrichies en radon grâce au dispositif construit à la COGEMA de Razès (Haute-Vienne, France).
- approfondissement de l'études des effets génétiques du SO^2.
- évaluation *in situ* des effets génétiques d'atmosphères diversement polluées.

III. Progress achieved:

1/ Etude de la forme de la réponse dose-effet génétique autour des sources de cobalt 60 installées dans la salle d'irradiation de la Section de Toxicologie expérimentale et de Cancérologie expérimentale de l'IPSN-DPS-SPE, CEA, de Bruyères le Chatel (France).

Des plants de Tabac portant le système génétique marqueur *a1+/a1 a2+/a2* ont été cultivés à différentes distances (1.87 m, 2.30 m, 2.80 m et 4.00 m) de trois sources de cobalt 60 caractérisées par des activités radioactives de respectivement 200 mCi, 20 mCi et 2 mCi, ceci déterminant par le jeu des différentes combinaisons [activité des sources-distance à la source] 12 valeurs de débits de dose comprises entre 46.5 mrd/h (soit 0.0465 cGy/h) et 0.126 mrd/h (soit 1.26×10^{-4} cGy/h).
Le tableau ci-dessous donne une vue d'ensemble sur les résultats globaux obtenus dans trois expériences réalisées autour des trois sources successivement installées dans la salle d'irradiation de Bruyère le Chatel :

Première expérience :

Activité de	Débits de dose		Taux moyens de	
la source			réversion ($x10^7$)	
(mCi)	cGy/h	mrd/h	/dose	/expér.
	0.046500	46.500	119	
200	0.032900	32.900	109	129
	0.022800	22.800	220	
	0.013700	13.700	67	
témoin	bruit de fond naturel		-	

Deuxième expérience :

Activité de	Débits de dose		Taux moyens de	
la source			réversion ($x10^7$)	
(mCi)	cGy/h	mrd/h	/dose	/expér.
	54.5×10^{-4}	5.450	84	
20	41.0×10^{-4}	4.100	94	95
	30.5×10^{-4}	3.050	190	
	17.5×10^{-4}	1.750	11	
témoin	bruit de fond naturel		-	

Troisième expérience :

Activité de la source	Débits de dose		Taux moyens de réversion ($x10^7$)	
(mCi)	cGy/h	mrd/h	/dose	/expér.
	$5.13x10^{-4}$	0.513	101	
2	$3.43x10^{-4}$	0.343	47	50
	$2.18x10^{-4}$	0.218	28	
	$1.26x10^{-4}$	0.126	23	
témoin	bruit de fond naturel		28	

On constate :

- une baisse des valeurs moyennes par expérience allant dans le même sens que celle des valeurs moyennes des débits de dose créés par les sources ;
- des relations moins évidentes entre débits de dose et valeurs moyennes des taux de mutation dans le cadre de chaque expérience.

Ces résultats seront à analyser en tenant compte des conditions dans lesquelles se sont effectuées les manipulations et des difficultés surgies à différentes reprises expliquant en particulier l'absence de valeurs témoins pour les taux de mutation dans les deuxième et troisième expériences. Un rapport détaillé sera fourni dès que toutes les données techniques et scientifiques seront disponibles.

2/ Etude des effets génétiques du radon avec des atmosphères diversment enrichies en radon grâce au dispositif construit à la COGEMA de Razès (Haute-Vienne, France).

Des plants de Tabac portant le système génétique marqueur *a1+/a1 a2+/a2* ont été cultivés dans des enceintes conçues pour la culture de ces végétaux dans des conditions contrôlées de température, d'humidité, de lumière et par ailleurs de teneur en radon des atmosphères entourant les plants. Trois lots ont été respectivement cultivés dans des atmosphères caractérisées par des activités radioactives de 1 100 000 bq/m^3, 400 000 bq/m^3 et 38 000 bq/m^3 soit respectivement 99 C.M.A. (Concentrations maximales admissibles), 36 C.M.A. et 3.5 C.M.A.

Le tableau ci-dessous donne une vue d'ensemble sur les résultats globaux obtenus. Les données détaillées qui ont permis de les établir, donneront lieu à une analyse statistique complète dans les premières semaines de février 1990.

Activité radioactive des atmosphères (bq/m^3)	C.M.A.	Taux de réversion ($x10^7$)
1 100 000	99.0	332
400 000	36.0	265
36 000	3.5	138

Dans des atmosphères beaucoup moins enrichies en radon que celles utilisées dans une précédente expérience, la mutagénicité du radon est confirmée mais encore une fois, il ne semble pas y avoir proportionnalité entre les valeurs des activités radioactives des atmosphères et celles des taux de réversion estimés à partir du repérage des variations somatiques sur les feuilles de Tabac.

3/ Les difficultés rencontrées pour réaliser les expériences décrites au point 1/ ont eu des répercussions sur l'ensemble des travaux ce qui nous a conduit à demander une prolongation du contrat de un an. Celle-ci a été obtenue. De ce fait les recherches relatives aux objectifs suivants seront effectuées pendant l'année 1990 :

- approfondissement de l'études des effets génétiques du SO^2.
- évaluation *in situ* des effets génétiques d'atmosphères diversement polluées.

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

- Laboratoire de Pathologie Pulmonaire Expérimentale de la COGEMA, Razès, Haute-Vienne, France
- Section de Toxicologie et Cancérologie Expérimentale de l'IPSN-DPS-SPE, CEA, Bruyères le Chatel, Essonne, France
- Laboratoire de Chimie-Energie-Environnement de l'Ecoloe Nationale de Chimie de l'Institut National Polytechnique de Toulouse, 118 Route de Narbonne, Toulouse, France
- Laboratoire de Mutagenèse de la Station d'Amélioration des Plantes de l'INRA, F-2014, Dijon Cedex, France
- Union Technique de l'Automobile, du Motocycle et du Cycle, Autodrome de Linas-Monthléry, Monthléry, France

V. Publications:

En préparation :

DELPOUX M. et M.A. DALEBROUX - Effets génétiques sur le système *a1+/a1 a2+/a2* du Tabac d'atmosphère diversement enrichies en radon.

DELPOUX M., M.A. DALEBROUX, H. DULIEU et A. LEONARD - Indicateurs biologiques et qualité de l'environnement

TORRES L., M. HAZIZA, S. MURATET O. VERNET et M. DELPOUX - Générateur d'atmosphères étalons pour l'étude de l'action de traces de polluants sur les végétaux.

MURATET S., O. VERNET, M. DELPOUX et M.A. DALEBROUX - Premières évaluations de l'impact génétique global de l'environnement à l'aide d'un dispositif mobile de culture *in situ*.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-F-298-D

Deutsches Krebsforschungszentrum
Institut für Radiologie
und Pathophysiologie
Im Neuenheimer Feld 280
D-6900 Heidelberg 1

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

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Inst. Radiologie & Pathophysiologie
Deutsches Krebsforschungszentrum
P.O Box 101949
D-6900 Heidelberg 1

Telephone number: (6221) 48 45 63

Title of the research contract:

Thorotrast - investigations to evaluate the long term effects caused by artificial radiation in man (Thorotrast patients) - Follow-up study.

List of projects:

1. Thorotrast - investigations to evaluate the long term effects caused by artificial radiation in man (Thorotrast patients) - follow-up study.

Title of the project no:

Thorotrast - investigations to evaluate the long term effects caused by artificial radiation in man (Thorotrast patients) - Thorotrast follow-up study.

Head(s) of project:

Prof.Dr. G. van Kaick, coordinator of the study
Prof.Dr.Dr.h.c. H. zur Hausen, chairman and Scientific Member of the German Cancer Research Center,
Prof.Dr. W.J. Lorenz, director of the Institute of Radiology and Pathophysiology (former: Institute of Nuclear Medicine)

Scientific staff:

Dr. H. Lührs, Dr. D. Liebermann

Statistical evaluation:

Dr. H. Wesch.

I. Objectives of the project:

The aim of the German Thorotrast study is to uncover the late effects of incorporated colloidal thoriumdioxide by epidemiological observation and clinical and biophysical examination of the patients; to compare the results of those of a corresponding control group and to assess the relationship between late effects and radiation dose. Furthermore we try to offer appropriate diagnostic and if possible therapeutic facilities and to give advise to the family physicians of the patients.

II. Objectives for the reporting period:

Research activities:
- Clinical, biochemical and radiological examinations of the Thorotrast patients and the control group followed by a report to the family physician
- Biophysical examinations to calculate the tissue dose due to the Thoriumdioxide deposits and their radioactive daughter products
- Identification of the causes of death of Thorotrast patients and members of the control group
- Statistical evaluation of the epidemiological, clinical and biophysical data.

III. Progress achieved:

The German Thorotrast Study is running since more than 20 years. The aim of the study was to uncover the late effects of incorporated colloidal thorium dioxide by epidemiological observation and clinical and biophysical examination and to compare the results with those of a corresponding control group. Furthermore it was part of the program to offer appropriate diagnostic and therapeutic facilities to the patients.

Patients and methods

Most of our patients were intravascularly injected with Thorotrast in the time between 1937 and 1947. Names and addresses of more than 5000 patients who had cerebral arteriography (70%) or arteriography of the upper and lower limbs (30%) with Thorotrast were obtained from records of different hospitals in the Federal Republic of Germany (van Kaick et al., 1984). The control group was selected from patients who were treated at the same time in the same hospital and whose surname began with the letter "B". The control patients were matched by sex and age disregarding the underlying diseases.

In 1968 when the study was started a large number of the Thorotrast patients had already died. The causes of death of those patients were clarified by hospital records, post mortem examinations etc.. Patients who died already in the first 3 years after Thorotrast injection were excluded from the evaluation to minimize the influence of the underlying diseases (van Kaick et al., 1983, 1986).

Deleting patients who died within the first 3 years, patients not traceable and patients not responding, the German Thorotrast Study comprises 2,326 Thorotrast patients and 1,890 control patients. 2,151 Thorotrast patients and 1,493 control patients have died up to 1989 (Table I).

The group of the examined patients is followed up every 2 years by out-patient-examination including biophysical measurements, radiological and clinical examination. Currently we are following up the remained 175 Thorotrast and 397 control patients.

Results and discussion

The final fate of the Thorotrast patients is the most important parameter for the calculation of the Thorotrast late effects. In the following we discuss 3 groups of diseases leading to death:

1. Diseases with high excess rate in the group of Thorotrast patients;
2. Diseases with possible excess rate;
3. Diseases without apparent excess rate.

1. Diseases with high excess rate

A significant excess rate was observed in malignant liver tumors, liver cirrhosis, myeloid leukaemias and bone marrow failures (Table II). A patient was only one time listed with his most important disease. Therefore we have put in brackets the neoplasia occured in combination with another disease leading to death.

The relative risk of malignant liver tumors for all Thorotrast patients is about 200. The latency period of malignant liver

tumors ranged from 16 to more than 45 years. 65% of the tumors were classified as carcinomas and 35% as hemangiosarcomas. Cholangiocellular carcinomas were predominant compared to the hepatocellular carcinomas. In 5 patients sarcoma and carcinoma simultaneously manifested in one liver. Cirrhosis was present in about 30% of liver tumor patients and in about 10% of the non liver tumor patients.

Table I German Thorotrast Study - Evaluated Patients

Patients' Status	Thorotrast	Control
Examined	899	662
Still living	175	397
Deceased	724	265
Not examined	1427	1228
Total	2326	1890

Table II
German Thorotrast Study - Diseases with High Excess Rate

Status '89 Cause of Death	Thorotrast n=2,326		Control n=1,890
Liver cancer	405	(17.4)	2 (0.11%)
Liver cirrhosis	186 [+171]	(15.35%)	47 [+3](2.65%)
Myeloid leukaemia	36 [+3]	(1.68%)	4 (0.21%)
Bone marrow failure	29	(1.25%)	4 (0.21%)

[] Additional cases with another disease leading to death

The cumulative rate of malignant liver tumors and leukaemias (Figure 1) was calculated with the sum-limit method by putting each case of liver cancer in relation to the number of individuals still at risk; this fraction is summed up year by year. The advantage of this procedure is that the effect of competing risks especially in the old aged patients can be minimized. The frequency of malignant liver tumors in male patients is significantly higher than in female patients. However, there was no difference between male and female patients with regard to age at injection, mean volume of injected Thorotrast and exposure time; so we must state that males are more sensitive than females to Thorotrast-induced liver cancer.
About 6% of our liver cancer patients suffered from a second neoplastic disease (Table III).

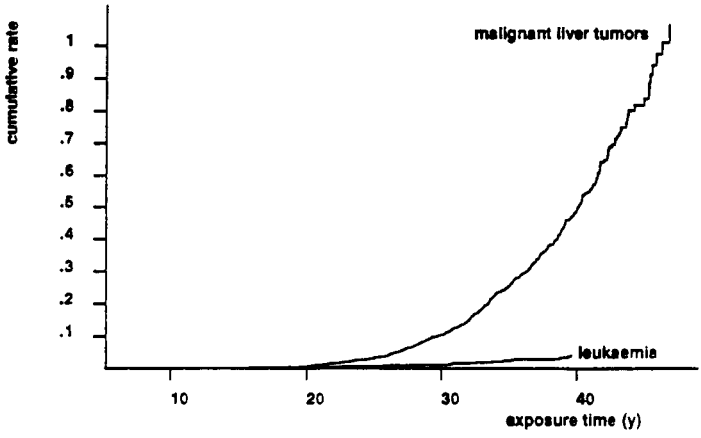

Figure 1
Cumulative rate (sum-limit method) of malignant liver tumors and leukaemias in all Thorotrast patients.

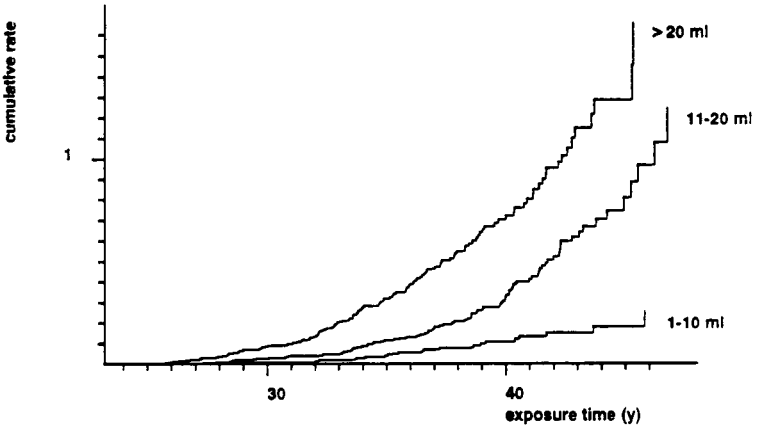

Figure 2
Cumulative rate (sum-limit method) of malignant liver tumors in Thorotrast patients who had whole body counting and whose area of paravascular deposits was less than 10 cm^2 (n=661). Three cohorts were formed according to the volume of intravascular injected Thorotrast.
a) 1-10 ml (n=159, 0.11 Gy/y) b) 11-20 ml (n=269, 0.18 Gy/y)
c) >20 ml (n=233, 0.27 Gy/y).

Table III
Liver Cancer Combined with other Neoplastic Diseases (n=25)

Second liver cancer	5
Ca. extra hepat. bile duct	3
Myeloid leukaemia	3
Plasmacytoma	2
Non-Hodgkin Lymphoma	1
Osteosarcoma	1
Lung cancer	2
Ca. G.I. tract	4
Other cancers	5

The induction of malignant liver tumors is caused by the chronic irradiation and not by the foreign body effect (Wesch et al., 1983). For the calculation of the relationship between dose rate and effect (Kaul et al., 1978) three cohorts were formed being injected with 1-10 ml, 11-20 ml, and more than 20 ml Thorotrast (Figure 2). The correlation between the dose rate to the liver expressed by the mean volume of administered Thorotrast and the cumulative rate of malignant liver tumors is quite evident.
Four cohorts were formed to study the frequency of liver tumors as a function of the age at injection (1-14; 15-29; 30-44, 45-59 years). The cumulative rate of liver tumors shows the same pattern for all cohorts (Figure 3). In each age group a marked increase in the liver tumor rate occurs 30 to 40 years after injection. The curve of the oldest cohort can be seen only in the beginning, as these patients cannot reach the exposure time and the accumulative dose, which are both necessary for the full induction of the malignant liver tumors.

Figure 4 shows the risk estimates for malignant liver tumors in the German Thorotrast Study. The calculation was done for all patients that had either whole body counting or the injected Thorotrast volume recorded in the patient file with the following assumptions:
1. Patients who have died within the first 15 years of exposure were excluded from the evaluation as they are not at risk for malignant liver tumors.
2. The dose delivered during the last 10 years before clinical manifestation of the malignant liver tumors will be looked upon as "wasted" dose, because the tumor already exists growing from microscopical to clinical dimensions! The calculation was done for each patient separately! Thorotrast uptake of 59% was assumed for the 1.8 kg liver of reference man and the 1.4 kg liver of reference woman. Only the alpha-particle energy that escaped from the Thorotrast aggregates was used to calculate the dose (Kaul and Noffz, 1978). The total risk was calculated for each year by taking the cumulative number of malignant liver tumors up to that year as the numerator and the accumulative dose of all patients up to 10 years before that year as the denominator. Those figures were plotted by the years after injection. There is a clear difference between the risk for male and fe-

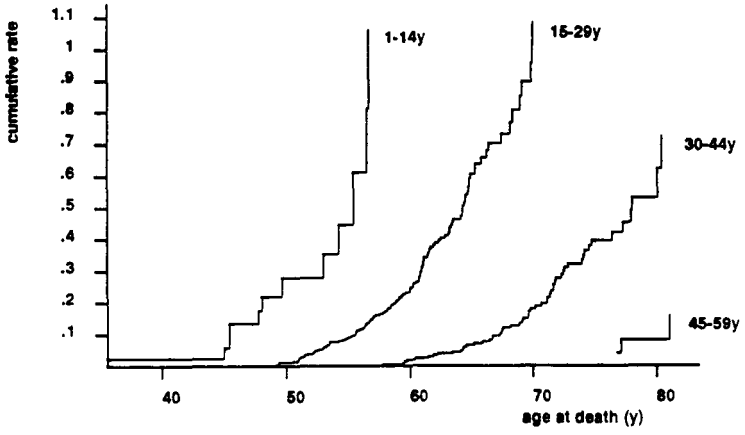

Figure 3
Cumulative rate (sum-limit method) of malignant liver tumors in Thorotrast patients of the examined group. Four cohorts were formed according to the age at injection:
a) 1-14 years b) 15-29 years c) 30-44 years d) 45-59 years.

Figure 4
Risk estimates for malignant liver tumors (MTL) correlated to the years of exposure separated in male and female patients. Patients of the examined group were combined with patients of the non examined group whose volumes of injected Thorotrast were documented (calculation see text).

male patients, which comes up after 40 years to 500 and 300 malignant liver tumors per 10^4 person Gy, respectively. In the Thorotrast patients with liver cancer, the 3 lowest doses at 10 years before diagnosis were 1.88, 2.65 and 2.71 Gy in female and 1.98, 2.06 and 2.30 Gy in male patients.

Myeloproliferative diseases (n=37) were observed about ten times more often in the Thorotrast group as compared to the control group. The majority were classified as acute myeloid leukaemias (25 cases); the reminder were described as erythroleukaemia (3 cases), monocytic leukaemia (5 cases) and chronic myeloid leukaemia (4 cases). The relatively high number of erythroleukaemia, which was also described in the Japanese Thorotrast study is remarkable (Hatakeyama et al., 1988). The shortest latency period in a leukaemia case was 5 years. A close correlation between dose rate in the bone marrow and frequency of leukaemias could not yet be proved. The 3 smallest accumulative doses to the red bone marrow at time of death in myeloid leukaemia patients were 0.23, 0.69, and 0.73 Gy.
The term bone marrow failure was used collectively for aplastic anaemia, agranulocytosis and thrombocytopenia. It is well known that these diseases may be caused by a variety of drugs, however, they are clearly more frequent in the group of Thorotrast patients.

2. Diseases with a probable excess rate
An excess rate of some neoplastic diseases in the Thorotrast group is possible (Table IV). However, we have to keep in mind, that twice the number of control patients are still alive so that these figures may change in the following years.
The last years have shown an increase of carcinomas of the extrahepatic bile ducts including carcinoma of the gall bladder. Daughter products in the bile and irradiation of the gall bladder wall by thorium aggregates in the surrounding liver could be the source of irradiation.
The excess rate of oesophageal and pancreatic cancer is difficult to explain. The excess of laryngeal cancer, however, might be related to the exhaled thoron.
There is a constant small excess of the Non-Hodgkin lymphoma during the last years. The same is true with regard to bone sarcomas; these 4 patients were injected at the end of their skeletal growth phase and had long exposure times of more than 30 years.
According to our results induction of plasmacytomas by internal irradiation can be possible.
Malignant mesotheliomas of the pleura and the peritoneum occured only in the Thorotrast group and not in the control group. The diagnoses of these patients are histologically confirmed. Of special interest are the peritoneal mesotheliomas arising from the peritoneum surrounding liver and spleen. The tumors could be explained by the fact that the adjacent layer of peritoneal cells can be reached by the alpha particles emitted from the thorium aggregates of liver and spleen. These tumors appeared after a long latency period of more than 30 years.

Table IV
German Thorotrast Study - Diseases with Possible Excess

Status '89 Cause of death	Thorotrast n=2,326	Control n=1,890
Ca ext. bil. ducts	16 [+2] (0.69%)	3 (0.16%)
Ca. gallbladder	11 [+1] (0.47%)	3 (0.16%9
Ca. pancreas	18 (0.77%)	5 (0.26%)
Ca. esophagus	7 [+1] (0.34%)	1 (0.05%)
Ca. larynx	6 [+1] (0.30%)	1 [+1](0.11%)
Non Hodgkin lymphoma	14 [+1] (0.64%)	4 (0.21%)
Bone sarcoma	4 (0.17%)	1 (0.05%)
Plasmocytoma	7 [+2] (0.39%)	1 (0.05%)
Mal. Mesothelioma		
Pleural	4 (0.17%)	0
Peritoneal	2 (0.09%)	0

[] Additional cases with another disease leading to death

A problem which needs a special comment are the paravascular deposits. In the group of the examined patients we detected 256 paravascular deposits of very different extension. 146 patients suffered from late effects after more than 15 years of latency: paralyses of nerves, chronic aches, and inflammation were most frequent followed by obturation of veins, abscesses, chronic fistula, narrowing of ureter, trachea and esophagus as well as rupture of arteries. Tumors close to the deposits, however, are extremely rare. Only one sarcoma of the soft tissue was observed which was located at the edge of a large Thorotrast granuloma with chronic inflammation and fistula of the upper thigh. In two other cases a causal connection to the thorium deposits is possible but uncertain.

3. Diseases without apparent excess rate
Two subgroups of organs have to be considered: (a) organs, which are exposed to a small dose rate (Table V), and (b) organs which are exposed to an extremely low dose rate (Table VI), at least the dose arising from the daughters in the blood stream (Dudley, 1967).

Ad 1: There is no excess rate with regard to chronic lymphatic leukaemias. One case of acute lymphatic leukaemia should be mentioned.
Morbus Hodgkin is found equally distributed in the Thorotrast and in the control group.
An important result of the study is the similar number of lung cancer in both groups, though the bronchi are exposed to chronic alpha irradiation by the exhaled Thoron. Hornik et al. (1989) reevaluated the calculations of the dose to the bronchi. The results give an explanation of the fact that up to now there is no excess of bronchogenic carcinomas in the Thorotrast group.

Table V
German Thorotrast Study - Diseases without Apparent Excess

Status '89 Cause of death	Thorotrast n=2,326	Control n=1,890
Chron. lymph. leukaemia	2 (0.08%)	3 (0.16%)
Acute lymph. leukaemia	1 (0.04%)	0
M. Hodgkin	3 (0.13%)	2 (0.11%)
Lung cancer	49 [+2] (2,19%)	48 (2.54%)
Kidney cancer	6 [+2] (0.34%)	5 (0.26%)
Urin. bladder Ca.	5 [+3] (0.34%)	4 [+1] (0.27%)
Adrenal Cancer	2 (0.086%)	1 (0.052%)

[] Additional cases with another disease leading to death

The kidneys are exposed to a mean dose of 40 mGy/year. No increase in renal cancer could be observed. Cancer of the urinary bladder and the adrenals (which are part of the reticuloendothelial system) have the same frequency in both groups.

Ad 2: The other neoplastic diseases are comming from organs at extremely low dose from the incorportated Thorotrast. Therefore no excess rate can be expected as for example: tumors of the G.I.-tract, prostate, breast, ovary and brain. The figures of Table VI demonstrate the high quality of the control group which was handled with the same engagement compared to the Thorotrast patients.

Table VI
German Thorotrast Study - Diseases without Apparent Excess

Status '89 Cause of death	Thorotrast n=2,326	Control n=1,890
Stomach cancer	30 [+3] (1.42%)	44 (2.33%)
Colon cancer	10 [+3] (0.56%)	17 (0.90%)
Rectal cancer	8 [+3] (0.47%)	13 [+1] (0.69%)
Prostate cancer	18 (0.77%)	13 (0.69%)
Breast cancer	8 (0.34%)	17 (0.90%)
Ovary cancer	5 (0.22%)	4 (0.21%)
Brain cancer	18 (0.77%)	11 (0.58%)

[] Additional cases with another disease leading to death

Life spans of Thorotrast patients

During the past years there is a constant trend that Thorotrast patients die earlier compared to patients of the control group (Fukutomi et al., 1988). This phenomenon is depending on the

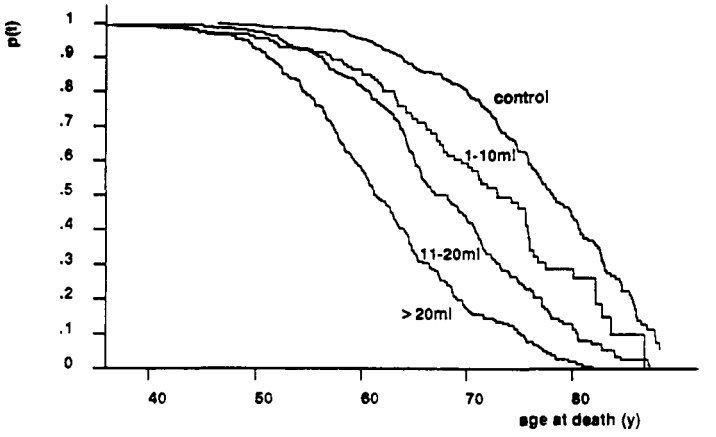

Figure 5
Kaplan-Meier survival curves of all control persons of the examined group and Thorotrast patients (same as in Figure 2) injected with different amounts of Thorotrast. The standard deviations are omitted to keep the graph readable.

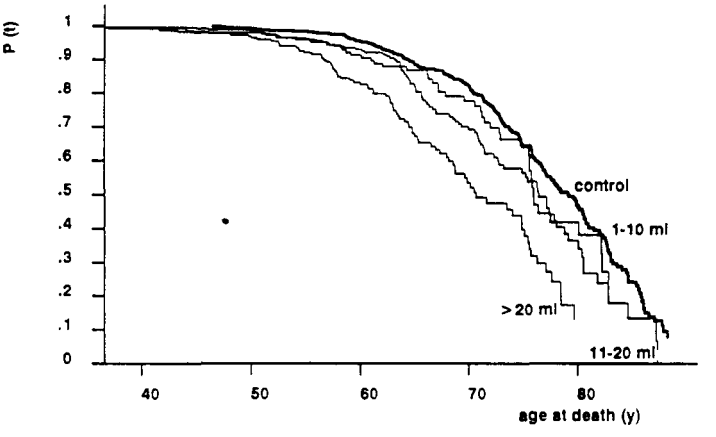

Figure 6
Kaplan-Meier survival curves of the same controls and Thorotrast patients as in Figure 5 excluding Thorotrast specific diseases.

amount of Thorotrast injected (Figure 5). However, as the frequency of malignant liver tumors increases with the incorporated volume of Thorotrast, this result could be caused by the high numbers of liver cancer.
Excluding in the analysis those patients who have died by Thorotrast specific diseases (liver cancer or leukaemias or liver cirrhosis) we have similar results (Figure 6) in dose rate dependent life-shortening as in Figure 5.
So we can state that there is a Thorotrast volume dependent influence to the age at death with regard to neoplastic and non-neoplastic diseases, but we are not yet able to give a fundamental explanation

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V. Publications:

Dalheimer, A.R., Kaul, A., Sid, M.D., Riedel, D.: Investigation on the Effect of Incorporated Radioactive and nonradioactive Particles and their Synergism by Long-Term Animal Studies - Physicochemical and Biokinetic Properties of Zirconium Dioxide Colloids. In: Gössner, W., Gerber, G.B., Hagen, H., Luz, A.(Edts.): The Radiobiology of Radium and Thorotrast, 167-171. Urban & Schwarzenberg, München - Wien - Baltimore 1986.

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RADIATION PROTECTION PROGRAMME
Final Report

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)]:

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Title of the research contract:

Development of fundamental data for radiation protection.

List of projects:

1. Development of fundamental data for radiation protection.

Title of the project no.: 1

Head(s) of project:

Dr H Smith

Scientific staff:

I. Objectives of the project:

Evaluating the biological basis of radiation-induced effects and the metabolism and dosimetry of incorporated radionuclides are integral parts of the recommendations in radiation protection and their implementation. In this respect, the Main Commission requires an input from its Committees, aided by task groups, who critically evaluate all available data at the request of the Main Commission. Reports prepared by committees are considered by the Main Commission and, if adopted, are published by the Secretariat. The head of the project plays a coordinating role in this process.

II. Objectives for the reporting period:

To review and adopt reports already prepared for the Main Commission, to continue with the development of additional fundamental data and to identify and discuss topics relevant to the revision of the ICRP Basic Recommendations.

III. Progress achieved:

1. INTRODUCTION

Task Groups are the functional units by which the Main Commission of the ICRP addresses a wide variety of issues in radiological protection. These Task Groups are composed of specialists in a particular area of science or medicine who are not necessarily members of the Committees of the ICRP. Reports from the Task Groups are peer reviewed by the appropriate Committee and returned to the Task Group for modifying if necessary. The final draft is submitted to the Main Commission and if considered suitable for adoption, will appear as an edited version in the Annals of the ICRP as a recommendation from the Main Commission.

During the period 1985 - 1990 several reports have been published and other reports are in the process of preparation by Task Groups. Much of the data contained in these reports will be used in the preparation of new basic recommendations in radiological protection that will supersede the 1977 recommendations in ICRP Publication 26.

2. PUBLISHED REPORTS

ICRP Publication 45

QUANTITATIVE BASES FOR DEVELOPING A UNIFIED INDEX OF HARM

In 1977, the International Commission on Radiological Protection issued ICRP Publication 27, Problems Involved in Developing an Index of Harm. That report discussed the difficulties of making an appropriate comparison of radiation and other effects and suggested a quantitative index to take account of the length of life or full activity lost as a result of occupational causes. This new report substantially extends the scope of ICRP Publication 27, by including new data on occupational accident risks as well as a consideration of radiation-induced non-fatal cancers, non-stochastic effects and hereditary detriment. An appendix discussing assessments of detriment in sections of the general public due to exposure to ionising radiation is also included.

ICRP Publication 46

RADIATION PROTECTION PRINCIPLES FOR THE DISPOSAL OF SOLID RADIOACTIVE WASTE

The application of the system of dose limitation recommended by the ICRP to the disposal of solid radioactive waste involves consideration of two special factors: the probabilistic nature of future exposures and the long timescales involved. This report takes into account the variable probabilities by generalising from a system of dose limitation to a system of risk limitation and showing how this can be applied. The long timescale involved in solid radioactive waste disposal are discussed in terms of truncation of calculations of collective dose equivalent, the weight to be assigned to future detriments and the use of utility values in quantifying the significance of exposures with a low probability of occurrence. The report also includes a discussion of exemption rules to be used in deciding whether a waste stream should be subject to control and of operational aspects of solid radioactive waste disposal.

ICRP Publication 47

RADIATION PROTECTION OF WORKERS IN MINES

This report describes the principles and applications of methods by which radiation hazards may be controlled in mines. Although primarily

directed to the uranium mining industry, the information presented in this report may be applied in varying degree to all mines.

Miners are exposed to airborne radon, thoron, and their short-lived decay products, to ore dust and, in some mines (particularly uranium and thorium mines) to external gamma and beta radiations.

The recognition of the role of radon and its decay products in the induction of lung cancer in uranium miners led to exposure limitation guides. The establishment of a limit for the inhalation of radon and its decay products has encountered substantial problems during the last 30 years. The Commission has recommended limits which have evolved during recent years with the improvement of knowledge. For the purpose of radiation protection in mines, the Commission recommended in ICRP Publication 24, a limit for the annual average concentration of ^{222}Rn in equilibrium with its short-lived decay products.

The general principles of monitoring for radiation protection of workers have been established by the Commission in ICRP Publication 35. These principles provide the basis for establishing monitoring programmes which contribute to meeting the objectives of the Commission's recommendations both effectively and economically. The main functions and the various forms of monitoring are analysed with particular attention given to the design of a monitoring programme and the interpretation of results for external radiation and for surface, air, skin and internal contamination. The requirements for both ambient and personnel monitoring in mines should conform with these general principles.

The scope of this report excludes the treatment of the other hazards, mechanical and toxic, that are characteristic of all mining operations. However, the protective measures developed to control radiation exposures may influence the situation regarding other hazards. The high ventilation rates usually needed to control the concentration of radon decay products are likely to be more than sufficient for the dilution of toxic air contaminants, but will also tend to dry surfaces in a normally wet mine, thereby enhancing the dispersion of dust, possibly containing silica or other harmful agents in addition to radioactive nuclides, unless appropriate measures are taken.

ICRP Publication 48

THE METABOLISM OF PLUTONIUM AND RELATED ELEMENTS

This report reviews, updates and extends the information on the metabolism of plutonium, neptunium and the trivalent actinides, previously reviewed in ICRP Publication 19, with special reference to the absorption from the gastrointestinal tract, the retention times in liver and skeleton, the influence of bone remodelling on the microdistribution within the skeleton and the relation of actinide metabolism to that of other radionuclides. Considerable emphasis has been placed on the new, or expanded, human data now available. The report is intended to complement, rather than replace ICRP Publication 19. The report considers not only occupational exposure to plutonium and other actinides, but also the possible exposure of the general population, including infants and fetuses, in the event of releases of these elements from nuclear installations.

The suggested changes in the metabolic parameters have some implications for the values of ALI given in ICRP Publication 30. For isotopes of plutonium of long physical half-life, the ALI for the ingestion of unknown or mixed compounds will need to be decreased by almost a factor of ten. However, the ALIs for ingestion of long-lived isotopes of plutonium as nitrates or oxides, as well as those for the ingestion of isotopes of short physical half-life, where the dose to the intestinal mucosa is of overriding importance, will need little change. Similarly, the effect on

ALIs for inhaled isotopes of plutonium will also be small, since, in this situation, transfer from gastro-intestinal tract to blood is very small in comparison with transfer from lung to blood. Values of ALI for ingested americium, curium and californium will be much less affected, since the proposed change in f_1 values is only a factor of two. For ^{239}Np, the ALI by ingestion will need to be increased by about an order of magnitude.

ICRP Publication 49

DEVELOPMENTAL EFFECTS OF IRRADIATION ON THE BRAIN OF THE EMBRYO AND FETUS

The developing mammalian brain is substantially more susceptible to teratogenic insults than most other embryonic and fetal structures; presumably this reflects its architectural complexity, its long developmental (and hence sensitive) period, the vulnerability of the undifferentiated neural cell as compared with the developed neuron, the fact that neuronal function is contingent upon position and that neuronal cells do not proliferate in the cortex, but must migrate there over substantial distances, and the inability of the brain to replace lost neurons. Clinical investigations of the effects of post-fertilisation pelvic irradiation have demonstrated a damaging effect of such exposure upon the development of the embryonic and fetal brain and an increased prevalence of severe mental retardation has been seen in children who were prenatally exposed to the atomic bombing of Hiroshima and Nagasaki.

A recent reassessment of these latter data has suggested that the highest risk of forebrain damage occurred in the 8th through the 15th week after fertilisation. This corresponds to the time when the most rapid proliferation of neuronal elements and substantial migration of neurons to the neocortex from their proliferative zones near the cerebral ventricles are occurring.

The objectives of this report are to give a critical evaluation of the data relating to radiation-induced effects on the central nervous system, especially radiation-induced mental retardation, assessing the gestational age at risk and the quantitative risk at low doses; to analyse these effects in the light of what is known about cell survival, proliferation, repopulation and differentiation in the development of the fetal brain; and to identify the needs for future research.

ICRP Publication 50

LUNG CANCER RISK FROM INDOOR EXPOSURES TO RADON DAUGHTERS

This report relates specifically to the risk associated with indoor exposure, particularly that resulting from inhaled ^{222}Rn-daughters. This type of exposure contributes the largest fraction of the natural radiation dose to populations living in the temperate regions of the world. A major part of this indoor exposure depends strongly on social factors and individual living habits. For this controllable fraction of natural radiation exposure, the principles for limiting exposure of the public to natural sources of radiation which have been recommended by the Commission in ICRP Publication 39 should be observed. In this context, the results presented in this report may provide guidance to the competent national authorities for the setting of action levels in existing houses and for the optimisation procedure in the planning of future houses.

The risk analysis described should be regarded as an attempt to quantify the possible lung cancer risk associated with the natural exposure to radon daughters. The results indicate that, although it is considered that cigarette smoking remains as the major cause of lung cancer in many countries, a significant fraction of the observed lung cancer frequency in populations may be attributed to the indoor exposure to ^{222}Rn daughters.

Further investigations are necessary to confirm, or to improve, this risk assessment. In several countries, epidemiological pilot studies on lung cancer among population groups exposed to enhanced ^{222}Rn levels in houses have been started or are planned. The main problems of such studies concern the retrospective estimation of the exposure and the competing influence of other occupational and environmental pollutants, and particularly of smoking.

ICRP Publication 51

DATA FOR USE IN PROTECTION AGAINST EXTERNAL RADIATION

ICRP Publication 21 contained data for protection against ionising radiation from external sources. The data were of two kinds, one on the relationships between various radiation quantities, the other on the shielding properties of various materials. Some revised shielding data are now in ICRP Publication 33 which deals with external sources used in medicine: the other kind of data is considered here, but is not intended to apply to the irradiation of patients.

The main reason for this revision is to adapt the data and the underlying approach to the Recommendations of the International Commission on Radiological Protection in ICRP Publication 26 and later relevant modifications. It is also necessary to take account of the report on radiation quantities and units from the International Commission on Radiation Units and Measurements and a subsequent report on the determination of dose equivalents. The third reason is to improve the original publication by amending or replacing some data.

This report provides information and advice on the relationships between radiometric, dosimetric and radiation protection quantities for external radiation and on their practical utilisation. Because of the large amount of data available for some types of radiation and the high rate at which they are being generated, change and refinement are inevitable, and responsible authorities should not hesitate to use more appropriate data as they become available.

ICRP Publication 52

PROTECTION OF THE PATIENT IN NUCLEAR MEDICINE

Because of its special relationship with the International Society of Radiology, and because of its contacts with the medical profession generally, the International Commission on Radiological Protection has traditionally provided detailed guidance on radiation protection in medicine. The Commission's most recent recommendations concerning nuclear medicine appeared as ICRP Publication 17 entitled "Protection of the Patient in Radionuclide Investigations". That report dealt with protection of the patient with respect to some of the principal diagnostic applications current in 1971. Since then, the range of available radiopharmaceuticals has widened, the instruments for the collection of data and the processing of information have become more complex, and more data have become available about pharmacokinetics.

This report is concerned with exposures of patients resulting from the administration of radiopharmaceuticals for diagnostic, therapeutic and research purposes. It does not discuss the irradiation of the staff involved in the administration of radiopharmaceuticals to patients; this topic is the subject of ICRP Publication 53. However, some recommendations on protection of the patient's family are included.

The establishment of measures for patient protection, as recommended in this report, should in no way impede the continuing development of nuclear medicine. On the contrary, such measures should contribute to higher standards of clinical practice in nuclear medicine. However, where

because of limitation of resources both in material and personnel the recommendations in this document cannot yet be fully met, patients should not be denied the benefit of necessary diagnostic examinations or treatment, provided that these are justified.

The aims of the report are:

(i) to advise nuclear medicine physicians, radiologists, medical physicists, technologists and others concerned with the practice of nuclear medicine on the factors that influence absorbed doses (and hence radiation risks) to patients from different types of nuclear medicine examinations;

(ii) to indicate ways by which these risks can be minimised without detriment to intended medical benefits.

Together with ICRP Publications 34 and 35 it completes a series of three reports dealing with protection of the patient exposed to ionising radiation in medicine.

ICRP Publication 53

RADIATION DOSE TO PATIENTS FROM RADIOPHARMACEUTICALS

The administration of radioactive substances to humans for diagnosis, therapy or research purposes is a well-established and developing branch of medical practice, and is, in most countries, recognised as a medical speciality under the name of nuclear medicine. New methods and new radiopharmaceuticals are continually being introduced. With regard to dose calculations, important basic material has been published in several ICRP Publications as well as in reports from the International Commission on Radiation Units and Measurements. Several absorbed dose catalogues and collections of published values have also appeared. Of special importance is the work of the Medical Internal Radiation Dose (MIRD) Committee of the United States Society of Nuclear Medicine and the dosimetry work performed at the Oak Ridge National Laboratory, Tennessee, USA. Extensive use has been made of the information and material available from these sources.

This report presents biokinetic models and best estimates of biokinetic data for some 120 individual radiopharmaceuticals, giving estimated absorbed doses, including the range of variation to be expected in pathological states, for adults, children and the fetus. Absorbed dose estimates are needed in clinical diagnostic work for judging the risk associated with the use of special radiopharmaceuticals, both for comparison with the possible benefit of the investigation and to help in giving adequate information to the patient. These estimates provide guidance to ethics committees having to decide upon research projects involving the use of radioactive substances on volunteers who receive no individual benefit from the study. This report supplements current and forthcoming ICRP reports, in particular ICRP Publication 52, Protection of the Patient in Nuclear Medicine.

ICRP Publication 54

INDIVIDUAL MONITORING FOR INTAKES OF RADIONUCLIDES BY WORKERS: DESIGN AND INTERPRETATION

This report provides the Commission's recommendations for the design of individual monitoring programmes and the interpretation of results of measurements of intakes of radionuclides by radiation workers. It follows the Commission's general principles of monitoring for radiation protection of workers and should be used in conjunction with those principles. The main objective of the document is to give specific guidance on the design of monitoring programmes, the calculation of derived reference levels and the interpretation of monitoring results. Since models are required to provide a link between the measured quantity and the appropriate limits or

reference levels, the document also gives general guidance on the models used. Finally, specific values of derived reference levels are given for radionuclides chosen for their potential importance in occupational exposure; these levels have been calculated for intake by inhalation only since this is the most likely route of intake. The Commission wishes to emphasise that the parameters used and the reference levels calculated in this report are appropriate for occupationally exposed adults only and that they are not intended to be used to evaluate exposure of members of the general public.

For several reasons, including improved data for the metabolic and dosimetric models, the values of ALI now recommended by the Commission differ from those implied by the earlier maximum permissible concentrations in air and water. One of the major changes of principle is the use of a limit on the effective dose equivalent and a non-stochastic limit on dose equivalent to any organ. The application of the annual limits on effective dose equivalent or, in cases when it is more restrictive, the non-stochastic limit in any organ leads directly to the ALI.

For most radionuclides, these changes have little effect on programmes of individual monitoring, apart from the way in which the results are evaluated. However, for a few materials of long effective half-life in the body and low values of ALI, the effect has been greater. Over the years it has become the practice to control the short term intake by monitoring in the work place, with individual monitoring programmes being aimed at compliance with the maximum permissible body burden. The replacement of the maximum permissible body burden by the ALI has caused a change of emphasis. If the short term (1 year) control of intake is to be transferred from work place monitoring to individual monitoring, a considerable increase in sensitivity will be required in individual measurements of materials of long effective half-life. In practice, this increase is difficult to achieve and the balance between individual and work place monitoring may have to be reconsidered. Special consideration is given in this report to these long-lived radionuclides, notably the isotope of plutonium.

ICRP Publication 55

OPTIMIZATION AND DECISION MAKING IN RADIOLOGICAL PROTECTION

In 1984 the Main Commission of ICRP established a Task Group of Committee 4 to produce a report on methods for optimization of protection other than cost-benefit analysis. As the work of the task group progressed it became clear that it would be more useful to produce a report on the entire field of application of optimization, mainly to show how the various techniques, including cost-benefit analysis, could be applied appropriately to problems at different levels of complexity.

Given the initial assumption, which is both plausible and prudent, that there is some additional risk from any increment of dose above that from natural sources, the system of radiological protection has to include some elements of the kind here called the optimization of protection - the use of all reasonable procedures to reduce exposures. The techniques described here are all of an essentially practical nature and range from common sense to advanced but conventional quantitative techniques for aiding the process of making decisions. They take account of the fact that extraneous factors, often totally unconnected with the magnitude of the radiation doses, have also to be taken into account, and may sometimes overwhelm the radiological protection aspects of the final decision. The techniques of this report will often identify and clarify such situations.

The Commission hopes that the growing use of these techniques will improve the standards of protection in areas of work where this is

desirable, while not imposing unnecessary constraints in areas where protection is already adequate.

ICRP Publication 56

AGE DEPENDENT DOSES TO MEMBERS OF THE PUBLIC FROM INTAKE OF RADIONUCLIDES. PART 1

The Commission in ICRP Publication 30 recommended dosimetric models and biokinetic data of radionuclides for estimating committed organ dose factors and the effective dose equivalents for the occupationally exposed adults from intakes by inhalation and ingestion. These values were not intended to be applied to members of the public following releases of radioactive materials into the environment.

To provide dose factors for members of the public, considering age and the transfer of radionuclides to the developing fetus, it is necessary to understand how age dependent biokinetic data and age related changes in organ masses, form and spatial separation within the body, influence the behaviour of radionuclides and the dose after entry into the body from the environment by inhalation and ingestion pathways.

A Task Group was established to be responsible for developing age-dependent dose factors for members of the public and for the fetus. Initially, this task was limited to the incorporation of those radionuclides which might be released to the environment under normal operating conditions and in cases of accidents at nuclear sites, thus leading to radiation exposure of members of the public via ingestion. These studies will be continued and extended to include radionuclides of natural origin. Dose factors are calculated for inhalation of radionuclides until the ICRP Task Group on Respiratory Tract Models has published its report.

This report gives ingestion and inhalation dose factors for infants, children, and adults. Dosimetric data for the embryo and fetus will be presented in a later report, after dosimetric and biokinetic models for estimating corresponding dose factors have been further developed.

The report describes biokinetic data and the dosimetric models to be used for computation of age-dependent dose per unit intake by ingestion following the release of radionuclides in the environment. These age-dependent biokinetic data have also been used in the calculation of inhalation dose factors. Data are presented for radioisotopes of the following elements: hydrogen, carbon, strontium, zirconium, niobium, ruthenium, iodine, caesium, barium, cerium, plutonium, neptunium, and americium. Age-dependent doses from intakes of radiopharmaceuticals are given in ICRP Publication 53.

ICRP Publication 57

RADIOLOGICAL PROTECTION OF THE WORKER IN MEDICINE AND DENTISTRY

The International Commission on Radiological Protection has been functioning since 1928, when it was established by the Second International Congress of Radiology to give guidance on the safe use of radiation sources in medical radiology. As a result of rapid developments in the field of nuclear energy and natural radiation, the Commission has subsequently expanded its area of guidance to cover more widespread uses of radiation sources, while strengthening its traditional relationship with the medical field.

This report furthers this objective by providing advice on the radiological protection of the worker in medicine and dentistry. It is consistent with the basic recommendations of the Commission in ICRP Publication 26 and in subsequent statements and with other ICRP publications in the medical fields, notably ICRP Publications 33, 34, 44

and 52. It supersedes ICRP Publication 25.

In establishments for medical diagnosis, treatment and research, widespread use is made of ionising radiations from X-ray and other machines and from radionuclides. This report discusses the safety measures applicable to workers involved either directly or indirectly in such uses; in particular, it deals with the safety measures applicable to radiologists, radiation oncologists, nuclear medicine physicians, medical physicists, radiographers, scientists, technicians, radiopharmacists, engineers, nurses and others (such as cardiologists and orthopaedic surgeons), when their work involves exposure to radiation.

This report is directed particularly towards the managing authority in each hospital or medical establishment and to the workers involved in work with radiation at such establishments. However, the report is also drawn to the attention of the relevant statutory authorities, whether national, regional or local, that are responsible for the enforcement of safety standards and for establishing training standards for workers.

The report is also intended for those responsible for the planning and provision of medical and associated technical services, since safety can only be assured if adequate standards are incorporated in the initial planning and design stage of a facility and if there is proper provision of equipment and of adequately trained staff.

ICRP Publication 58

RBE FOR DETERMINISTIC EFFECTS

The evidence reviewed in this report clearly indicates that RBE values of high-LET radiations for deterministic (i.e. non-stochastic) effects depend on many factors and vary widely. The type of radiation and the type of tissue effect are of prime importance. In addition, RBE values generally increase with decreasing dose, dose per fraction and dose rate. Maximum values derived by extrapolation to very low doses are denoted RBE_m to distinguish them from RBE_M values derived for stochastic effects.

Values of RBE_m, with a single exception for kidney damage by 2.5 MeV neutrons, are lower than 10 and therefore are considerably smaller by a factor in the range of 2 to 5 than values of RBE_M for various types of stochastic effects. A similar conclusion also applies to the "equal effectiveness ratios" of α-particle and β-particle emitting radionuclides in the lung for deterministic effects compared with cancer induction.

RBE values for actual exposures to mixtures of high-LET and low-LET radiations can be derived using a mathematical formalism developed in the report. Application of RBE_m values will yield estimates of maximum values of equivalent doses and these should only be applied for planning medical interventions if the contribution from high-LET radiation is small. Because RBE_m values for deterministic effects are considerably smaller than the RBE_M values for many stochastic effects upon which the assessment of Q values has been based, the application of Q values in cases where deterministic effects are important would result in an overestimate of the contribution from high-LET radiation. The present Q factors are certainly adequate for the establishment of those ALI's which are determined by deterministic effects.

ICRP Publication 30 Part 4

LIMITS FOR INTAKES OF RADIONUCLIDES BY WORKERS: AN ADDENDUM

In 1979 the International Commission on Radiological Protection issued ICRP Publication 30; Limits for Intakes of Radionuclides by Workers. In 1981 the Commission established a Task Group on review, update and extend the information on the metabolism of plutonium, neptunium and trivalent actinides as previously reviewed in 1972 in ICRP Publication 19. Specific

attention was directed towards information regarding absorption from the gastrointestinal tract and the distribution between, and retention within, the skeleton and the liver. In 1986 the report of this Task Group was issued as ICRP Publication 48. Because Publication 30 had relied heavily upon ICRP Publication 19, the information presented in ICRP Publication 48 regarding the behaviour in the body of plutonium, neptunium, and the trivalent actinides has implications for the values of the secondary limits presented in ICRP Publication 30. In this addendum to ICRP Publication 30, secondary limits for isotopes of neptunium, plutonium, americium, curium, berkelium, californium, einsteinium, fermium, and mendelevium are presented, based on the recommendations of ICRP Publication 48.

Secondary limits are also presented for a member of isotopes of elements other than those discussed in ICRP Publication 48. For these radionuclides, nuclear decay data were not available at the time the calculations for the element were performed, but details were later tabulated in ICRP Publication 38. For this reason secondary limits are presented here for the first time for ^{82}Sr, ^{95m}Tc, ^{95}Tc, ^{116}Sb, ^{246}Pu and ^{250}Cm. With the exception of the latter two radionuclides, the metabolic models used in this calculation are those given in ICRP Publication 30. The data for ^{246}Pu and ^{250}Cm are presented with the revised secondary limits for plutonium and curium.

3. PROGRESS ON THE COMMISSION'S TASK GROUPS:

Task Group on Revision of the Basic Recommendations

Major progress was made on the main text during the 1989 Oxford meeting and the eighth draft will be considered by the Task Group responsible for drafting the new recommendations in January 1990. The ninth draft together with annexes will be widely circulated thereafter for comment.

There will be several chapters in the main text to cover the history of the Commission and its recommendations, and the scope of the new recommendations; the physical quantities used in radiological protection; biological aspects; the conceptual framework of radiological protection; the system of protection for normal situations and its practical implementation; the system of protection in pre-existing situations where remedial action may be needed and in accidents and emergencies and the practical implementation of the recommendations in emergency situations. Annexes on physical quantities used in radiological protection, biological effects and criteria for judging the significance of the effects of radiation will provide supporting material.

Task Group on Risk Estimates for Cancer and Genetic Effects

The report from the Task Group will rely heavily on the recent reviews by UNSCEAR and BEIR. The next draft will be submitted to Committee 1 at its 1990 meeting. In the meantime the Task Group responsible for the revision of the basic recommendations will incorporate the available information into the main text and its biological effects annex.

Nominal probability (risk) coefficients will be based upon an age specific multiplicative projection model and a reduction factor reflecting extrapolation from high dose, high dose rate to low dose, low dose rate conditions of exposure. Weighting factors to be taken into account will probably include the greater loss of life expectancy from leukaemia compared with other cancers (due to short latency); and a weighting for mortality versus morbidity will be considered for some cancers with high cure rates.

For genetic disorders, a probability coefficient which includes an

estimate of the multifactorial disorders will probably be included.

Thus total nominal probability (risk) coefficients several times higher than current ICRP risk coefficients will probably be used which may be applied separately to a population of all ages and a working population.

Task Group on Biological Basis for Dose Limitation to the Skin

The report of the Task Group is in its final stages and one more meeting is anticipated before presentation to the Main Commission for its adoption.

Radiation induced skin cancer could be considered to have a low mortality, about 2×10^{-4} Sv^{-1}. The cancer incidence has increased compared with previous estimates and risks are significantly higher in areas of skin exposed to ultraviolet radiation in light skinned races. However prevention of deterministic effects is still the overriding factor in radiological protection.

In order to prevent late detrimental cosmetic effects due to chronic dermal exposure an annual dose limit of 0.5 Sv per year could be recommended, the dose being evaluated at a depth of 300 - 500 μm. Early acute ulceration from small radioactive particles deposited on skin should be prevented if the dose to a small area, delivered over a few hours, is restricted to about 1 Sv at a depth of 100 - 150 μm.

Task Group on Respiratory Tract Models

The objective of the Task Group is to develop a comprehensive, scientifically justifiable model of the human respiratory tract for use in radiation protection for calculation of ALI's as well as for individual and population dose assessments. The dosimetric model will probably be based on a three compartment system; extra-thoracic (ET), tracheo-bronchiolar (TB) and the alveolar-interstitial (AI) with competitive mechanical and translocation clearance. Mechanical clearance rates will be assumed to apply to all materials and all individuals. The rate constants for translocation (absorption into blood) will be derived from experimental data obtained from humans and animals. Where such data are not available, default values will be recommended. The Task Group will probably propose values for model parameters applicable to the reference worker for calculation of ALI's and other values for use in calculating dose equivalent accounting for age and gender differences applicable to the general public.

The model will provide for calculating radiation-sensitive weighted dose equivalents to regions of the respiratory tract which can be summed to give a value of dose equivalent to use with risk coefficients and tissue weighting factors for the total respiratory tract.

It is now anticipated that a draft acceptable to Committee 2 will be available by October 1990 for consideration by the Main Commission at its next meeting thereafter.

Task Group on Reference Man

The Task Group has decided that the most appropriate age, height and weight for "new reference man" should cover a range of 10 - 50 years with a reference single age of 35 years; a height of 176 cm and a weight of about 73 kg. A final draft containing extensive data on gross and elemental composition, intakes and losses from the body, balance for individual elements and details of each of the major systems of the body is expected to be presented to Committee 2 at its October 1990 meeting.

It was noted that the extensive data on Chinese and Japanese populations were so similar that they could be combined into a single population; but that this population represented about 60% of the global

population. Thus "new reference man" representing western populations will be unrepresentative of the majority of the world population. The task group will briefly address the differences and the Commission may recommend that a further study of Asian Man be undertaken by another organisation.

Task Group on Age Dependent Doses to Members of the Public from Intake of Radionuclides

The first report in the process of publication contains information on 12 elements. The second report will contain data on S, Co, Ni, Zn, Mo, Tc, Ag, Te, Pb, Po, Ba and Ra and should be completed in early 1991. The third report containing data on Sb, Pr, Nd, Fe, Bi, U, Th and possibly Ru will be completed in 1992/93.

It is intended to have a chapter in Part 3 on the uncertainties of the dose coefficients based upon sensitivity analyses and - if agreed by Committee 2 - a chapter on "needs for future work". In addition, or as a separate part, dose coefficients will be prepared for pregnant women (dose to embryo and fetus, per unit intake by the mother).

Task Group on Revision of ICRP Publication 40: Protection of the Public in the Event of Major Radiation Accidents: Principles for Planning

Conceptual material from the report is to be included in the Main Text of the revision of the Basic Recommendations. The report will include advice on procedures to be adopted after exposure of workers and the public in the early phases of an emergency, and will also consider longer times after an accident and at greater distances than has been addressed in the ICRP Publication 40. The Task Group is to meet in March 1990 and expects to submit a first draft document to the June 1990 meeting of Committee 4.

Task Group on Probabilistic Exposures

Conceptual material from the report is to be included in the Main Text of the revision of the Basic Recommendations, possibly in a chapter entitled "System of radiological protection for potential exposures". When the 9th draft of the Recommendations incorporating this material becomes available, a decision will be made as to whether it is necessary to convene a further meeting of the Task Group.

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

See reference to Task Groups in the Introduction.

V. Publications:

ICRP Publication 45, Quantitative Bases for Developing a Unified Index of Harm. Annals of the ICRP 15 (3) 1985.

ICRP Publication 46, Radiation Protection Principles for the Disposal of Solid Radioactive Waste. Annals of the ICRP 15 (4) 1985.

ICRP Publication 47, Radiation Protection of Workers in Mines. Annals of the ICRP 16 (1) 1986.

ICRP Publication 48, The Metabolism of Plutonium and Related Elements. Annals of the ICRP 16 (2/3) 1986.

ICRP Publication 49, Developmental Effects of Irradiation on the Brain of the Embryo and Fetus. Annals of the ICRP 16 (4) 1986.

ICRP Publication 50, Lung Cancer Risk from Indoor Exposures to Radon Daughters. Annals of the ICRP 17 (1) 1987.

ICRP Publication 51, Data for Use in Protection Against External Radiation. Annals of the ICRP 17 (2/3) 1987.

ICRP Publication 52, Protection of the Patient in Nuclear Medicine. Annals of the ICRP 17 (4) 1987.

ICRP Publication 53, Radiation Dose to Patients from Radiopharmaceuticals. Annals of the ICRP 18 (1-4) 1987.

ICRP Publication 54, Individual Monitoring for Intakes of Radionuclides by Workers: Design and Interpretation. Annals of the ICRP 19 (1-3) 1988.

ICRP Publication 30 Part 4. Limits for Intakes of Radionuclides by Workers: An Addendum. Annals of the ICRP 19 (4) 1988.

ICRP Publication 55, Optimization and Decision-Making in Radiological Protection. Annals of the ICRP 20 (1) 1989.

STATEMENTS IN THE ANNALS OF THE ICRP

Statement from the 1985 Paris Meeting of the ICRP 15 (3), i-ii, 1985.

Statement from the 1987 Washington Meeting of the ICRP 17 (2/3), i-iii, 1987.

Statement from the 1987 Como Meeting of the ICRP 17 (4), i-v, 1987.

ICRP Progress Report on the Preparation of the New Recommendations 19 (4) 1988.

Statement from the 1989 Paris Meeting of the ICRP 20, (1), 1989.

SOME FORTHCOMING ICRP PUBLICATIONS

Summary of the Current ICRP Principles for Protection of the Patient in Diagnostic Radiology. (To appear in the Annals as a Note.)

Age-Dependent Doses to Members of the Public from Intake of Radionuclides: Part 1. (To appear as ICRP Publication 56).

Radiological Protection of the Worker in Medicine and Dentistry (to appear as ICRP Publication 57).

RBE for Deterministic Effects (to appear as ICRP Publication 58).

The following section lists some of the reports being prepared by task groups of the ICRP. These have yet to be adopted by the Commission in their final form.

Recommendations of the ICRP (will supercede ICRP Publication 26 and subsequent Statements).

Risk Estimates for Cancer and Genetic Effects.

Biological Basis for Dose Limitation in the Skin.

Human Respiratory Tract Models.

Reference Man (Revision of ICRP Publication 23).

Protection of the Public in the Event of Major Radiation Accidents: Principles for Planning (Revision of ICRP Publication 40).

Reference Radiations for Use as a Basis for Quality Factors for Ionizing Radiation Protection.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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

Contract no.: BI6-F-138-NL

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

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151 Lange Kleiweg
NL-2280 HV Rijswijk

Telephone number. 015/13.69.40

Title of the research contract:

Absorbed dose assessments in diagnostic radiology, nuclear medicine and radiotherapy with respect to the female breast.

List of projects:

1. Absorbed dose assessments in diagnostic radiology, nuclear medicine and radiotherapy with respect to the female breast.

Title of the project no.:

Assessment of absorbed dose in the female breast in diagnostic radiology

Head(s) of project:

Prof.dr. J.J. Broerse and Dr. J. Zoetelief

Scientific staff:

Prof.dr. J.J. Broerse, N.J.P. de Wit and Dr. J. Zoetelief

I. Objectives of the project:

Because of the high incidence of mammary cancer in the member states of the European Communities, screening projects employing mammography are carried out. In the Netherlands (and the UK), nation wide population screening is planned to be implemented in 1988. Therefore, it is essential that absorbed doses in mammography can be determined in relation to the image quality of the mammograms. Such studies will be required to formulate protocols for quality assurance as well as to provide recommendations for dose reduction in mass-screening programs. Absorbed dose values in the breast have to be derived from measurement 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 quality assurance protocol will continuously be tested at the Comprehensive Cancer Centre Rotterdam with emphasis on different mammography units. Investigations of film/-screen combinations in dependence on film processing will be performed in terms of absorbed dose and image quality. In addition, characteristics of AEC units will be studied.
The investigations of the influence of compressed breast tissue composition on absorbed dose in relation to the mAs values will be continued for actual mammograms.

III. Progress achieved:

Survey of absorbed dose in the female breast due to mammography in relation to physical image quality for several Dutch hospitals.

The risks of mammography (i.e. tumor induction) are dependent on the absorbed dose in the tissue at risk, whereas the benefits are related to the efficacy of the detection of small malignant lesions in the breast, which is dependent of the physical image quality. It was therefore of interest to perform a survey of absorbed dose and physical image quality in several Dutch hospitals to obtain information on the state-of-the-art of mammography in the Netherlands.

Methodology

Measurements of absorbed dose and dose distributions were made with an 0.6 cm^3 thimble type Baldwin Farmer (BF) 2571 graphite ionization chamber. The BF chamber was calibrated at several radiation qualities at the primary standardization laboratory of the Netherlands. For conversion from exposure to absorbed dose a factor of 8 mGy/R was used according to Hammerstein et al. (Radiology, **130**, 585-491, 1979). A displacement correction factor for measurements in-phantom with the BF-chamber of 0.79 was used according to Hofmeester et al. (Proc. ICRR, section E2-18, 1983). In addition, measurements were made with thermoluminescent (TL) dosemeters.

The survey was carried out at twelve institutes with fourteen mammography units for irradiation conditions similar to those employed in routine mammography. The diagnostic image quality was assessed with a Wisconsin Mammographic Random Phantom and judged independently by four diagnostic radiologists.

Results and discussion

The average absorbed dose to the breast per mammogram as derived from the ionization chamber measurements differed greatly among the different hospitals, i.e. from 0.8 to 4.2 mGy (see, Zuur et al., 1985).

It was expected that the scores for image quality would positively correlate with the mean absorbed dose. The results obtained (Zuur, et al., 1985), however, demonstrate no dependence of image quality on the absorbed dose. This indicates that a dose reduction could be possible at various hospitals without loss of physical image quality by improving the mammography procedures.

In addition, a dose discrepancy at the entrance surface of the phantom between TLD and BF ionization chamber measurements was found i.e. D(TLD)/D(BF) of about 0.6 ± 0.1.

In view of this dose discrepancy, measurements have been made with TLD in various irradiation geometries at the surface relative to the surface dose derived from a Weichstrahl ionization chamber (Zoetelief et al., 1989b). It was shown that the discrepancy is most likely to be attributed to the attenuation of the low energy X-rays in the material used for encapsulation.

Evaluation of screening frequency in mammography

The need for regular mammographic screening is generally accepted, however, the required frequency is still under discussion.

Methodology

For a fixed detection threshold and a defined screening interval, the detected breast tumours will show a size distribution dependent on the growth rate of the tumours. Geul and Van Bekkum (IKR Bulletin **8**, 3, 1984) calculated the frequencies of the diameters of tumours after 12 and 24 months for tumours with a diameter doubling time of 5 months. The diameter of the diagnosed tumours is one of the most important parameters to determine the survival prognosis of the patient (Adair et al. Cancer **33**, 1145, 1974). The sum of the products of the different tumour diameters and the survival percentages for these diameters results in the relative number of surviving women.

Results and discussion
The relative number of surviving women for the relevant survival period was calculated for screening with interval periods of 12 and 24 months. The extra benefit of yearly screening can be calculated (Zuur and Broerse, 1985) as 69 more cancer patients cured and 1520 years life time gained per 1000 patients.
For screening with an annual average absorbed dose of 1 mGy, the extra risk for induction of breast cancer of women between 35 and 75 years is about 1 per cent of the natural incidendence. The extra induced mortality caused by annual mammography is very small compared to the benefit. Moreover, these induced tumours will be detected when they are relatively small, because the women are participating in the screening program and will benefit from the increased cure rate for participants in programs for early detection of breast cancer.

Displacement correction factors of 0.6 cm³ BF ionization chambers for measurements in-phantom at various radiation qualities
Absorbed dose is a macroscopical quantity and is defined at points, while the ionization chambers used for dose determinations are of finite dimensions. The reading of an ionization chamber will be proportional to an absorbed dose averaged over the sensitive volume and this average value must be related to the absorbed dose at a specific point, i.e. the effective point of measurement. Inside a phantom, the effective point of measurement can be different from free-in-air due to the replacement of phantom material by the cavity of the ionization chamber causing changes in attenuation and scattering of the radiation. The displacement correction factor, k_d, is to be applied when the geometrical centre of the chamber is taken as the point of measurement.
For X-rays as used in radiobiology (150-300 kV), k_d is equal to 1, however, for low energy X rays as used in mammography (see, e.g. Hofmeester et al., Proc. ICRR, section E2-18, 1983) k_d can be considerably different from 1.

Methodology
Measurements have been made with three spherical ionization chambers (cavity radii 4, 8 and 16 mm) and a cylindrical Baldwin Farmer (BF) 2571 chamber in polymethylmethacrylate (PMMA) phantoms for 30, 42, 50, 58, 74 and 90 kV X rays. Extrapolation of the readings of the spherical chambers (centred at the same depth) as a function of cavity radius to a radius of zero gives the dose value in the phantom without cavity, from which the displacement correction factors can be derived.

Results and discussion
The results of k_d for the BF chamber for various types of radiation derived from linear extrapolation of the readings to zero cavity radius (Zoetelief et al., 1989b) are given in Table 1. It is evident that k_d for mammography X rays is considerably smaller than that at higher tube voltages. Hofmeester et al. assumed that a Weichstrahlkammer would have no displacement. In case they would have extrapolated their values for cylindrical chambers to a cavity radius of zero, their value of k_d for the BF chamber would have been 0.67 instead of 0.76, which is in agreement with the value in Table 1. It should be stressed that k_d values inside other phantom materials can be considerably different. It should furthermore be recognized that non-linear extrapolation would lead to different displacement correction factors and their associated errors. This requires further investigation.

Depth-dose measurements in phantoms of different materials and thicknesses at various mammography radiation qualities
Information on absorbed dose in mammography is essential to assess the risks related to the irradiation.
For an optimal dose specification, the absorbed dose and the masses of the tissue at risk

should be determined. Stanton et al. (Radiology, **150**, 577-584, 1984) determined for an average breast composition $\overline{D}_g/\overline{D} = 0.95 \pm 0.05$ for screen/film mammography, where $\overline{D}_g$ is the average dose in mammary gland tissue and $\overline{D}$ is the average whole breast dose.

Table 1: Displacement correction factors of BF 2571 cylindrical ionization chambers for measurements in PMMA phantoms irradiated with X rays of different quality.

tube voltage (kV)	HVL (mm Al) first	second	k_d (BF 2571 chamber)
30	0.29	0.33	0.67 ± 0.04*
			0.71 ± 0.04**
28	0.35	--	0.76 ± 0.01***
42	1.5	1.7	0.93 ± 0.01
50	1.6	2.1	0.94 ± 0.01
58	1.8	2.3	0.94 ± 0.01
74	2.2	3.0	0.95 ± 0.01
90	2.5	3.7	0.96 ± 0.01

* 15.3 mm depth in phantom
** 30.5 mm depth in-phantom
*** Hofmeester et al., 1983 (22 mm depth)

Methodology

Absorbed dose measurements have been made with a BF 2571 ionization chamber in PMMA phantoms of various thicknesses, employing a displacement correction factor slightly varying with depth. The obtained depth dose distributions, using an exposure to absorbed dose conversion factor of 8 mGy/R, were fitted by the following expression:

$$D(x) = D(0)\,(SSD/(SSD + x))^2\, e^{-\mu x}\,[1 + A\,(\mu x)^4]$$

where D(x) represents the absorbed dose in mammary gland tissue at depth x in-phantom, SSD is the source-to-surface distance, μ is an effective attenation coefficient and the coefficient A is applied to account for changes in attenuation and radiation quality with depth in-phantom. The results obtained for various irradiation conditions were analyzed employing individual fits for each depth-dose curve and by using common fit parameters for μ and A for each tube voltage. It was concluded that a fit for each voltage with common μ and A gives results which are not significantly different from that of individual fits, thus indicating the usefulness of the fit function.

Fits to the measured depth-dose distribution were also made with a fixed value of A of $9.5\ 10^{-4}$ indicated as μ^*. In Table 2 the ratios of entrance, average and exit doses derived from the two mathematical procedures i.e. the two parameters (μ and A) fit and the one parameter (μ^*) fit, are given. The ratios are not significantly different from one. This idicates that fits with a common value of A of $9.5\ 10^{-4}$ are providing adequate results.

Results and discussion

Values of the parameter μ^* obtained from least-squares fits to the depth-dose data measured with the Baldwin-Farmer ionization chamber inside 60 mm thick PMMA phantoms at two types of mammography units are given in Table 3 for several tube voltage settings.

For the same tube voltage setting the values of μ^* for the Mamex DC Mag are lower than for the Senographe 500 TS. This is due mainly to the differences in tube voltage generation. For the Mamex DC Mag, high-frequency transformation is applied, whereas for the Senographe 500 TS, classical transformation is employed which provides a lower effective potential than

the peak voltage value. At a given tube voltage, the variation in the means of μ^* for the Mamex DC Mag over a 9-month period is smaller than that for the Senographe 500 TS in a period of 11 days (see Zoetelief et al., in press). This is also related to the principles followed for high-voltage generation.

Table 2: Comparison of results of fits with two parameters μ and A to fits with one parameter μ^* with a fixed value of A of 9.5×10^{-4}. Given are the ratios of the entrance, average and exit doses, D_{en}, $\overline{D}$ and D_{ex}, respectively.

X-ray unit	setted tube voltage (kV)	D_{en} (ratio)	$\overline{D}$ (ratio)	D_{ex} (ratio)
Mammodiagnost U-M	25	0.988	0.997	0.98
	28	1.003	1.000	1.01
	30	0.997	1.000	1.00
	31	1.018	1.005	1.02
Mamex DC Mag	25	1.003	1.000	1.00
	27	0.997	1.000	0.99
	28	0.994	0.999	0.99
	30	1.000	1.000	1.00

Table 3: Fit parameter μ^* of depth-dose measured in 60 mm thick PMMA phantoms for various tube voltage settings for two mammography units.

Mammography unit	Tube voltage setting (kV)	μ^* ($\pm$ SD) (10^{-2} mm^{-1})
Mamex DC Mag	25	8.59 ± 0.09
	26	8.31 ± 0.08
	27	8.11 ± 0.14
	28	7.89 ± 0.05
	29	7.73 ± 0.05
	30	7.56 ± 0.04
	31	7.42 ± 0.04
Senographe 500 TS-1	27	8.39 ± 0.19
	28	8.22 ± 0.24
	29	7.90 ± 0.06
	30	7.71 ± 0.08
	31	7.56 ± 0.07

Absorbed dose measurements made in phantoms of various materials with a thickness of about 5 cm employing a displacement correction factor slightly varying with depth and phantom material are shown in Figure 1. The phantom materials employed were 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).

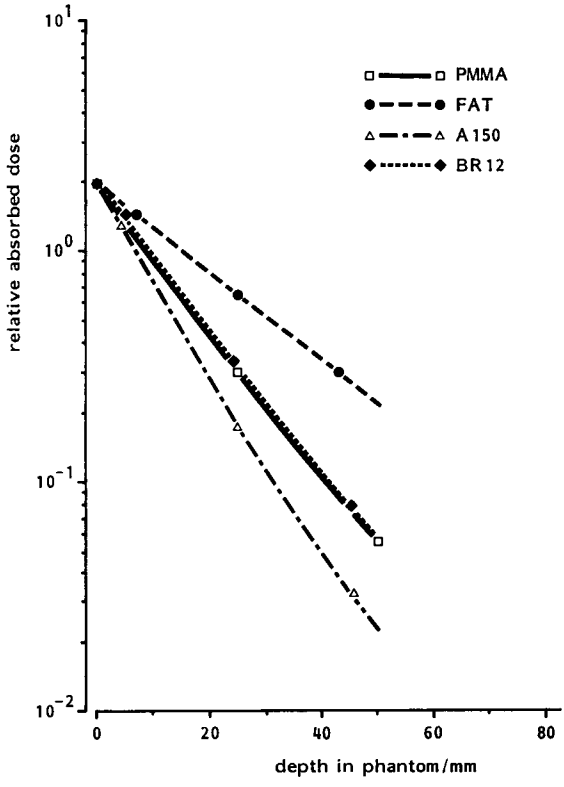

Figure 1: Central axis depth-dose curves in 50 mm thick phantoms of various materials irradiated with 31 kV X rays (first HVL: 0.33 mmAl; H = 0.80) at an SSD of 526 mm.

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).

Output and automatic exposure control (AEC) units of several mammography units

To assess the average absolute absorbed dose values in phantoms, the absorbed dose at a

specific point should be established in addition to the information on relative dose distributions. Monitoring of the output of the X rays tubes through measurement of the focal charge can be related to the entrance dose when corrections for source-to-surface distance are made. Mammography units are generally equipped with automatic exposure control (AEC) devices which in principle determine the value of the exit dose and the average density of the radiographs.

Methodology

By measurement of the focal spot charge (mAs) information can be obtained on the output of the X ray tubes. The output, Y, is defined here as the absorbed dose at the entrance surface of the phantom per unit of focal spot charge and expressed in μGy/mAs. Measurements with phantoms of various thicknesses indicate that the values of Y corrected for source-to-surface distance (SSD) are approximately constant (standard deviation: about 2 per cent). This indicates that the back scatter factor is not varying considerably with phantom thickness.
To investigate the appropriateness of AEC units, exit dose values were derived by measurement of the focal spot charge, output as given in Figure 2 and the parameterization of depth dose data described. In addition, the photographic density of radiographs of phantoms of various thicknesses were measured.

Results and discussion

In Figure 2, Y (normalized to an SSD of 600 mm) is given on a logarithmic scale as a function of tube voltage for the four mammography units investigated. The measured values of tube voltage are used for all units except the Mammodiagnost U-M.

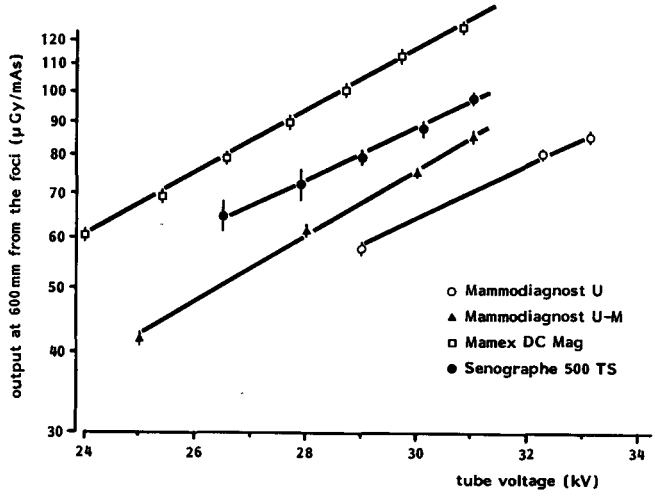

Figure 2: Output, Y, at 600 mm distance from the foci as a function of tube voltage for four mammography units. The output is defined as the entrance dose (μGy) at a PMMA phantom per unit of focal spot charge (mAs).

The use of the focal spot charge for monitoring of the entrance dose at a given SSD, requires calibration for each mammography unit. The output of the Mamex DC Mag did not vary more than about 2 per cent in use during a period of half a year. This unit is also equipped with a monitoring device in terms of μJ/cm^2. The entrance dose corrected for SSD per

$\mu J/cm^2$ is approximately constant (standard deviation about 1.3 per cent for the measured tube voltage range from 24 to 30.8 kV).
The results of exit absorbed dose and average photographic density for radiographs of PMMA phantoms of various thicknesses employing AEC units are given in Table 4 for two mammography units. It is concluded that the use of the automatic exposure control devices for monitoring of the dose at the image receptor plane and for obtaining constant photographical density of radiographs has serious restrictions. The AEC unit of the Senographe 500 TS seems superior with regard to obtaining a constant photographic density for a given tube voltage at various phantom thicknesses, whereas the AEC unit of the Mamex DC Mag seems to provide a more constant photographical density for a given phantom thickness at various tube voltages.

Table 4: Exit absorbed dose (D_{ex}) and average photographic density ($\bar{d}$) for radiographs of PMMA phantoms of various thickness at several tube voltages at two mammography units employing automatic exposure control.

Senographe 500 TS, focus-to-film distance 618 mm

measured tube voltage	26.5 kV		27.5 kV		28.8 kV		29.9 kV		30.9 kV	
phantom thickness (mm)	D_{ex} (μGy)	$\bar{d}$	D_{ex} (μGy)	$\bar{d}$	D_{ex} (μGy)	$\bar{d}$	D_{ex} (μGy)	$\bar{d}$	D_{ex} (μGy)	$\bar{d}$
20	81	1.24	82	1.25	78	1.20	73	1.06	65	0.98
30	86	1.07	88	1.14	81	1.11	68	1.03	61	0.98
40	89	1.05	78	1.02	76	0.99	68	0.87	58	0.84
50	108	1.07	89	0.95	84	0.89	66	0.73	59	0.69
60	146	1.14	122	0.98	79	0.90	71	0.63	57	0.50
Average	102	1.11	92	1.07	83	1.02	69	0.86	60	0.80
St.dev.(%)	26	7	19	12	10	13	4	22	5	26

Mamex DC Mag, focus-to-film distance 601 mm

measured tube voltage	24.0 kV		25.4 kV		26.6 kV		27.7kV		28.7 kV		29.7 kV		30 kV	
phantom thickness (mm)	D_{ex} (μGy)	$\bar{d}$	D_{ex} (μGy)	$\bar{d}$	D_{ex} (μGy)	$\bar{d}$	D_{ex} (μGy)	$\bar{d}$	D_{ex} (μGy)	$\bar{d}$	D_{ex} (μGy)	$\bar{d}$	D_{ex} (μGy)	$\bar{d}$
20	103	1.59	102	1.64	101	1.70	109	1.81	108	1.84	110	1.89	111	1.90
30	90	1.22	86	1.28	88	1.37	85	1.37	83	1.45	88	1.45	83	1.47
40	80	0.91	77	0.99	76	1.03	73	1.00	71	1.08	68	1.05	67	1.07
50	73	0.60	69	0.61	70	0.68	64	0.68	60	0.77	59	0.77	57	0.80
60	60	0.45	57	0.42	59	0.52	58	0.57	55	0.56	52	0.53	49	0.59
Average	81	0.95	78	0.99	79	1.06	78	1.09	75	1.14	75	1.14	73	1.17
St.dev(%)20	49		21	50	21	46	26	47	28	45	31	48	33	45

Monitoring of absorbed dose to the breast in actual mammography

Methodology
Measurement of the focal spot charge or energy fluence might be employed to monitor the entrance dose at a PMMA phantom at a given source-to-surface distance (SSD). It is therefore of interest to investigate whether, for actual mammogaphy conditions, measurement of energy fluence combined with the data obtained from PMMA phantoms at a given SSD can be employed for monitoring of average breast doses.

Results and discussion
Energy fluence values ($\mu J/cm^2$) during a series of actual mammograms as a function of breast thickness were compared with the energy fluence values obtained for PMMA phantoms of various thicknesses (Zoetelief et al., in press). It was concluded that the energy fluence values for a given compressed breast thickness show considerable variations. This must be due to differences in compressed breast composition, since repeated measurements for a PMMA phantom show only variations in the order of 2 per cent. For relatively thinner breasts, i.e. smaller than 50 mm thickness during compression, the variation in composition seems larger than for thicker breasts. For thinner breasts the energy fluence values are considerably larger than the values for PMMA phantoms of the same thickness; for intermediate breasts (about 5 cm) they are about equal; and for thicker breasts smaller.
Average absorbed doses derived during actual mammography employing effective attenuation coefficients based upon energy fluence values and data for PMMA phantoms vary from about 0.4 to 1 mGy per radiograph (Zoetelief et al., in press).

Quality control of technical and dosimetric aspects

To maintain an optimum in the risk/benefit balance, a quality control program concerning the technical and dosimetrical aspects of mammography has to be implemented. A quality control protocol was drafted, which includes constancy checks at different time intervals.

Methodology
- For each woman the compressed breast thickness and focal spot charge (mAs) are registered;
- Daily checks of the film processing are carried out using sensitometry/densitometry; daily a radiograph is made of a reference phantom (for which the focal charge is recorded and the average density is determined);
- Approximately three-monthly, absorbed dose measurements are made with an ionization chamber inside a PMMA phantom with reference to the automatic exposure control unit (AEC); the radiation quality is assessed and the focal spot size is determined.

Results and discussion
The preliminary quality control protocol was implemented and tested at the mammography unit of the Comprehensive Cancer Centre Rotterdam (BOC-IKR) (see Zoetelief et al., 1989).
- Concerning film processing it was found that sensitometry provides adequate information. The photographic density at a sensitometer step which provides a D-value of about 1 provides a reasonable indication of the constancy. A limiting value of $\pm$ 0.15 D was derived.
- To check the total mammography procedure a daily radiograph of a reference phantom proved to be essential. A limiting value for the photographic density of $\pm$ 0.2 D and for the focal spot charge (mAs-value) of $\pm$ 20 per cent were determined. It was found that the average density of the radiograph of the reference phantom and sensitometry results and/or focal spot charge are correlated.
- Variations in absorbed dose values are related to the stability of the film processing but also strongly dependent on the constancy of the radiation quality i.e. tube voltage. A limiting value of $\pm$ 0.5 kV was derived for tube voltage. The focal spot size should be measured instead of using values from the manufacturer.

- The determination of compressed breast thickness and the focal spot charge (mAs) value provide information on absorbed doses for actual mammograms.

Absorbed dose to the female breast due to radiotherapy

It is generally assumed that in patients treated for malignant disease by curative radiotherpay, radiation induction of a second primary cancer in the target volume is rare, probably occurring in less than 0.1 per cent of the long term survivors (Beentjes, Klinische Fysica, **3**, 116-121, 1987).

Outside the target volume there is direct evidence of radiation carcinogenesis with cumulative absorbed doses in excess of 0.1 Gy (ICRP, report 44, 1985). The dose outside the target volume is caused by leakage radiation and scattered radiation. Calculations of organ doses due to scattered radiation are made by e.g. Williams et al. (GSF-Bericht S 1054, 1984) and given in Table 5, for absorbed dose to the breast per unit of absorbed dose in various target volumes irradiated during treatment (lower values refer to 25 MV X rays; higher values to ^{60}Co). The relative frequencies of irradiation of different target volumes, excluding bronchus (low survival), skin and mammary irradiation, according to Beentjes are also given in Table 6. Beentjes estimates 6900 women to receive radiotherapy in the five target volumes irradiated with total absorbed doses of 60 Gy and a cure rate of about 0.65. This results in an annual collective absorbed dose of about 500 to 1000 woman-Gy to the female breast for a total population of about 7.3 10^6 women (in 1984). Appropriate 40 per cent of the female population is in excess of 40 years of age (i.e. 2.9 10^6 women).

Table 5: Estimate of absorbed dose to the female breast due to radiotherapy for various target volumes in Dutch women.

target organ	fraction of absorbed dose to the breast (mGy/Gy)	relative frequency of therapy*	annual number of patients irradiated	absorbed dose in target area** (Gy)	annual collective absorbed dose to the breast (woman - Gy)
Neck	0.2 - 0.4	0.06	414	40	3.3 - 6.6
Thorax	10 - 18	0.046	310	40	127 - 229
Pancreas	6 - 12	0.150	1035	40	248 - 497
Gall bladder	3 - 8	0.087	600	40	72 - 192
Pelvic area	0.2 - 0.4	0.657	4533	40	36 - 72
Total		1.00	6900		486 - 997

* excluding bronchus, skin and mammary irradiation

** product of absorbed dose and surviving fraction

Assuming that radiotherapy is applied only for woman older than 40 years, this would result in a value of absorbed dose to the breast of about 0.16 - 0.34 mGy per woman per year. In mammography, absorbed doses to the breast vary from about 0.5 to 3 mGy per annual or biannual investigation.

Absorbed dose to the female breast due to nuclear medicine

Measurement of absorbed dose to various organs were performed by Camps, Zuur, Blokland, Broerse and Pauwels (Eur. J. Nucl. Med. **14**, 529-532, 1988) for ^{81m}Kr lung ventilation studies employing a breathing lung phantom. In this study performed with 300 kcnts, a conversion factor of 0.15 μGy/GBqs for absorbed dose to the breast per cumulated activity of ^{81m}Kr in the lungs was derived. Variation of the flow rate from 0.5 to 3.5 l/min results in cumulated activities of about 30 to 60 GBqs in the lung corresponding to absorbed doses to

the breast of about 4.5 to 9 μGy.
Average employed activities are generally a factor of three larger (Beekhuis, Health Phys. **54**, 287-291, 1988), but the values are still small compared to those found in mammography or radiotherapy. The same holds for the mean effective dose equivalent per caput due to nuclear medicine in The Netherlands of 0.037 mSv (Beekhuis) and the mean effective dose equivalent per patient of 2.7 mSv.

Conclusions

- A survey of absorbed dose and physical image quality in mammography performed in several Dutch hospitals revealed a variation in absorbed dose by a factor of about 5 without correlation with physical image quality.
- From an evaluation of risks and benefits of mammography it was concluded that annual screening is advantageous compared to biannual screening.
- The displacement correction factor ,k_d, of a 0.6 cm^3 BF chamber for measurement inside a PMMA phantom was experimentally determined by extrapolation of measurement results of chambers of various sizes and appeared to be significantly different from one. Further studies are required to confirm the extrapolation procedure and to investigate the influence of phantom material composition.
- A mathematic expression was obtained to characterize depth-dose distributions in-phantom for mammography qualities by a single parameter μ^* which is dependent on radiation quality and phantom material. The value of μ^* shows smaller variations at a setted tube voltage for mammography units in which high voltage is obtained from high frequency transformation.
- From studies with different phantom materials it is concluded that the steepest depth dose curve is found for A-150 plastic, an intermediate and almost equal dependence is observed for PMMA and BR-12 and the shallowest curve is measured for fat.
- Measurement of the focal spot charge (or energy fluence) can be used to determine entrance doses. Calibration of the output should be made for individual units.
- The AEC units studied, showed serious shortcomings in providing constant photographic density of radiographs and exit absorbed doses for different phantom thicknesses and/or different tube voltages.
- Employing energy fluence and measurement of compressed breast thickness, absorbed dose during actual mammography can be monitored. The use of PMMA pantoms provides a good simulation for the average breast composition at average thickness but not for thick or thin breasts. At BOC-IKR average absorbed dose values per radiograph vary from about 0.4 to 1 mGy, dependent on breast composition and thickness.
- A preliminary quality control protocol for technical and dosimetric aspects of mammography was investigated. Limiting values were derived for various relevant parameters. Further studies are needed to evaluate the usefulness in common practice.
- Absorbed dose to the female breast due to radiotherapy of other organs is significant, i.e., about 0.16 - 0.34 mGy per woman (with an age in excess of 40 years) per year, compared to mammography. The absorbed dose to the breast due to nuclear medicine is marginal.

IV. Objectives for the next reporting period:

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

- Dr. J.J. Paulides. Comprehensive Cancer Centre Rotterdam, Rotterdam, The Netherlands.
- Dr. A.H.L. Aalbers. National Institute for Public Health and Environmental Hygiene, Bilthoven, The Netherlands.
- Dr. H.W. Julius, Radiological Service TNO, Arnhem, The Netherlands.
- Dr. F. van der Meer. Department of Radiology. Medical Faculty of the University of Rotterdam, Rotterdam, The Netherlands.

VI. Publications:

1. C. Zuur, J. Zoetelief, A.G. Visser and J.J. Broerse. Absorbed dose from mammography in several Dutch hospitals. Brit. J. Radiol. Suppl. **18**, 110-114, 1985.
- C. Zuur and J.J. Broerse. Risk- and cost-benefit analysis of breast screening programs derived from absorbed dose measurements in The Netherlands. Diagn. Imag. Clin Med. **54**, 211-222, 1985.
- 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.
- J. Zoetelief. Mammografie. In: Grondbeginselen stralingsfysica en radiobiologie voor medische toepassingen (Eds. J.J. Broerse, L.A. Hennen, A.F. Hermens en J. Zoetelief). IRS-TNO, pp. 596-612, 1987.
- J. Zoetelief, N.J.P. de Wit and J.J. Broerse. Technical and dosimetric aspects of quality control in mammography. In : Technical and Physical Parameters for Quality Assurance in Medical Diagnostic Radiology: Tolerances, limiting values and appropriate measuring methods (Eds.: B.M. Moores, F.E. Stieve, H. Eriskat and H. Schibilla). BIR Report 18, 143-146, 1989a.
- J. Zoetelief, N.J.P. de Wit and J.J. Broerse. Dosimetric aspects of film/screen mammography: In-phantom dosimetry with thimble-type ionization chambers. Phys. Med. Biol. **34**, 1169-1177, 1989b.
- J. Zoetelief, J.J. Broerse and D.W. van Bekkum. Stralingsdosis en beeldkwaliteit in de mammografie. IKR-Bulletin **13**, 12-14, 1989.

- J. Zoetelief, N.J.P. de Wit and J.J. Broerse. Geabsorbeerde dosis en beeldkwaliteit in de mammografie. In: Workshop stralingsbescherming van de "consument" van medische stralingstoepassingen. Ministerie van Welzijn, Volksgezondheid en Cultuur, Report no VDB-273, 66-79, 1989.
- J. Zoetelief, N.J.P. de Wit and J.J. Broerse. Technique for monitoring absorbed dose to the breast in mammography (NRPB-CEC workshop, September 1988, Oxford). In press.

2. J.J. Broerse, C. Zuur and J. Zoetelief. Mammografie voor vroegtijdige detectie van borstkanker. Proceedings Symposium on Medical Technology Assessment TNO, Leiden, 1986, p. 28.
- 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.
- W.A. Hummel: Systeemanalytische beschrijving van het beeldvormende systeem voor mammografie. Afstudeerverslag, TU Delft, January 1988.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-F-139-I

Università degli Studi di Pisa
Lungarno Pacinotti 43/44
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Head(s) of research team(s) [name(s) and address(es)]:

Prof. L. Donato
Istituto di Patologia Spec. Med.
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I-56100 Pisa

Telephone number: 050/47231

Title of the research contract:

Limitation of patient exposure to radiation from emerging medical diagnostic procedures in high morbidity disease areas.

List of projects:

1. Nuclear cardiology versus two-dimensional echocardiography for detection, quantitation and follow-up studies of myocardial disease.
2. Diagnostic efficacy of immunoscintigraphy with radioactive monoclonal antibodies (versus bidimensional echography).

Title of the project no.: 1

Nuclear cardiology versus two-dimensional echocardiography for detection, quantitation and follow-up studies of myocardial disease.

Head(s) of project:

C. Contini, Associate Professor of Clinical Physiology, University of Pisa.

Scientific staff:

D. Neglia, M.D., A.Distante, M.D., O. Parodi, M.D., D. Levorato, M.D., C. Arlotta, M.D., P. Marzullo, M.D., E. Picano, M.D.

I. Objectives of the project:

The project is aimed at the investigation of radiation exposure to patients and operators resulting from nuclear cardiac blood pool gating and other nuclear cardiology procedures largely used to screen suspects of cardiomyopathies (primary or secondary). Moreover the project will attempt to evaluate the role of ultrasounds as an alternative procedure.

II. Objectives for the reporting period:

To assess the he role of functional indexes, derived from radionuclide angiography (RNA), to diagnose early myocardial disease in patients with complex ventricular arrhythmias (CVA) and/or left bundle branch block (LBBB) but without clinical evidence of heart failure or other cardiac and systemic diseases in comparison with radiological invasive, and non radiological non invasive procedures.

The presence of organ-specific cardiac autoantibodies was checked, in view of developments in the possible future developments in the field of radioimmunoscintigraphy of myocardial disease.

III. Progress achieved:

1. Basal radionuclide angiography in the diagnosis of early ventricular dysfunction in patients with complex ventricular arrhythmias

Study Protocol

Twentyseven patients (group 1), referred to our Institution because of symptomatic or asymptomatic ventricular arrhythmias, were selected according to the following criteria: 1) presence of CVA (Lawn class IVa-b) at 24-hour Holter monitoring; 2) no clinical evidence of heart failure (NYHA class I) and of other cardiac or systemic diseases.

In this group, RNA was performed to provide basal indexes of regional and global biventricular function: wall motion score indexes (WMI), ejection fraction (EF) values. Results were compared with those of 20 normal individuals . After the noninvasive evaluation, in 24/27 patients of group 1, who had shown regional dyssynergies, and in the 10 patients of group 2 with atypical chest pain, cardiac catheterization and coronary angiography were performed.

Results

In the patients selected for the present study, severe ventricular arrhythmias could not be explained by routine clinical evaluation: in no case signs or symptoms of cardiac failure were evident at admission and other cardiac or systemic diseases were reasonably excluded by clinical, biohumoral and instrumental criteria. Ischemic heart disease was also excluded by means of exercise stress test, ergonovine maleate test and coronary angiography.

In this population, basal RNA allowed to evidence regional myocardial dysfunction in a high percentage of patients (89%) involving, with similar frequencies, the right, the left or both ventricles. Contrast angiography could confirm the diagnosis of left ventricular dyssynergies but was less sensitive in the detection of right ventricular wall motion abnormalities.

Biventricular global systolic function, as evaluated by RNA EF measurements, was depressed in the study population as compared with normal subjects, and the degree of ventricular global impairment was correlated with the extent of regional dyssynergies. However, the prevalence of pathologic EF values in arrhythmic patients (59%) was low when compared with that of wall motion abnormalities and only RV function appeared to be severely depressed in some of these patients. Scintigraphically assessed ventricular dysfunction did infrequently correspond to pathologic hemodynamic measurements.

These results suggest that severe ventricular arrhythmias are associated with myocardial disease also in absence of clinically evident left heart failure. Myocardial damage is mainly regional, may impair right ventricular function but still spares global LV performance in basal conditions.

Conclusions

CVA may be associated with subclinical myocardial disease: in this population a basal evaluation by RNA allows the diagnosis of early regional and/or global ventricular dysfunction and may be used, together with 2D-Echocardiography, to follow the possible progression of the disease. The invasive radiological procedures do not add any useful information in this early stage and should be recommended only when functional deterioration is apparent.

2. Assessment of early impairment of myocardial functional reserve in patients with CVA or LBBB by radionuclide angiography during stress and pharmacological interventions:

Study protocol

As previously shown, patients with CVA or LBBB, and without clinical evidence of heart failure or other cardiac/systemic diseases, may show regional ventricular dysfunction and normal or moderately depressed left ventricular (LV) EF. One possible approach to better define if these patients have or not an early stage myocardial disease and to separate them from normals would be the demonstration of depressed LV functional reserve during stress.

Aim of this study was to evaluate if RNA, combined with non-invasive aortic blood pressure monitoring, could provide useful indexes to assess left ventricular (LV) functional reserve during stress or pharmacologial interventions in this population.

Eighteen patients with CVA (Lawn class IVa-b) and/or LBBB at basal ECG and 24-hour Holter monitoring were selected according to the following criteria: 1) no clinical evidence of heart failure (NYHA class I-II) or of other cardiac and systemic diseases; 2) angiographically normal coronary arteries and normal central hemodynamics.

RNA and non-invasive aortic blood pressure monitoring were simultaneously performed in basal conditions (BAS), during maximal atrial pacing tachycardia (PAC), during submaximal isometric exercise (handgrip=HG) and after intravenous injection of isosorbide dinitrate (NITR). The following LV functional indexes were computed: end-diastolic, end-systolic volume index (EDVi, ESVi)(cc/m^2); EF (%); peak systolic pressure/end-systolic volume ratio (PSP/ESVi) ($mmHg/cc/m^2$). Moreover the relationship between PSP/ESVi and EDVi was analysed as a possible indicator of LV performance independently of heart size. Results were compared with those obtained in 14 normal individulas matched for age and sex.

Results

All patients showed regional wall motion abnormalities at basal RNA. According to basal LV volumes and EF values, they could be subdivided in 2 groups: group A, including 10 patients with normal EF (within 1SD from the mean values of controls); group B, including 8 patients with abnormal EF.

In basal conditions, controls and group A patients could not be separated and were significantly different from group B patients for all functional indexes (see following table) (*=p<.01 vs normals and A).

	EF	EDVi	ESVi	PSP/ESVi
Normals	46 ± 5	78 ± 16	42 ± 9	3.40 ± 0.92
Group A	44 ± 5	78 ± 10	43 ± 6	2.98 ± 0.44
Group B	23 ± 7 *	117 ± 16 *	89 ± 12 *	1.45 ± 0.24

During stresses and after nitrates administration LV function remained significantly depressed in group B patients as compared to normals and group A patients, due to an impaired reserve. Group A patients were indistinguishable from normals during HG and after NITR; neverthless, during PAC these subjects could be differentiated from controls because of a lower functional reserve (figure 1).

In each group of patients and in controls, basal PSP/ESVi and EDVi values showed inverse relationships best fitted by hyperbolic curves (r values ranging from .64 to .84). The corresponding linear functions, obtained by substituting 1/EDVi to EDVi, did not differ between controls and group A patients (slopes = 273 vs 199, ns) but showed a significantly lower slope in group B patients as compared to controls (123 vs 273, p<.05).

When all the conditions (BAS, PAC, HG, NITR) were analysed together, PSP/ESVi and EDVi values still showed hyperbolic relationships in each group (r values ranging from .83 to .88). In this case, however, the corresponding linear function could clearly separate all the 3 groups showing slopes significantly (p<.01) decreasing from normals (336), to group A (218) and group B (135) patients (figure 2).

As a working hypothesis, the slope of this relationship may express the functional state of the heart and its capability to respond to stresses independently of loads and heart size.

Conclusions

Patients with CVA and/or LBBB and without clinical evidence of heart failure may show an abnormal LV functional reserve during stress or pharmacological interventions unregardness of basal LV function. This behaviour is probably expression of a diffuse myocardial disease not yet clinically apparent. The non-invasive evaluation of LV response to different stresses by means of RNA and blood pressure monitoring may provide new functional indexes, independent of loads and heart size, which improve sensitivity in the assessment of early myocardial impairment.

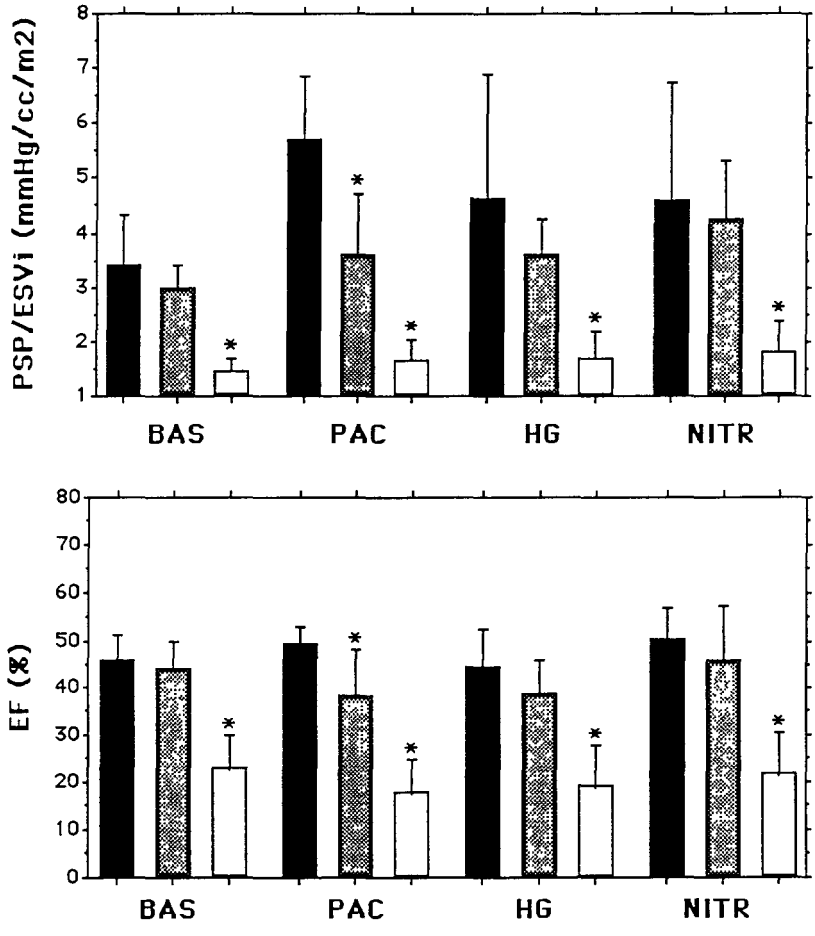

Figure 1: Mean ± SD values of LV PSP/ESVi and EF are represented in normals (black bars), group A (grey bars) and group B (white bars) patients in the 4 study conditions. Group B patients are always clearly separated from the other 2 groups (*=p<.01 vs normals and group A). By contrast, group A patients, which show a fairly normal LV function in 3 conditions, can be separated from controls during pacing because of a lower increase in PSP/ESVi and a decrease in EF.

3. Novel organ-specific circulating cardiac autoantibodies in dilated cardiomyopathy

(Study performed in cooperation with the Dept. of Cardiological Siences, St. Georges's Hospital Medical School and Dept. of Immunology, University College and Middlesex School of Medicine of London)

Study protocol

In dilated cardiomyopathy (DCM) an autoimmune etiology has been proposed and cardiac autoantibodies have been described, but their organ and disease-specificity remain controversial if this were confirmed, the possibility of early diagnosis of myocardial disease by radioimmunoscintigraphy might because practical interest.

To establish wether organ-specific cardiac autoantibodies exist in dilated cardiomyopathy, we used the reference technique, indirect immunofluorescence on human atrium, ventricle and skeletal muscle. Sera were tested from: 1) 65 patients with DCM; 2) 42 with ischemic chronic heart failure; 3) 208 with other cardiac disease; 4) 200 normals.

Results

Three immunofluorescence patterns were observed, termed, respectively, organ-specific, cross-reactive 1 and 2.

Organ-specific cardiac antibodies were more frequent in DCM (26%) than in other cardiac disease (1%, $p<.001$), heart failure controls (0%, $p<.001$) or in normals (3.5%, $p<.001$). Organ-specific cardiac antibodies were more common in DCM patients with fewer symptoms (NYHA class I) and with more recent onset of disease (< 2 years). Conversely, cross-reative antibodies were detected in similar portions of subjects in all groups.

Conclusions

The presence of organ-specific cardiac antibodies provides a novel serological marker of cardiac autoimmunity in DCM and sets the basis for future application of Radioimmunoscintigraphy.

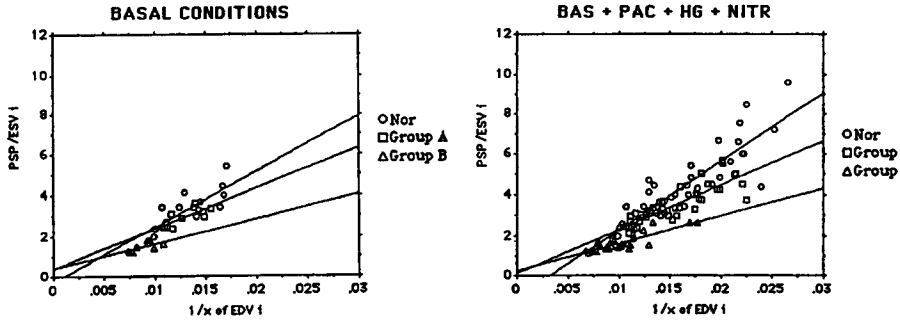

Figure 2: PSP/ESVi vs EDVi relationships in the 3 groups of subjets are analysed in basal conditions (upper panel) or in all the study conditions (lower panel). See text for details.

Conclusive remarks

The main objectives of the project were to evaluate the cost-benefit ratio in the utilization of techniques of nuclear cardiology, as compared to other noninvasive (Electrocardiography, 2D-Echocardiography) or invasive methods, for screening patients with suspected "primitive" myocardial disease but without clinical evidence of heart failure.

The main results of this five years study show that some electrocardiographic patterns (i.e. complex ventricular arrhythmias and/or left bundle branch block) are associated, with high frequence, to early myocardial dysfunction. Also if not conclusive, these data suggest that the ECG evaluation (24-hour Holter monitoring) may serve as a first screening of patients with arrhythmias or conduction disturbances and without other evidence of cardiac or systemic disease.

In these patients, 2D-Echocardiography is able to document regional myocardial dysfunction with a sensitivity similar to that of radionuclide angiography or contrast angiography. The main limitations of the technique, as compared to scintigraphy, appear to be in the evaluation of the right ventricle and in the difficulty to provide informations on global ventricular performance in basal conditions and during stress. Neverthless, together with electrocardiography, 2D- Echocardiography is able to detect the majority of patients with early myocardial dysfunction among patients with arrhythmias and is particularly suitable for the follow-up of this population.

The invasive evaluation, including endomyocardial biopsy, did not seem to add useful information to non-invasive techniques in patients with early stage dilated cardiomyopathy and without evidence of possible myocarditis. It should be recommended when the deterioration of ventricular function is apparent, rising the possible decision of heart transplant.

One of the main advantages of radionuclide angiography, the use of which should be limited because of patients and operators exposure, is its good sensitivity for the detection of regional myocardial dysfunction, in particular when it involves mainly the right ventricle. However, the field in which this technique appears to be fundamental is in the evaluation of global ventricular performance and its response to stress (myocardial functional reserve). It easily provides indexes of ventricular function which closely describe the intrinsic characteristics of the myocardium and may consequently identify, in a very early stage, diffuse abnormalities. This approach is particularly suitable to evaluate the possible effects of ttherapy and the progression of the disease.

As a final statement, we can conclude that patients with suspected myocardial disease should be first screened by ECG and 2D-Echocardiography. If the suspicion remains, a scintigraphic evaluation is recommended both in basal conditions and during stress. All these patients should be followed by conventional techniques and resubmitted to scintigraphic evaluation as soon as any functional change occurs.

Finally, the development of immunoscintigraphy could play an important role in the study of so called "primitive" myocardial diseases in which a possible autoimmune component is coming to great attention.

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

C.N.R. - Institute of Clinical Physiology of Pisa, Pisa (Italy).

V. Publications:

1) C. Arlotta, A.M. Piacenti, D. Levorato, S. Berti, C. Contini: Holter monitoring in patients with right cardiomyopathy. III International symposium Holter monitoring, Vienna 1988, p.251 (Abs n. 198)

2) D. Levorato, C. Arlotta, S. Berti, A.M. Piacenti, L. Paperini, A. Pozzolini, M.T. Baratto, M.G. Bongiorni, C. Contini: Cardiac arrhythmias in early and advanced myocardial damage. III International symposium Holter monitoring, Vienna 1988, p.135 (Abs n. 82).

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4) C. Contini, S. Berti, D. Levorato, M.G. Bongiorni, M.T. Baratto, C. Arlotta, A.M. Piacenti, A. Pozzolini, L. Paperini, G. Kraft: Myocardial damage and ventricular arrhythmias: an early step toward dilated cardiomyopathy. Eur. Heart J., 9: 106, Suppl. 1, 1988 (Abs n. 558).

5) Berti S., Levorato D., Neglia D., Bongiorni M.G., Baratto M.T., Arlotta C., Piacenti M., Paperini L., Pozzolini A., Kraft G., Contini C.: Role of radionuclide angiography in the diagnosis of initial dilated cardiomyopathy. New Trends in Cardiomyopathies, Florence, 1989, p. 58 (Abs n. C12).

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Eur. J. Nucl. Med. 15: 471, 1989 (Abs n. 292)

8) Neglia D., Palombo C., Parodi O., Sambuceti G., Marabotti C., Genovesi-Ebert A., Nudi F., Ghione S., L'Abbate A.: Noninvasive detection of early myocardial dysfunction in patients with left ventricular hypertrophy secondary to high blood pressure.
Eur. J. Nucl. Med. 15: 480, 1989 (Abs n. 325)

9) Neglia D., Berti S., Pelosi G., Levorato D., Parodi O., Baratto M.T., Arlotta C., Marzilli M., L'Abbate A., Contini C.: Do the invasive procedures play any role in the early diagnosis of dilated cardiomyopathy?
Eur. Heart J. 10: 61, 1989 (Abs n. P304)

10) Berti S., Neglia D., Parodi O., Piacenti M., Bongiorni M.G., Nudi F., L'Abbate A., Contini C.: Diagnosis of early myocardial damage in patients with complex ventricular arrhythmias.
Eur. Heart J. 10: 258, 1989 (Abs n. 1340)

11) Parodi O., Neglia D., Marcassa C., Salvadori P., Bellina R., Camici P., Fusani L., Franceschini R., Riva A., Sorace O.: Regional myocardial blood flow response to pacing tachycardia and dipyridamole infusion in patients with subclinical dilated cardiomyopathy: a quantitative assessment by positron emission tomography.
International Teach-in for promoting scientific basis of Cardiology, Rome, October 1989 (abs).

12) Neglia D., Parodi O., Palombo C., Sambuceti G., Nudi F., Marcassa C., Berti S., L'Abbate A.: Depressione della funzione globale e della riserva contrattile del ventricolo sinistro in pazienti con sospetta cardiomiopatia dilatativa.
Cardiologia, 34, Suppl. 2: 99, 1989 (Abs n. P129).

13) Parodi O., Neglia D., Marcassa C., Salvadori P., Bellina R., Camici P., Fusani L., Franceschini R., Riva A., Sorace O.: Regional myocardial blood flow response to pacing tachycardia and dipyridamole infusion in patients with subclinical dilated cardiomyopathy: a quantitative assessment by positron emission tomography.
JACC , 1990 (Abs) (in press)

Papers

1) Berti S., Neglia D., Levorato D., Bongiorni M.G., Baratto M.T., Arlotta C., Piacenti M., Paperini L., Pozzolini A., Kraft G., Contini C.: Role of radionuclide angiography in the diagnosis of early ventricular dysfunction in patients with omplex ventricular arrhythmias.
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Summary of 1985-1988 activity

1985

The task for the first year of activity was the definition of the methodological aspects of the study aimed at assessing the relative role of ultrasound (echocardiography, 2D-Echo) and ionizing radiations (radionuclide ventriculography, RNA) for the evaluation of myocardial disease.

The main results were in the definition, by a retrospective analysis of patients with dilated cardiomyopathy, of early clinical predictors of future development of the disease: 1) complex ventricular arrhythmias at high incidence; 2) contractile impairment of one or both ventricles also if segmentary; 3) chest pain with or without ST segment alterations.

Echocardiographic and Radioisotopic methodological approaches were standardised.

1986

The task of the second year of activity was the collection of patients without clinical evidence of myocardial disease but at higher risk to develop cardiomyopathy according to the clinical criteria defined above. Repeated 2D-Echo and RNA were performed in this population and their diagnostic value compared.

Fortyfive patients were recruited. The results showed that nuclear and echocardiographic techniques present different characteristics as far as sensitivity and specificity in the diagnosis of early myocardial impairment. In particular RNA has some advantage in the evaluation of the right ventricle and in the quantitation of global ventricular function. On the other side, 2D-Echo allows to obtain better informations on regional kinetics of the left ventricle. The two techniques appeared to be complementary.

1987

The task of the third year of activity was to compare the informations obtained by the combined RNA and 2D-Echo evaluation with those desumed from cardiac catheterization and endomyocardial biopsy in patients showing deterioration of myocardial function by non-invasive methods.

Comparing 2D-Echo and RNA results with ventricular contrastographic data, the invasive method, initially assumed as the reference approach, was in fact less sensitive of both non-invasive techniques. Endomyocardial biopsy results showed aspecific but highly frequent histologic alterations similar to those found in advanced dilated cardiomyopathy.

As a conclusion, 2D-Echo and RNA seemed to be the best methods to diagnose and follow-up patients with initial myocardial disease.

1988

The task of the fourth year of activity was to draw conclusive results concerning the diagnostic reliability of 2D-Echo and RNA as compared to the angiographic, hemodynamic and bioptic evaluation and to assess possible evolution of early myocardial disease by a short term follow-up consisting of clinical and echocardiographic controls every 6 months.

Non-invasive techniques were confirmed as valuable and complementary clinical tools to document subclinical myocardial disease in patients suspected of early cardiomyopathy. While echocardiography is particularly usefull in a preliminary screening of patients, the scintigraphic approach allows a definite evaluation of the extent of biventricular global impairment. The invasive study can confirm non-invasive findings but seem to add nothing in patients with early cardiomyopathy. Clinical and echocardiographic follow-up was able to document worsening of the disease in about half of the patients.

If the progression of early myocardial dysfunction in patients with arrhythmias could finally lead to overt heart failure cannot be stated at present. However, the results of this study show that complex ventricular arrhythmias are infrequently isolated manifestations of heart disese. In this patients an early non-invasive evaluation of myocardial function and a prolonged follow-up is mandatory.

Title of the project no.: 2

Diagnostic efficacy of immunoscintigraphy with radioactive monoclonal antibodies (versus bidimensional echography).

Head(s) of project:

G. Mariani, Associate 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 radiolabelled anti-tumor monoclonal preparations.

2) To assess the diagnostic efficacy of this novel medical procedure as compared with a conventional non-ionizing technique (bidimensional echography).

II. Objectives for the reporting period:

1985: To perform preliminary studies on the in vivo tissue distribution kinetics of anti-tumor monoclonal ^{131}I-tracers in patients bearing tumors different from those for which the tracers were expected to be specific, to assess non-specific radioactivity uptake at various sites. The plan for the first year envisaged screening of at least two types of monoclonal preparations specific for CEA.

1986: To assess, on the basis of the in vivo distribution of the tracers, the organ exposures resulting from the use of monoclonal tracers for RISG.

1987: To compare, in patients with lung and gastrointestinal adenocarcinoma, the diagnostic efficacy of RISG with that of standard x-ray, CT and ultrasound (echography and/or echotomography).

1988: To compare the diagnostic sensitivity of RISG with that of standard x-ray in patients with malignant melanoma.

1989:To continue the analysis of RISG sensitivity in comparison with other noninvasive diagnostic techniques.

III. Progress achieved:

1st year-1985:

A total of 14 cancer patients were studied, all with normal serum CEA levels: three anti-CEA monoclonal tracers were testeo, respectively the intact FO23C5-IgG_1 (in four patients), the bivalent $F(ab')_2$ (in four patients) and the monovalent Fab (in six patients) fragments.

The anti-tumor monoclonal tracers never showed any uptake at known tumor sites, thus confirming their anti-CEA specificity. The average whole-body radioactivity retention curves and plasma disappearance curves obtained for the three monoclonal tracers tested are comparatively plotted in Figure 1, corrected for isotopic decay. A highly homogenous kinetic pattern was observed for each type of tracer, particularly at early times after injection, whereas significant differences were found among the three different preparations.

On the basis of the overall kinetic and tissue distribution results obtained in this study, it was decided to select the FO23C5-F(ab')2 monoclonal preparation (either labeled with ^{131}I of with ^{111}In) for a clinical trial in patients bearing cancer characterized by a significant expression of CEA by the tumor cells.

2nd year-1986

During the second year of the contract, a total of nine in vivo tissue distribution kinetic studies of the anti-adenocarcinoma monoclonal antibody ^{131}I-KC4-IgG_3 were performed in cancer patients, excluding adenocarcinomas. The data obtained for each case study (plasma disappearance curve of the injected tracer, whole body radioactivity retention curve, activity/time curve for each region of interest) were fed into a computer, using the CAMIRD III program for the computation of radiation dosimetry to the whole body and critical organs (targets, bone red marrow and gonads) (see Tables 1 and 2).

3rd year-1987

The study was coordinated by the National Research Council of Italy and involved 200 patients selected from a total of 542 patients, submitted to RISG.

The main findings were the high proportion of tumor lesions detected at RISG (over 80% of the localisations in CEA-seropositive patients) and the high number of unexpected tumor lesions imaged at RISG (34%) in patients previously classified as tumor-free.

The overall efficacy of RISG was 82.2%, resulting from the combination of a 79% sensitivity and a 96.7% specificity.

The overall predictive value of a negative RISG was 50.6% (29.3% in CEA-seropositive, 65.1% in CEA-seronegative patients), while the predictive value of a positive RISG result was 99% (99.2% in CEA-seropositive, 94.2% in CEA-seronegative patients) (see Tables 3, 4 and 5).

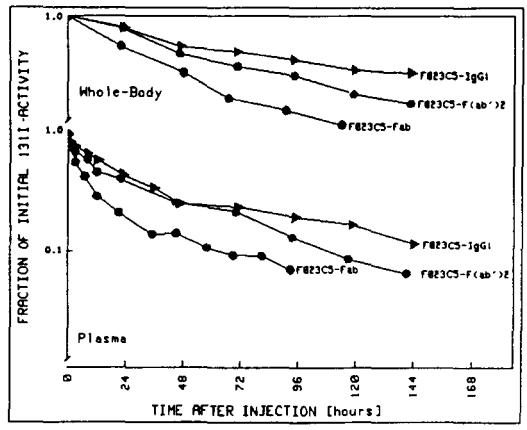

Figure 1

Table 1

RESIDENCE TIMES OF ^{131}I-MONOCLONAL ANTIBODIES

STUDY	WHOLE BODY (hrs	LIVER (hrs)	SPLEEN (hrs)	BLOOD (hrs)
32	59.30	11.20	3.98	34.37
35	70.80	5.78	2.01	40.48
36	83.29	7.65	2.47	40.40
37	72.76	7.77	3.48	36.33
40	83.12	6.84	3.80	36.47
41	93.42	9.95	4.05	53.65
45	67.97	2.71	.78	35.96
46	52.25	6.49	.79	28.99
55	62.38	15.95	4.11	39.96
min	52.25	2.71	.78	28.99
max	93.42	15.95	4.11	53.65
mean	71.70	8.26	2.83	38.51
S.D.	13.09	3.76	1.37	6.73
C.V.	18.26%	45.57%	48.35%	17.47%

Table 2

ORGAN DOSES FROM ^{131}I-MONOCLONAL ANTIBODIES

STUDY	WHOLE BODY rad/mCi	LIVER rad/mCi	SPLEEN rad/mCi	RED MARROW rad/mCi	TESTS rad/mCi	OVARIES rad/mCi
32	.58	3.55	10.72	.33	.26	.30
35	.70	1.99	5.61	.48	.43	.47
36	.82	2.58	6.86	.61	.54	.59
37	.72	2.58	9.47	.50	.44	.48
40	.82	2.33	10.35	.63	.56	.61
41	.92	3.31	11.06	.61	.52	.58
45	.67	1.07	2.38	.52	.47	.51
46	.51	2.13	2.33	.35	.30	.33
55	.61	4.97	11.07	.29	.21	.25
min	.51	1.07	2.33	.29	.21	.25
max	.92	4.97	11.07	.63	.56	.61
mean	.71	2.72	7.76	.48	.41	.46
S.D.	.13	1.11	3.61	.13	.13	.13
C.V.	18.28%	40.92%	46.50%	26.72%	30.88%	29.12%

Table 3 - x-rays vs. RISG

	Lesions ≤ 2 cm			Lesions > 2 cm		
	x-ray+	x-ray-	x-ray±	ray+	x-ray-	x-ray±
Lung Cancer						
RISG+	13	13	4 (76.9%)	15	3	7 (83.3%)
RISG-	4	2	0 (15.4%)	1	2	0 (10.0%)
RISG±	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 RISG sentitivity: 76.9% x-ray sensitivity: 43.6% Concordant results: 43.6%			Patients n.: 30 RISG sensitivity: 83.3% rays sensitivity: 60% Concordant results: 56.7%		
Gastrointestinal Cancer						
RISG+	17	15	7 (100%)	40	6	6 (69.6%)
RISG-	0	0	0 -	8	4	1 (14.1%)
RISG±	0	0	0 -	6	5	4 (16.3%)
	(43.6%)	(38.5%)	(17.9%)	(55.7%)	(29.4%)	(11.9%)
	Patients n.: 39 RISG sensitivity: 100% x-ray sensitivity: 43.6% Concordant results: 48.7%			Patients n.: 64 RISG sentitivity: 69.6% x-ray sensitivity: 58.7% Concordant results: 75%		

Legends:

Patient 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 4 - CT vs. RISG

	Lesions < 2 cm			Lesions > 2 cm		
	CT+	CT-	CT±	CT+	CT-	CT±
Lung Cancer						
RISG+	2	1	1	4	0	1
RISG-	0	0	1	1	0	0
RISG±	0	0	0	0	0	0
Gastrointestinal Cancer						
RISG+	11	2	4	21	3	1 (65.8%)
RISG-	0	0	0	3	1	0 (10.5%)
RISG±	0	0	0	4 (73.7%)	2 (15.8%)	3 (23.7%) (10.5%)

Legends as in Table I.

Patients with CT: 65

Sensitivity of RISG lower than CT (73.7% versus 65.8%) in gastrointestinal cancer patients with tumor lesions larger than 2 cm.

Concordant results: 65.8% in gastrointestinal cancer patients with tumor lesions larger than 2 cm.

Table 5 - ECHO vs. ISG

	Lesions < 2 cm			Lesions > 2 cm		
	US+	US-	US±	US+	US-	US±
Lung Cancer						
RISG+	1	2	1	2	2	0
RISG-	0	0	0	0	0	0
RISG±	0	0	0	0	1	0
Gastrointestinal Cancer						
RISG+	4	14	5 (100%)	22	4	4 (63.8%)
RISG-	0	0	0	2	2	1 (10.6%)
RISG±	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: 81 (patients with Echography: 29; patients with echotomography: 52).

GI cancer patients with tumor lesions 2 cm.:
- 17.4% concordant results
- 100% RISG sensitivity
- 17.4% US sensitivity

GI cancer patients with tumor lesions 2 cm:
- 57.5% concordant results
- 63.8% RISG sensitivity
- 66.0% US sensitivity.

4th year-1988

A group of 46 patients was selected out of 300 melanoma patients submitted to RISG with the monoclonal tracer 22.28S-F(ab')$_2$: 26 cases of choroidal melanoma, 14 of lung melanoma and 6 of axillary lymph node metastases.

Based on the threshold size limite, the results of each test (RISG or x-ray) could only be either true-positive or false-negative, in terms of patients classification. The statistical evaluation of each test was, therefore, performed solely in terms of sensitivity with respect to the presence of tumor. The diagnostic sensitivity of RISG resulted to be 82.6% in the overall cases, significantly higher than that observed for x-rays (58.7%, $p<0.025$) (see Table 6).

5th year-1989

A group of 200 patients were selected from a total of over 800 patients included in two multicenter clinical trials of the diagnostic accuracy of tumor RISG with the anti-CEA tracer F023C5-F(ab')$_2$ or with the anti-melanoma tracer 225.28S-F(ab')$_2$. Patients with all tumor lesions either larger or smaller than a pre-set size limit and all proven independently from RISG and standard x-ray were selected for this study; the size limit was set at 2 cm for 131 patients with gastrointestinal (GI) and 69 patients with lung cancers, while it was set a 1 cm for 26 patients with choroidal melanoma.

In the GI cancer patients, RISG sensitivity was significantly higher than that of x-ray for tumor lesions both smaller than 2 cm (100% vs. 61.5%, $p.<0.001$) and larger than 2 cm (85.9% vs. 70.7%, $p<0.05$). In the lung cancer group, RISG performed significantly better than x-ray only in patients with smaller tumor lesions (84.6% vs. 59%, $p<0.05$); 90% vs. 83% for lesions larger than 2 cm). In the melanoma patients RISG sensitivity was much higher than that of x-ray for tumor lesions smaller than 1 cm (63.6% vs. 9.1%, $p<0.05$), while the difference was not significant for lesions larger than 1 cm (93.3% vs. 80%) (see Table 6).

The results of the analysis confirm the remarkably good diagnostic performances of tumor RISG and strongly support its use, complementary to other techniques, for the early detection of tumor recurrences in the staging and follow-up of cancer patients.

Table 6

GASTROINTESTINAL CANCER (No. 131)

Anti-CEA F023C5-F(ab')$_2$

Tumor lesions 2 cm (No. 39)			Tumor lesions 2 cm (No. 92)		
RISG+	39/39	(100.00%)	RISG+	79/92	(85.87%)
x-ray+	24/39	(61.54%)	x-ray+	65/92	(70.65%)
P<0.001					

LUNG CANCER (No. 69)

Anti-CEA F023C5-F(ab')$_2$

Tumor lesions 2 cm (No. 39)			Tumor lesions 2 cm (No. 30)		
RISG+	33/39	(84.62%)	RISG+	27/30	(90.00%)
x-ray+	23/39	(58.97%)	x-ray+	25/30	(83.33%)
P<0.05					

CHOROIDAL MELANOMA (No. 26)

Anti-melanoma 225.28S-F(ab')$_2$

Tumor lesions 1 cm (No.11)			Tumor lesions 1 cm (No.15)		
RISG+	7/11	(63.64%)	RIS+	14/15	(93.33%)
x-ray+	1/11	(9.09%)	x-ray+	12/15	(80.00%)
P<0.05			N.S.		

IV. Other research group(s) collaborating actively on this project [names(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 at the University of Pisa, Pisa (Italy).

V. Publications:

A cost/benefit analysis of nuclear medicine diagnostic procedures performed with 99mTc radiopharmaceuticals.
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Int. Symp. Monoclonals and DNA Probes in Diagnostic and Preventive Medicine, p. 136, Florence, 1986 (Abstract).

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In: Technectium in Chemistry and Nuclear Medicine, Nicolini M., Bandoli G., Mazzi U. eds., p. 305, New York, Raven Press, 1986.

Size-related diagnostic sensitivity of radioimmunoscintigraphy versus soft-tissues x-ray in patients with ocular melanoma.
Mariani C., Rosa C., Siccardi A.S.
Eur. J. Nucl. Med., 15, 546, 1989 (Abstract).

Size-related diagnostic sensitivity of tumor radioimmunoscintigraphy (RIS) versus standard x-rays.
Mariani G., Rosa C., Siccardi A.S.
J. Nucl. Med., 30, 749, 1989 (Abstract).

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Mariani G., Fatigante L., Mazzuca N., Molea N., Rosa C.
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Mariani G., Ferrante L., Rescigno A.
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A protocol for screening tumour radioimmunoscintigraphy agents.
Mariani G.
In: Lectures and Symposia, 14th Int. Cancer Congr., vol. 6: Epidemiology, Prevention, Diagnosis, Lapis K. and Eckhardt S. eds., p. 329, Budapest, Akadeamiai Kiado, 1987.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

CAATS-INSERM Unité 240
Ctre d'Eval.l'Assur.de qualité des
Appl.Techn.dans le dom.de la Santé
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Contract no.: BI6-F-132-F

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

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Telephone number: 01/46.54.74.67

Title of the research contract:

Analysis of the patient exposure to radiation from medical diagnosis: exposure data, quality assurance and risk asessment.

List of projects:

1. Assessment of the somatic dose and risks related to medical radiodiagnosis.
2. Quality assurance in medical diagnostic radiology, expert systems.
3. Optimization of image quality criteria.

Title of the project no.:

Assessment of the somatic dose and risks related to medical radiodiagnosis.

Head(s) of project:

C. Maccia

Scientific staff:

M. Benedittini, F. Fagnani, C. Le Faure, C. Maccia.

I. Objectives of the project:

- To complete previous evaluations of the absorbed dose due to diagnostic radiology procedures in France carried out within the framework of the 1982-1985 CEC Radiation Protection Programme in order to estimate the collective risk associated with the use of ionizing radiation in medicine (conventional diagnostic examinations).

II. Objectives for the reporting period:

- To publish the obtained results in scientific journals.
- To stimulate all possible scientific actions (training, technical booklets, etc.) aiming at informing practitioners, medical physicists and, possibly, patients about doses received during both conventional x-ray examinations and computed tomography.

III. Progress achieved:

INTRODUCTION

In many countries medical exposure of patients represents the greatest man made contribution to the collective dose imparted to the population.

In France, as in other countries, it is generally considered that medical exposure equals approximately half the exposure from natural sources of ionizing radiation, and much effort should be devoted to its reduction. Many publications have already investigated the various aspects and levels of such radiation sources, and both the UNSCEAR and the BEIR have issued very extensive reports on this subject.

As far as France is concerned, the last available assessment of gonadal doses and of the consequential genetic risks of diagnostic radiology examinations was carried out on a national scale longer than 30 years ago (1957). Since then, numerous studies dealing with dosimetry of certain categories of x-ray examinations have been performed, but none evaluated the collective dose received in France. Furthermore, since 1957 both diagnostic imaging techniques and medical practices have changed considerably, and new dosimetry technologies also have been introduced and applied in diagnostic radiology.

Considering this, a survey was conducted on a national scale in France by the CEPN-INSERM in 1982. The main objectives of this survey were : to learn about the staffing and facilities for diagnostic radiology; to establish the frequency of x-ray examinations and the age and sex distribution of patients; to ascertain current levels of exposure for patients; and to evaluate the radiation risks from the various radiological procedures.

This final report gives the main results of this survey and describes (**a**) the assessment of the collective effective dose equivalent associated with the different types of radiological examinations practiced in France in 1982, (**b**) the distribution of collective patient doses absorbed in particularly radiosensitive organs, and (**c**) the genetically significant dose (GSD).

MATERIAL AND METHODS

a) The National CEPN-INSERM Survey (1982)

Detailed methodology used in carrying out the 1982 survey of the radiological activity in France has already been published elsewhere. To summarize briefly, the survey was conducted in two separate phases (the survey excluded dental radiology, mass chest screening practices, Ministry of Defense Hospitals, independent fluoroscopy units, C.T. scanners and nuclear medicine). Initially, about 500 radiology departments, private clinics and offices practicing diagnostic radiology were selected throughout the country by a stratified sampling procedure (average sample rate of 1/10); the stratification was based on a rough evaluation of their annual x-ray film consumption. All the necessary information about the provision of staff and facilities for diagnostic radiology in terms of numbers of radiologists, radiographers and x-ray sets available was finally gathered in 386 public

hospitals and private practices that actually participated in the survey. In a second phase, in order to estimate the total number of x-ray exams annually practiced in France, broken down by age and sex of patients, a questionnaire was sent to a sub-sample selected among the x-ray sets (549 out of 1,372) that equipped the 386 radiological departments mentioned above. Technical data about each of 13,000 x-ray examinations such as the number of films, the fluoroscopic screening time, the x-ray beam projection, the applied potential (kVp), the tube current (mA) and the exposure time (s) were thus collected over a specified one week period in June 1982.

In all, 45.4 million x-ray examinations were estimated for France in 1982 by extrapolating the weekly figures taking account of the seasonal radiological activity variations.

b) Dosimetry

Concerning the dose evaluation problem, measurements were performed either on an anthropomorphic phantom in 1984 or directly on the patient by using thermoluminescent dosimeters in 1985,1986, and 1987 (lithium borate pellets) which previously were calibrated with a standard source of ^{60}Co. The general protocol used is hereafter described.

1. Adult phantom dosimetry

In the case of the measurements on the phantom, three major problems were to be solved.

a) Selection of the parameters to be used in experimental measurements.

Almost 1,500 configurations, expressed in terms of combination of physical and anatomical parameters such as kVp, mAs, film size, x-ray beam projections and centering point position were actually observed in the survey. In order to limit the number of experimental measurements, this number was reduced by considering the following two criteria: either the relative low frequency in the actual practice, or the likely low contribution to the collective population dose (limb x-rays were not included); in doing so, the configuration number falls into 37.

It can be noticed that, in order to complete the set of the dosimetric measurements two extra configurations have been considered for the simulation of the fluoroscopy radiation mode. This enabled us to cover in a more realistic manner the radiological practice, and to add useful information about the dose associated with fluoroscopy.

b) Selection of a representative x-ray table.

This point concerned the selection of an x-ray table considered representative of the most routinely operating x-ray equipment in France, on which dosimetry measurements were to be performed. For practical reasons, 17 different x-ray tables, remote controlled, conventional and specialized ones, installed in five hospitals which participated in the dosimetry survey were selected. Free in air exposure variations were checked as a function of kVp, mAs and quality of the detector (standard film and rare earth screen-film) by using ionization chambers and TLDs. For the same kVp and mAs values, exposures were found to range by a factor of 1 to 3, depending on the x-ray equipment considered. Finally, the selected x-ray table, having the closest values of technical parameters (filtration, HVL etc.) as compared to the average was a model made by GE.

c) Organ dose assessment methods.

One problem related to the selection of particularly radiosensitive organs inside the Rando-man phantom. Keeping in mind the objectives of the dosimetry study i.e. the assessment of the dose received by all the organs recommended by the ICRP, two different procedures were followed in measuring organ doses.

Concerning lenses (6 TLDs), lungs (57 TLDs), testes (6 TLDs), ovaries (9 TLDs), mammary glands (9 TLDs) and thyroid (3 TLDs), TLDs were inserted into the corresponding organ positions and systematically irradiated 30 times to obtain significant dose values, especially at different points outside the primary beam. This was repeated as many times as the number of the configurations considered. In addition to those dosimeters, three TLDs were also attached to the phantom's surface at the center of each x-ray field, in order to assess the entrance skin dose.

Concerning the red bone marrow, the bone surface and the "remainder" organs, the estimation of doses was mainly based on the entrance skin dose measurements carried out for each radiograph. This has been achieved through Monte Carlo calculations using standardized x-ray field size and positions, corresponding to the 37 configurations, and an idealized mathematical phantom (MIRD) representing the patient. To be coherent and also to validate this procedure, a comparison between the Rando phantom dose and the MIRD dose has been carried out in terms of factors relating organ doses to the entrance skin dose for a wide range of x-ray field sizes, positions and projections. Comparative organ dose data are shown in Table 1.

As one can notice, CEPN-INSERM conversion factors, derived from the experimental measurements carried out on a Rando phantom, are generally lower than the correspondent calculated MIRD conversion factors. Nevertheless, reasonable agreement is obtained between the two sets of organ dose conversion factors when the beam sizes are the same. In those cases, the National Radiological Protection Board (NRPB) and CEPN-INSERM factors agree to within 30% for all except the abdomen exam in AP projection, the chest exam in PA projection and the skull exam in AP projection.

2. Patient Dosimetry for children.

In order to complete the previous set of dosimetry measurements, carried out exclusively on an adult Rando phantom, some in-vivo measurements were also performed on a sample of young patients (less than 10 years old) for a limited number of x-ray examination types. In accordance with the strategy adopted for assessing both somatic and genetic risk associated with the radiological practices, the following five common types of x-ray examinations were considered as the most representative ones: pelvis, intravenous urography, abdomen, lumbar spine and barium meal. Measurements were then carried out on patients undergoing the selected examinations by using TLDs attached to the patient's skin. Thyroid, gonads (only for the boys) and lung doses were estimated directly from skin dose measurements: one TLD over the thyroid, one TLD over the testes and two TLDs, respectively, on the front and back of the thorax. Doses for other organs

were, as in the case of the adult patients, deduced from measurements of entrance skin dose (including backscatter) per radiograph using Monte Carlo conversion factors adapted to a pediatric phantom.

ORGANS	PROJECTIONS	ANATOMICAL LANDMARK		FILM SIZE		CONVERSION FACTOR *		RATIO
		NRPB	CEPN	NRPB	CEPN	NRPB (1)	CEPN (2)	(1)/(2)
OVARIES	LATERAL	ABDOMEN	ILIAC CREST	36x43	36x43	0.04	0.057	0.7
	A/P	ABDOMEN	ILIAC CREST	36x43	36x43	0.15	0.08	1.81
	A/P	PELVIS	ILIAC CREST	36x43	30x40	0.116	0.053	2.18
	LATERAL	PUBIS	PUBIS	36x43	24x30	0.056	0.054	1.04
	A/P	PUBIS	PUBIS	30x40	30x40	0.26	0.23	1.13
	LATERAL	LUMBAR SPINE	LUMBAR SPINE	30x40	24x30	0.019	0.013	1.46
LUNGS	A/P	CHEST	XYPHOID BONE	36x43	24x30	0.23	0.2	1.15
	A/P	CHEST	CHEST	36x43	35x35	0.39	0.41	0.95
	LATERAL	CHEST	CHEST	36x43	35x35	0.21	0.3	0.7
	P/A	CHEST	CHEST	36x43	36x43	0.46	0.32	1.44
	A/P	CHEST	CHEST	36x43	36x43	0.39	0.31	1.26
	LATERAL	CHEST	CHEST	36x43	36x43	0.25	0.28	0.89
	A/P	LUMBAR SPINE	LUMBAR SPINE	30x40	24x30	0.013	0.01	1.3
HEAD	LATERAL	HEAD	CERVICAL SPINE	24x30	18x24	0.029	0.018	1.61
	A/P	HEAD	CERVICAL SPINE	24x30	24x30	0.03	0.018	1.67
	A/P	HEAD	HEAD	24x30	24x30	0.05	0.035	1.43

* Expressed as absorbed dose in the organ relative to the entrance skin dose.

Table 1. Comparative organ dose data between CEPN-INSERM and NRPB.

RESULTS

(a) Frequency of x-ray examinations

The CEPN-INSERM survey indicated that 45.4 million x-ray examinations were performed in France in1982. This corresponds to 820 examinations per thousand head of population. Figures of 23.9 million x rays and 21.5 million x rays were obtained respectively for male and female patients. Table 2 shows the weekly figures and the annual numbers of x-ray examinations broken down in 30 different categories.

EXAMINATIONS	OBSERVED NUMBER	ANNUAL ESTIMATED NUMBER (10^6)	%
CERVICAL SPINE	396	1.26	2.8
THORACIC SPINE	301	0.95	2.1
LUMBAR SPINE	643	1.84	4.1
SACRO-LUMBAR SPINE	226	0.69	1.5
PELVIS-HIP	919	3.39	7.5
ABDOMEN	422	1 59	3.5
IV UROGRAPHY	797	1.97	4.3
HYSTEROGRAPHY	62	0.18	0.4
CHOLECYSTOGRAPHY	266	0.67	1.5
SKULL	1076	4.02	8.9
BARIUM ENEMA	375	0.84	1.9
BARIUM MEAL	458	1.12	2.5
THORAX	3537	15.48	34.1
LIMBS (INFERIOR & SUPERIOR)	2543	10.04	22.1
MAMMOGRAPHY	150	0.26	0.6
CEREBRAL ANGIOGRAPHY	61	0.14	0.3
THORACIC ANGIOGRAPHY	63	0.13	0 3
ABDOMINAL ANGIOGRAPHY	37	0.07	0.2
INFERIOR LIMBS ANGIOGRAPHY	13	0.03	<0.1
PHLEBOGRAPHY	64	0.15	0.3
OBSTETRICAL ABDOMEN	125	0.32	0.7
PYELOGRAPHY	26	0.06	0.1
TOTAL	12591	45.35	100

Table 2 : Breakdown of x-ray examinations (France 1982).

The first general comment concerns the thorax examination number, which represents 34% of all x rays in France. The other most important examinations practiced in 1982 are limbs (22%), skull (9%) and pelvis-hip (7.5%).

Regarding the age and sex distribution of patients, Figure 1 shows the annual frequency of diagnostic radiology exams by age and sex per 1,000 patients of each age class.

For the first age class, (patients aged less than 1 year) the mean number of x rays all together, ranges from 2.2 to 3 per inhabitant and per year; while beyond the age of 60 years this value falls

into a range of 1 to 1.5. One can also notice that male patients are examined more frequently than the female, all age categories together, except in the case of the very young patients (less than 1 year old). In the latter, a reverse situation is observed, almost 3,000 x-rays are annually performed per 1,000 girls against 2,200 x rays per 1,000 boys. One of the most important reason for that being the high number of pelvic x-ray examinations taken for diagnosing the congenital hip dysplasia.

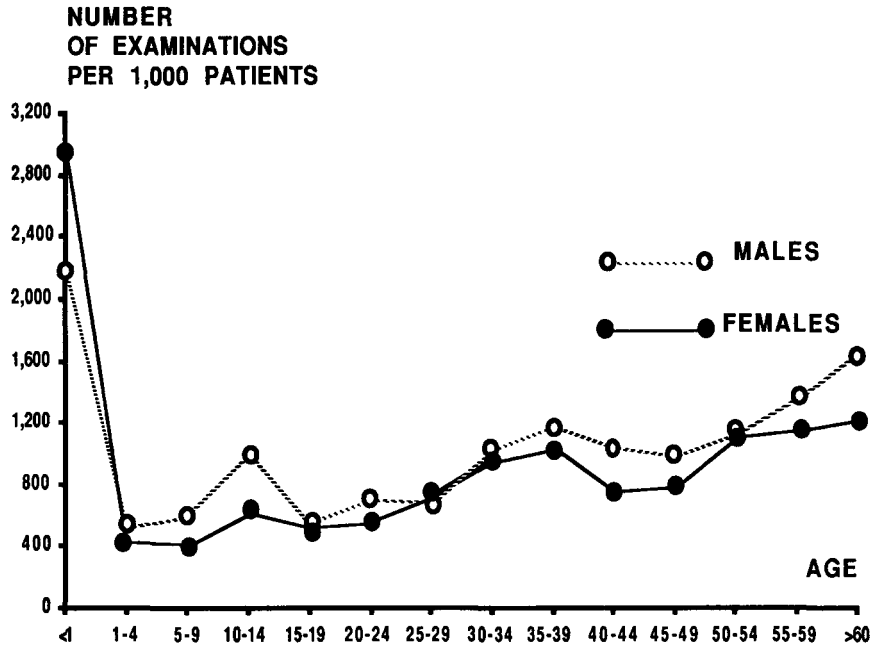

Fig. 1 Annual frequency of diagnostic radiology exams by age and sex per 1,000 patients of each age class (France 1982)

(b) Examination Organ Dose Estimates.

The entrance skin dose measurements carried out for each radiograph belonging to the previously mentioned CEPN-INSERM configurations have allowed the estimation of doses to all the major radiosensitive organs for each complete x-ray examination.

Table 3 shows the mean values of the total organ doses observed for each examination category considered. Values are expressed in terms of absorbed dose to ICRU muscle, apart from those for breast, which are calculated assuming a tissue composition of 50% water and 50% fat. As far as the "remainder organs" are concerned, figures represent the mean of the doses to the additional five most irradiated radiosensitive organs not otherwise specified in the table.

A first comment concerns the range of the gonad doses which vary from 0.03 mSv for a complete

examination of the thorax to 5 mSv for a lumbo-sacral spine exam. In this respect, two examinations seem to be the most irradiating ones: the abdominal angiography, which also delivers the highest dose to the total body all examinations considered, and the barium enema.

As far as the breast doses are concerned, the following three radiological procedures have to be mentioned as the most important ones: barium meal (36.43 mSv), thoracic angiography (10.01 mSv) and thoracic spine (9.98 mSv). In particular for barium meal, which necessarily involves the use of contrast media, a large number of films (9.5) and frequently fluoroscopy (81%), the breast dose value seems particularly high.

EXAMINATIONS	GON	BRE*	RBM	LUN	THY	BON	REM	EFF
CERVICAL SPINE	0.14	0.01	0.43	0.82	10.37	3.01	2.56	1.35
THORACIC SPINE	0.21	9.98	1.45	1.34	0.94	3.47	3.21	2.24
LUMBAR SPINE	1.32	0.13	1.41	3.21	0.29	4.09	12.42	4.72
LUMBO-SACRAL SPINE	5.01	0.04	2.88	1.79	0.07	3.33	9.55	4.73
PELVIS-HIP	1.28	0.03	0.81	1.17	0.01	1.02	3.41	1.59
ABDOMEN	0.99	0.05	0.74	2.67	0.03	2.35	6.16	2.56
IV UROGRAPHY	3.92	0.27	2.99	11.67	0.21	8.82	25.53	10.42
HYSTEROGRAPHY	3 77	0.03	3.98	0 59	0 06	2.47	11.38	4.78
CHOLECYSTOGRAPHY	1.18	0 25	2.63	0.62	0.06	5.19	21.91	7.21
SKULL	0.03	0.01	0.61	0.35	5.66	3.56	3.29	1.35
BARIUM ENEMA	4.95	0.13	4.14	10.79	0.11	7.56	22.72	9.96
BARIUM MEAL	0.98	36.43	3.99	5.25	7.06	9.32	8.82	6.73
THORAX	0.03	0.16	0 22	0.64	0.42	0.44	0.44	0.28
CEREBRAL ANGIOGRAPHY	2.14	0.07	2.84	10.98	159.32	19.41	16.54	12.33
THORACIC ANGIOGRAPHY	1.74	10 01	2.14	13.49	19.94	4.26	5.27	5.01
ABDOMINAL ANGIOGRAPHY	4.89	0.52	4.81	11.03	0.75	33.45	54 07	20 24
INFERIOR LIMBS ANGIOGRAPHY	4.32	0 08	2.73	8.05	0.08	9.61	24.05	9.88
PHLEBOGRAPHY	4.49	0.19	2.71	6.16	0.51	15.29	28 11	11 11
OBSTETRICAL ABDOMEN	2 11	0.03	1.88	0.95	0.06	1.71	6.34	2.83
PYELOGRAPHY	1.83	0.09	4 53	0.55	0.08	6.13	15.81	5.91

* For female patients only.
GON : Gonades; BRE : Breast; RBM : Red Bone Marrow; LUN : Lung; THY : Thyroid; BON : Bone Surface; REM : Remainder Organs; EFF : Effective Dose Equivalent.

Table 3. Organ doses by x-ray examination category (mSv).

Although it is difficult to precise effect of radiographic technique used in carrying out this examination on the radiosensitive organ such as the breast, it might be thought that it is not necessary for the breast to be directly irradiated during a barium meal. However, because of their anterior position in the body, the doses to this organ depend not only on the degree of field collimation but also on whether the radiation is predominantly AP or PA.The rather high proportion of remote control fluoroscopy units with under-couch image intensifiers operating in France probably leads to more AP projected x-ray beams in this examination and hence large breast doses.

In general, it can be argued that the complexity of the radiographic technique used in performing a given examination, (film number, type of projection, fluoroscopy...) is largely responsible for the highest doses estimated in the context of the methology followed. This is clearly illustrated by the

thyroid dose values for a cervical spine exam and for a conventional cerebral angiography. In the latter, the number of x-rays (46), the fluoroscopy used for selective arterial catheter placement (mean time of 482 s.), lead to a thyroid dose of about 160 mSv, whilst the dose to the same organ for a cervical spine is more than ten times lower i.e. 10 mSv.

As far as the red bone marrow doses are concerned, considerable variations are also apparent in comparing the mean values of dose obtained with those reported by different authors for some particular examinations. Discrepancies of up to a factor 10 between the mean doses for a given examination were found, probably due less to differences in radiographic practice than to important differences in the dosimetric technique employed. Similar discrepancies also occur in dose data reported for other organs. Regarding the remainder organ doses, their contribution to the effective dose equivalent has been found to range from 40% for a conventional cerebral angiography to about 90% for a cholecystography.

Concerning the effective dose equivalent to patients having x-ray examinations, not surprisingly, the largest mean values are associated with the 'complex' examinations (angiographies, intravenous urography, barium meal). Conversely, the figure from the survey for a chest examination, the most frequent of all radiological procedures conducted in France, is 0.28 mSv.

Although there are currently no recommended limits for the doses which patients may receive from medical radiological examinations, it can be pointed out that 11 % of the x rays annually performed in France result in higher effective dose equivalents than the limit of 5 mSv that any member of the public may annually receive from all other artificial sources of exposure, and the corresponding percentage of the examinations the dose of which is over 2 mSv (mean exposure due to the natural background in France) is about 48 %.

Collective Dose Estimates

The collective effective dose equivalent estimates obtained in combining both examination frequencies and the indivual effective dose equivalent mentioned above, are presented in Table 4 for each examination category. As it can be noticed, from the collective point of view, the most important examination is, by far, the IVU which leads to a collective exposure of about 20,500 person-sievert, the corresponding values for lumbar spine, barium meal and barium enema being closely equal to 8,000 person-sievert. Regarding the use of fluoroscopy technique, its contribution to the collective dose ranges from 3% for thorax to 70% for thoracic angiography, with an average figure of 15%.

Finally, the total collective effective dose equivalent received by patients who undergo diagnostic radiology examinations in France in 1982 is about 86,000 person-sievert, i.e. 158 person-sievert per million inhabitants.

EXAMINATIONS	COLLECTIVE EFFECTIVE DOSE EQUIVALENT (person-sievert)	FLUOROSCOPY PERCENTAGE (%)
CERVICAL SPINE	1,680	18
THORACIC SPINE	2,100	16.5
LUMBAR SPINE	8,580	13
SACRO-LUMBAR SPINE	3,400	7
PELVIS-HIP	5,350	3
ABDOMEN	4,120	6.5
IV UROGRAPHY	20,580	11.5
HYSTEROGRAPHY	810	17
CHOLECYSTOGRAPHY	4,860	34.5
SKULL	4,990	10
BARIUM ENEMA	8,210	21.5
BARIUM MEAL	7,460	31.5
THORAX	4,110	3
CEREBRAL ANGIOGRAPHY	1,780	15
THORACIC ANGIOGRAPHY	680	70.5
ABDOMINAL ANGIOGRAPHY	5,590	34
INFERIOR LIMBS ANGIOGRAPHY	280	15
PHLEBOGRAPHY	940	37
OBSTETRICAL ABDOMEN	930	8
PYELOGRAPHY	370	24

Table 4. Collective Effective Dose Equivalent by examination category (France 1982).

The Genetically Significant Dose (GSD)

The previous results concerning both frequencies and gonadal doses associated with each examination type have been combined with child expectancy of the patients to obtain the GSD. The total GSD to the population of France due to the diagnostic radiology examinations in 1982 is estimated to be 295 μSv. The male and female contribution to the GSD are 30.2% and 69.8%, respectively. The main contributors to the GSD are examinations of the urinary system and pelvis which acount almost 60% of the total GSD. Overall, fluoroscopy accounts for only 10%, while radiographic examinations contribute 90% of the GSD.

CONCLUSION

The main conclusions of this multiannual study are the following :

- As far as the Genetically Significant Dose is concerned, the figure obtained from the survey represents approximately 14% of the total GSD from natural background.
- Concerning the ICRP "remainder organs" their contribution to the effective dose equivalent associated with each examination category must be stressed. In particular, in almost 60% of the examinations considered, doses received by these organs represent more than 40% of the effective dose equivalent.
- Over 80% of the routine examinations yearly carried out in France have effective dose

equivalents that are more than the average per caput annual dose of 2 mSv due to the natural background radiation in France.

- Estimate of the collective effective dose equivalent associated to the radiological practice is 86,000 person-sievert i.e. an individual effective dose equivalent of 1.58 mSv per year (1.48 for females and 1.70 for males).

- Finally, there have been few comprehensive publications of organ dose data for diagnostic radiological procedures, which probably reflect the difficulties of achieving suitable dose estimates for many organs. Comparison of our organ dose data with results from similar surveys indeed indicates large discrepancies for certain organs and examinations, which must be due at least in part to the differences in dosimetric technique used. Although higher levels of patient exposure have been found for France (the British collective dose per 10^6 inhabitants is six time lower than the French collective dose), without comparison of the image quality as well as the patient doses, it is difficult to verify whether optimal conditions have been applied in other countries.

The introduction of quality assurance programs in diagnostic radiology, which is being encouraged in Europe by the CEC, might certainly help to establish and maintain optimal procedures in this field.

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- National Radiological Protection board (NRPB) Chilton Didcot - Oxforshire OX11 ORQ - U.K
- Ospedale S. Maria della Misericordia (Unità Sanitaria Locale n° 7) Udine, Italy.
- GSF-Institut für Strahlenshutz - Ingolstäter Landstr. 1 - München - Neuherberg. - FRG.

V. Publications:

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Training

Maccia, C. Doses to patients from diagnostic radiology in France. Advanced Seminar on Diagnostic Radiology Dosimetry. CEC Joint Research Centre Ispra-Italy. May 18-22, 1987.
Maccia, C. Doses to patients from diagnostic radiology in France. International Seminar on "Radiation Protection and Quality Assurance in Radiodiagnostic" Madrid 13-17 March 1989.

Title of the project no.:

Quality Assurance in medical diagnostic radiology, expert system.

Head(s) of project:

C. Maccia

Scientific staff:

S. Castellano, M. Benedittini, C. Lefaure, F. Corlobé, F. Fagnani, C. Maccia.

I. Objectives of the project:

- To evaluate the status of the radiological equipment currently used in mammography.
- To assess pediatric radiological practices in France and to evaluate the feasability of reducing dose levels in the case of newborn pelvis x-rays.
- To study the total population exposure due to the CT examinations.

II. Objectives for the reporting period:

III. Progress achieved:

a) The newborn pelvic x-rays.

The extrapolation of the statistical data obtained through the CEPN-INSERM national survey carried out in 1982 enabled us to estimate the total number of pelvis x-ray examination performed for 0-1 year old children aiming at diagnosing the congenital hip dysplasia (CHD). A figure of 725,400 pelvic x-rays was found which represents a rather high number of examinations per 1,000 inhabitants : 1,563 and 550 examinations for 1,000 girls and boys respectively.

Out of these data, only the examinations which were declared by the radiologists as "routinely" performed were selected (33% for males and 57% for females). The figures concerning this sub-population showed that, one out of three boys and two out of three girls undergo in the first year of their life a pelvic x-ray, what indicates a quasi-systematisation of this radiological practice although no official systematic CHD x-ray diagnosis programme exists in France. As far as the examined children's age distribution is concerned almost 22.5% of these examinations are carried out in the first ten days of life, slightly more than 40% between the third and forth month and 37.5% after the forth month. Knowing that before the third month of life the pelvic x-ray is not reliable method for CHD diagnosis, the routine x-ray done in the first few days may thus appear as *needlessly expensive and irradiant.*

Concerning the dose evaluation problem, measurements have been performed directly on 35 children aged between 3 and 5 months (16 girls and 19 boys) by using thermoluminescent dosimeters (lithium borate chips). In the case of male children, gonads were shielded, while for girls no special protection was set up. Only additional diaphragms were used to reduce the radiation field (Ø 10 cm). All studied examinations were carried out on "Titanos 50 " tables suitable for radiopediatry and with a three phased generator.

The x-ray tubes were equiped with a tungsten anode. The two available focal spots had a size of 0.6 mm and 1.2 mm respectively. The tube inherent filtration was of 2 mm Al and an additional filtration of 1 mm Al was always used.

The average physical and technical parameters are given below :

KV : 40 (small focus spot)	53 (large focus spot)
mAs : 16 (small focus spot)	7 (large focus spot)
number of films : 1 (girl)	1.2 (boys)
FFD : 110 cm	
Diaphragm diameter : 10 cm	
Film size : 18 x 24	
Patient thickness : 8 ± 2 cm	
No grid - Rare earth screen cassette	

The skin dose in the beam center was estimated : as far as the gonad dose is concerned, TLDs were on the same level with the iliac joints over the presumed location of the ovaries for girls, and on the scrotum for boys. The figures obtained were : 0.01 mGy for testes and 0.30 mGy for the ovaries.

If a systematic x-ray diagnosis program existed in France the resulting genetic risk could then be evaluated. According to the ICRP recommandations the risk of serious hereditary ill health within the two first generations following the irradiation of either parents is taken to be about 10^{-2} Sv^{-1}. Both parents being involved in a radiological mass screening and assuming there is no gonadal shielding in females, the genetic risk would be 1.02/695,000 i.e. 1.45 for 1 million births.

b) Quality Assurance in mammography

In France, as in many other developed countries, breast cancer remains the major cause of disability and death among women. The number of new cases of invasive breast cancer was estimated to be 275,000 in 1981 with a corresponding number of deaths of 8,900.

The most relevant way to reduce this incidence relies upon the early detection of infra-clinical lesions such as tiny microcalcifications or low contrast lesions which may be currently considered as indications of malignancy. It is also widely recognized that mammography is the most effective diagnostic imaging technique to be performed in order to detect such minute details and irregular borders of lesions.

In the last two decades many countries have carried out breast cancer screening programs on asymptomatic women by exclusively using this technique.

Nevertheless, many controversies have arisen in the form of scientific debates over both radiographic techniques to be used (type of detector, number of projections...) and epidemiological considerations (age of patients to be screened, dose risk relationship curve...).

As far as France is concerned, some local trials are being performed and some others, even national, are likely to be scheduled in the forthcoming years. Unfortunately all these initiatives are characterized by the absence of well defined quality control procedures. In this respect, it seemed important, from the physicist's point of view, to proceed towards a general overview of a sample of the radiological equipment which potentially could be used within the framework of such trials. In doing so, a pilot survey of 75 dedicated mammographic units installed in both public hospitals and private radiological offices all over in France was conducted and radiological techniques (kVp's setting, anti-scattering grid, magnification...), likely to influence both breast dose and image quality, were evaluated.

MATERIALS AND METHODS

In 1987, the CEPN-INSERM in close cooperation with the Comission de Radiodiagnostic of the Société Française des Physiciens d'Hôpitaux (SFPH), carried out, in 52 public hospitals and 23

private radiological offices, a pilot survey the aim of which was to create a statistical data base dealing with the radiographic techniques commonly used in mammography.

A questionnaire was sent to all radiological centres asking for several physical parameters which may be roughly split into the following four categories : those associated with the installation itself (geometry, x-ray tube, focal spot size, target materials, filtration ...); those inherents to "optional" devices which nevertheless affect patient dose (phototimer, anti-scattering grid, compression apparatus, field diaphragm ...); those connected with the image receptor (film, screen, film processing ...) and, finally, those related to the technical procedures (kVp, exposure time, mAs product, type and number of projections, practice modifications as a function of the breast density...).

From the collected data, three categories of units, corresponding to the most representative mammographic sets, were selected in order to perform both dose and image quality measurements. This selection was essentially based upon technical characteristics such as focus-to-film distance (FFD), focal spot size and target material (molybdenum or tungsten).

These three selected categories were a "500 T" and a "Sénographe I" both made by the Compagnie Générale de Radiologie (CGR) and a "Mammo diagnost" made by Philips. The Sénographe I contained a fixed Mo target with the beam filtered by 0.5 mm Al equivalent, and the two others were equiped with a rotating Mo target with a filtration of either 0.5 mm Al or 0.03 mm Mo. Concerning the focal spot size, Sénographe and Mammo Diagnost had only one focus of 0.6 mm, whilst two foci were available on the 500 T (0.1 mm and 0.3 mm). No grid was installed on the Sénographe and its FFD ranged from 10 cm to 50 cm, the other units having a fixed FFD of 60 cm.

Concerning the evaluation of the image quality, the RMI Model 152B, was used.

About 50 radiograms of the phantom, taken under different technical conditions (kVp, mAs ...), were randomly shown successively to six experienced radiologists in usual interpreting conditions (viewing box, magnifying glass). Credit concerning information on image quality was given for objects correctly identified by three viewers or more.

From the dosimetry point of view, two sets of measurements were performed using thermoluminescent dosimeters (TLD) ($Li_2 B_4O_7$/Mn pellets) which were previously calibrated against an X-ray beam for energy values ranging from 15 KeV to 100 KeV. The reading out system used was a "Toledo 654". The first set of measurements was carried out on a phantom made of different thicknesses of lucite which enabled estimates of entrance skin doses, exit doses and depth doses. The second set was carried out on 16 patients in order to compare the phantom doses with those actually received during an examination.

For practical reasons, all investigations related to dose and image quality were performed on six (two for each category) dedicated mammographic units chosen among those having participated to the survey. Since the objective of the survey was basically to simulate the real situation of mammography, no preliminary calibration of the selected x-ray units was performed and radiological

equipments associated to each installation (image recorder, processing, grids) were those routinely used.

Results.

- of the 75 units considered, about 85% were equipped with a Mo fixed or rotating anode (respectively 15% and 70%) and the remaining ones were equipped with a fixed or rotating W anode (respectively 6 % and 9 %).
- Three out of four x-ray tubes had only one focus, the size of which generally ranged from 0.3 to 1.2 mm, while, when a second focus is available, its size is of 0.1 mm.
- 40% of the installations did not allow mammograms to be taken with a FFD above 48 cm.
- only 60% of the practitioners actually used the phototimer, although 72% of the total number of units were equipped.
- an anti-scattering grid was installed on 47% of the mammography systems considered and on only one third of these magnification technique was possible.
- neither direct film mammography nor Xerox plate was used in the whole sample of participating centres and 12 screen-film combinations, often different from those suggested by manufacturers, have been found. In about a quarter of these centers, films were processed with equipment exclusively dedicated to mammography.
- kilovoltage settings for a 4 cm "average" breast shown large discrepancies and ranged from 19 kVp to 42 kVp with a mean value of 28 kVp all target materials together.
- practitioner's modifications of technique for a "dense" breast or a "fatty" one appeared as one of the most important sources of disparity encountered in the survey. In diagnosing a "fatty" breast for instance, some radiologists would tend to increase physical parameters namely : kilovoltage (16%), exposure time (5%) and phototimer sensitivity (6%). Conversely, some others, would tend to decrease the same parameters (9%, 12%, 12% respectively), 15% of radiologists made no modifications and the remaining 25% had no opinion.

Regarding a "dense" breast examination, almost all radiologists increased these parameters : kilovoltage (32%), exposure time (19%), both kilovoltage and milliampere-second product (28%), 10% keep parameters unchanged and, finally, 11% had no opinion.

Dose and Image Quality

Regarding entrance skin dose and optical density, findings obtained with the previously mentioned phantom of lucite when simulating the real range of kilovoltage are detailed in Table I.

Indicated values rely upon conditions "routinely" used in the considered radiological centres. As can be deduced from the table, the corresponding average entrance skin dose is 12.2 mGy while, according to the kilovoltage, doses may vary by a factor of five. However such discrepancies can be interpreted and linked to a series of physical parameters that may have an influence on the received dose, namely : speed of detector system, beam quality, choice of both exposure time and

kilovoltage, calibration of phototimer, type of anti-scattering grid, film processing.

	PHILIPS MAMMODIAGNOST-U		CGR SENOGRAPHE 500 T		CGR SENO I	
	I	II	I	II	I	II
21 kVp	n.m	n.m	n.m	6.8 *	26.7	16.2
				0.25 **	0.71	0.79
25 kVp	11.5	8.1	15.6	8.9	24.0	13.6
	1.00	1.50	1.33	0.73	1.85	1.08
28 kVp	8.6	4.6	18.3	7.8.	18.5	12.8
	1.20	1.40	1.90	0.92	2.13	1.28
31 kVp	5.5	2.9	13.2	6.2	14.7	11.5
	0.96	0.58	1.95	0.96	2.21	1.50
Film Screen	Kodak PE 205 Min - R	Agfa MR 3 Min - R	Kodak PE 205 Min - R	Kodak PE 205 Min - R	Min - R Min - R	Ortho - MA Min - R

routine operating conditions

* Entrance skin dose (mGy)

** Optical density.

Table I : Dose and optical density results as a function kilovoltage.

Patients		kVp	Thickness* (cm)	Entrance Skin Dose (mGy)	Exit Dose (mGy)	Optical Densities
	n° 1	32	3	24.9	0.71	1.3
	n° 2	31	2	12.8	0.44	1.2
5	n° 3	32	5	34.6	0.85	1.2
0	n° 4	30	2	9.2	0.93	1.4
0	n° 5	32	2	16.2	0.55	1.0
	n° 6	32	4	17.1	0.91	1.3
T	n° 7	32	5	26.4	0.71	1.2
	n° 8	32	4	12.6	0.57	1.2
	n° 9	28	4	9.8	0.39	1.1
	n°10	28	4	9.7	0.47	1.2
S	n°11	28	4	10.2	0.55	1.3
E	n°12	28	4	14.1	0.71	1.3
N	n°13	28	4	14.8	0.29	0.9
O	n°14	28	4	8.2	0.64	1.2
	n°15	28	3	9.4	0.51	1.2
I	n°16	28	5	17.1	0.77	1.3

* compressed breast.

Table II:"In vivo" dose measurements and film optical densities.

For each unit considered, depth dose measurements enabled us to estimate a mean breast dose figure as 12% of the entrance skin dose with a corresponding exit dose of 2%.
This latter value generally agreed with the "in vivo" dose measurement results (see Table II).
Concerning the image quality assessment, limits of detectability were deduced from the radiologist's observation of the RMI's phantom mammograms taken under routine interpreting conditions. In only one case (500 T unit No I), observers have detected the finest objects that are embedded in the phantom, (15 out of 16 wax blocks). In other cases, quality of the image is less accurate, and the resulting loss of information may be significant (only 5 out of 16 wax blocks are seen on Sénographe unit No I). As previously the impact of the kilovoltage applied on limiting detectability has been considered and has shown potential for improving the image quality (eg. sizes of the smallest detected microcalcifications: 0.20, 0.28 and 0.32 mm at respectively 25, 28 and 31 kVp on Mammo diagnost unit No I). In the same manner, using either anti-scatter grid or magnification techniques have improved detectability of the phantom structures with a corresponding increase of the entrance dose of 2.5 and 5 respectively.

DISCUSSION

A first general comment about the results deals with the necessity of harmonizing the various parameters which can interact with both dose and image quality. This might be achieved by introducing quality control procedures. Indeed, checking the beam quality seems the first action to be undertaken in order to ascertain for instance the reliability of kilovoltage.
Furthermore, estimates of optical densities associated with routine operating conditions suggest that the calibration of phototimer should be suited to the kilovoltage actually used as a function of the receptor sensitivity. In the same way, skin entrance dose values might be lowered by using exposure times shorter than one second. On the contrary, in spite of the increase in entrance skin dose, anti-scatter grids significantly improve the image quality and their use should be promoted on a larger scale.
Despite the small number of x-ray units considered in this pilot survey and the experimental character of this study, all these considerations, together with clinical aspects (eg. : examination techniques towards the variation of the breast density), call for a need for setting up a Quality Assurance programme in mammography in France. Besides quality control, such a programme might obviously include many activities like preventive maintenance, equipment calibration, training and in-service education of technologists and spreading of information. Only under such conditions, and particularly in the context of a breast mass screening project, the quality of mammograms would be guaranteed with a consequential real benefit for patients in terms of reliability of diagnosis and dose reduction.

c) C.T. examinations.

Diagnostic radiological practices in France have been strongly marked by the introduction of the computed tomography since the beginning of the "80"s. A national survey conducted by the Direction Générale des Hôpitaux related to about 650,000 C.T. examinations carried out in France already in 1985.

Since then, the total number of examinations yearly performed has rapidly increased being given the number of new C.T. machines installed each year (from 161 in 1985 to 345 in 1987 for instance).

Within this context, it seemed important to assess the effective dose equivalent associated with such a radiological technique as well as its impact on the patient collective dose.

As a first tentative attempt, a survey was therefore conducted In 1985 by the CEPN-INSERM to esthablish a statistical data base concerning 15 C.T. scanners operating in the PACA region (Provence Alpes Côte d'Azur). Originally, through this survey, C.T. technical related data (kilovoltage, tube current, filtration...), patient related data (age, sex, anatomical region examined...) and examination related data (slice number, slice thickness, exposure time....) were collected for about 700 different C.T. examinations. In a second step, to allow for variations in x-ray output, beam collimation and scanning geometry between all considered C.T. scanners and between different selections of the scanning parameters on a particular machine, the free-in-air axial dose profiles for a single slice at the centre of rotation were measured for each commonly used set of scanning parameters on each scanner. For such a purpose, a row of TLDs was used, with individually known sensitivities, spaced at approximately 1 mm intervals and of sufficient number to cover at least twice the nominal slice thickness and aligned along the axis of rotation .

Results of these dose measurements were matched with data collected during the survey, and used to assess, through a Monte Carlo simulation programme provided by the NRPB for the specific C.T. machines considered, the dose contribution of each scan slice to the dose received by each organ as a function of the anatomical region examined. Effective dose equivalents for all examinations were therefore evaluated by applying the ICRP weighting factors.

The dose range.

The free-in-air axial dose profiles measured at the centre of rotation were normalised to the nominal slice thickness to obtain the CT Dose Index (CTDI) for each single slice.

Considerable CTDI variations were observed in the survey depending on the slice thickness and on the C.T. machine studied. For instance, for the most commonly used radiological technique (1mm slice thickness, 130 kVp and 6.8 s.), CTDI values varied from 108 mGy to 340 mGy with an average value of 130 mGy : the latter being twice or three times higher when using the same technical conditions for 5 mm or 10 mm slice thickness respectively.

Besides such variations in dose, also large discrepancies were found concerning the technical protocols (number of slices) followed in performing different type of C.T. examinations as a function

of the part of the body examined : 11 to 13 slices, on average, during an head scan and 22 to 24 slices, on average, during an abdomen examination.

The assessment of the effective dose equivalent.

Average organ doses of each C.T. examination type observed in the survey and the corresponding effective dose equivalent values are detailed in Table 1 and Table 2 respectively.

EXAMINATIONS	GONADS	BREAST	R.B.M	LUNG	THYR.	BONE	REM
SCANNER (SKULL)	0.00	0.03	1 37	0 11	0.77	6.16	4 50
SCANNER (CERV. SP.)	0.00	1 72	1.22	1.21	27.80	3.63	0.93
SCANNER (LB.SPINE)	0.20	1.02	1.51	0.99	3.63	3.34	13.84
LIMBS	0.50	0.00	0.51	0.00	0.00	4.85	1.05
SCANNER (THOR.)	0 01	24 07	5.26	18.64	3.42	12 29	11 54
SCANNER (ABDOM.)	0 52	6 57	4.28	8.27	0.37	9.01	20.14
SCANNER (PELV.)	3.56	0.20	3.81	0.67	0.75	6 26	13 22

Table 1 : Average organ doses by C.T. examinations type.

	minimum EDE mSv	maximum EDE mSv	mean EDE mSv	Standard Deviation
Total body	0.20	29.84	4.80	4.42
Skull	0.48	7.51	1.73	0.87
Cervical spine	0.79	4.07	1.77	0.83
Limbs	0.20	1.86	0.71	0.64
Lumbar spine	0.25	10.76	4.90	2.15
Abdomen	1.03	29.84	9.09	4.70
Thorax	1.65	29.84	10.42	6.41
Pelvis	1.16	10.91	6.51	3.00

Table 2 : Effective dose equivalent (EDE) by C.T. examination category.

As can be seen average EDE values may range markedly depending of the examination type (factor of six between skull and thorax C.T. scan for instance). Furthermore, Table 2 gives maximum EDE values which may occur when particular examination techniques are used (high resolution mode, > 30 slices).This is particularly true for abdomen and thorax for which maximum EDE is about 30 mSv and the mean EDE is about 10 mSv.

Taking into account the relative frequency of each examination type estimated through the National survey previously mentioned, the collective effective dose equivalent associated with the C.T. practices was assessed combining statistical and technical data gathered in the PACA C.T. sanner sample togheter with the mean EDE of each examination type.

In all, a figure of 6,318 person-Sv. was found corresponding to 1,300,000 C.T. examinations performed in 1987 in France i.e. about 7% of the total collective effective dose equivalent attributable to diagnostic radiology practices.

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

- National Radiological Protection board (NRPB) Chilton Didcot - Oxforshire OX11 ORQ - U.K
- Ospedale S. Maria della Misericordia (Unità Sanitaria Locale n° 7) Udine, Italy.
- GSF-Institut für Strahlenshutz - Ingolstäter Landstr. 1 - München - Neuherberg. - FRG.

V. Publications:

Articles

Lefaure, C.; Maccia, C.; Corlobé, F. Cost-effectiveness and risk associated with infants' hip dysplasia screening in France. Ann. Radiol. 29 (3/4): 393-399; 1986.

Reports

1) Maccia C.; Castellano S. L'assurance de qualité en radiodiagnostic : le cas de la mammographie. Rapport CEPN 120. June 1987.

2) Castellano S. Optimisation de la dose et de la qualité d'image en mammographie. Thèse de Doctorat de 3ème cycle. Université P. Sabatier. Toulouse. Octobre 1987.

Title of the project no.:

Optimization of image quality criteria.

Head(s) of project:

C. Maccia

Scientific staff:

B. Husson, F. Corlobé, C. Maccia.

I. Objectives of the project:

- To provide practitioners with a provisional acceptable list of radiological, technical and dosimetric criteria which could be useful to judge the quality of the radiographs routinely undertaken in diagnostic radiology.
- To assess the relevance of these criteria for six types of x-ray examination commonly carried out in everyday radiological practice : chest, skull, lumbar spine, pelvis and sacrum, urinary tract and breast.
- To provide practical considerations on how to reduce and to optimise patient doses in diagnostic radiology.

II. Objectives for the reporting period:

- To complete the statistical data analysis of the trial conducted in different x-ray departments in Europe.
- To contribute to the elaboration of the final CEC document on quality criteria for radiographic images.
- To prepare the final report to be distributed to all the participating x-ray departments.

III. Progress achieved:

1. Introduction

In many countries, medical exposure of patients represents the greatest manmade contribution to the collective dose imparted to the population.

Many studies have already investigated the various aspects and level of such radiation sources, and have provided a baseline of dose data which can be used as a starting point towards the optimisation of radiological practices. Although the dose received by patient concept in it self, does not permit an evaluation of the quality of the diagnostic information imaged on a radiograph, it can be considered as one of the most relevant indicators of the quality of the radiological practice. Incidentally, for the optimization of the radiographic image, knowledge of technical characteristics such as image detector sensitivity or film-screen combination is required and physical quantities such as spatial resolution, optical density and signal to noise ratio can be directly measured.

In addition, there are some other methods to assess the image quality. One of these is the so-called R.O.C. curve analysis which is based on signal detection theory which provides a technique for measuring the discrimination between signal and noise, in a manner which does not depend on the observer's decision criteria. Unfortunately for the implementation of such an analysis a well established methodological framework is, almost always, needed. Radiologists in their daily practice, are faced with the crucial problem of establishing the most relevant diagnosis through the most suitable imaging technique while keeping the patient dose as low as reasonably achievable. In other words, they have to look, as reliably as possible, for a best compromise between "image quality" and "dose" using very simple criteria and methods.

As a first tentative attempt, a study group of the radiation protection programme of the CEC, initiated, in 1987, a project on the establishment of quality criteria for diagnostic radiographic images.

The main goal of this project was therefore to provide practitioners with a provisional acceptable list of **radiological, technical** and **dosimetric** criteria which could be useful to judge the quality of the radiographs routinely undertaken in diagnostic radiology.

This final report presents, on the one hand, the main results of the multinational trial set up by the CEC expert group to assess the relevance of these criteria and, on the other hand, provides practical considerations on how to reduce and to optimise patient doses in diagnostic radiology.

2. Method

In order to evaluate the suitability of the criteria listed in a draft document, the group set up a trial in different x-ray departments in Europe, the aim of which was to collect information on those types of x-ray examination included in the document.

A questionnaire was therefore circulated to 24 radiology departments which actively participated in the project by checking the suggested image quality criteria and commenting on their relevance.

Thermoluminescent dosemeters were also supplied for measuring the entrance surface dose for every radiograph assessed in the trial.

In addition to the very efficient collaboration of the personnel working in the participating hospitals, the trial deeply involved national laboratories and research centres responsible for the dosimetry and for the statistical data analysis respectively.

2.1. The questionnaire.

In structuring the questionnaire four main items were considered : the radiographic technique, the patient, the image criteria and the dose.

a) The radiographic technique

In performing a given x-ray examination type, radiological equipment characteristics as well as technical parameters may vary markedly from one hospital to another. Consequently, patient doses may also range significantly. It is therefore necessary to get all the relevant data which might have an influence on the patient exposure : type of equipment, total filtration, focal spot size, grid ratio and film-screen combination.

b) The patient.
Some patient related data such as height, weight, age and sex are of great interest. They can help correlate patient dose with the radiographic technique actually used and, they can also contribute to the improvement of the quality criteria definition.

c) Quality criteria for radiographic images.
In diagnostic radiology, the need to present the most relevant information in the image, in such a way that it is readily assimilated by the observer, is central to the problem. It is also clear that the quality of this information strongly depends upon different factors such as the size of the patient, radiographic technique (projection, positioning), x-ray beam quality and, finally, the visual perception of the observer (the radiologist). Now, within the context of the trial, and taking into account the general objectives of the CEC initiative, the radiologist members of the study group defined a provisional list of radiological criteria which refer, for each projection of each examination type, to important anatomical features that should be always visible, or visualized in a satisfactory manner, on a given radiograph. The compliance with all these criteria, would therefore allow the information imaged on the film to be much closer to the most suitable one which is needed to perform diagnosis for the patient.
This task has implicitly required that all radiologists participating in the study group agree on what and how medical information should actually be imaged in a "normal" x-ray film, and revealed the necessity of harmonizing the radiological terminology in Europe.
The radiologists deliberately excluded all pathological and/or abnormal considerations in establishing such criteria since they would not be avaliable for assessment in all images.
The x-ray projections for which the image criteria were defined are the following : chest (PA and Lateral), skull (AP, PA and Lateral), lumbar spine (AP, Lateral and Lateral projection of lumbo-sacral junction), pelvis (AP), urinary tract (AP before and after administration of contrast medium), and breast (unspecified).

d) Patient dose evaluation.
As far as the the dosimetry is concerned, Entrance Surface Dose (ESD) including backscatter was measured using thermoluminescent dosemeter chips directly stuck on the x-rayed patient's skin.

Examination Type	Entrance Surface Dose (mGy)				
	min.	1st Quartile	Median	3rd Quartile	Max.
Chest (PA)	0.03	0.13	0.18	0.26	1.43
Chest (Lateral)	0.14	0.49	0.99	1.46	10.6
Skull (PA)	1.82	3.26	4.25	5.49	13.1
Skull (AP)	0.73	2.97	4.02	4.97	13.9
Skull (Lateral)	0.36	1.42	2.19	2.85	9.09
Lumb. Spine (AP)	0.83	5.65	7.68	11.2	59.1
Lumb. Spine (Lateral)	2.38	12.7	19.7	30.1	108
L5-S1 junction	7.4	24	34.5	50.2	131
Pelvis (AP)	0.85	4.19	5.67	7.86	31.6
Urinary Tract (AP)	0.71	4.69	6.68	10.5	62.4

Table 1 : Entrance Surface Doses observed in British survey in 1983-84.
As a first tentative step in establishing ESD reference values, use was made of the extensive data

already collected during a national patient dose survey conducted in Great Britain in 1983-84. The spread of doses observed on 3,200 patients at 20 randomly selected hospitals is indicated by tabulating minimum, quartile and maximum values for each examination and projection included in the lists of quality criteria (see Table 1). Many of the doses in the upper end of the range may represent radiation levels that are unnecessarily high for optimal diagnostic usefulness. It is also true that many doses in the lower end of the ranges may result in poor image quality. However the quality criteria already contain requirements for acceptable images and these in themselves preclude the use of too low a level of radiation.
Guidance on an appropriate value of ESD was obtained from Table 2 which shows the percentage of hospitals in the British survey where the mean ESD measured on a sample of about 20 patients at each hospital falls below the various quartile values of Table 1.On average, 14% of hospitals fall below the first quartile, 40% below the median and 75% below the third quartile. It was therefore proposed that **provisional** reasonable ESD reference values should be based on the rounded third quartile values observed in the British survey. Because breast examinations were not included in this survey, the ESD reference value for mammography was derived from experimental measurements carried out at TNO Radiobiological Institute, taking into account that such a value should be based on a maximum average absorbed dose to the entire breast of 1 mGy.

Examination Type	Percentage of hospitals with mean dose less than : 1st Quartile	Median	3rd Quartile
Chest (PA)	15	30	75
Chest (Lateral)	17	55	77
Skull (PA)	20	40	73
Skull (AP)	20	45	70
Skull (Lateral)	25	40	60
Lumbar Spine (AP)	10	40	70
Lumbar Spine (Lateral)	15	45	80
L5-S1 junction	15	40	75
Pelvis (AP)	15	35	75
Urinary Tract (AP)	10	35	85

Table 2 : Percentage of hospitals in the British survey with mean dose on about 20 patients less than various quartile values.

2.1. The data collection.
In order to be able to collect all relevant data regarding the different radiographic techniques, examination modalities and compliance with the image criteria, all participating x-ray departments received from the CEC-DGXII a questionnaire. In addition to that, a package of thermoluminescent dosemeters was sent by three European dosimetry laboratories to all these x-ray departments to allow patient doses to be measured.
Actually, a minimum number of 10 average-sized adult patients were to be taken into consideration for each x-ray projection.
Examinations were to be undertaken using the radiological equipment under routine operating conditions without any previous quality control being performed specifically for the trial.

Individual patient dose received during a given x-ray projection, was to be measured using a TLD pellet stuck as close as possible to the centre of the x-ray field, on the patient skin surface at the entrance side.
Compliance of the radiological films with the image criteria was to be checked by radiologists who performed the examinations and reported on the questionnaires.
Once completed, all questionnaires were to be sent back to the laboratories responsible for the dosimetry namely : the NRPB (National Radiological Protection Board) in the United Kingdom, the GSF (Gesellschaft für Strahlen-und Umweltforschung) in the Federal Republic of Germany and the USL (Unità Sanitaria Locale n°7) in Italy.
The close collaboration established between these three laboratories, which also provided a preliminary dosimetry intercomparison to ensure reliability of the dose measurements and read out the hundreds of dosemeters sent throughout Europe, was the key element of the success of this delicate phase of the trial.
Finally, patient dose data, together with the completed questionnaires, were collected and centralized at the INSERM U240 (Institut National de la Santé et de la Recherche Médicale - Unité 240) in France for the evaluation of the trial.

3. Results - General statistics.
Despite rather complex management problems, the trial was successfully completed and a satisfactory number of questionnaires were collected for each x-ray examination category considered (see Table 3).
More than 900 patient examinations were therefore evaluated through the questionnaires and more than 1,200 dose measurements were actually performed in 24 different x-ray departments of 10 European countries.

Examination Type	Number of Countries	Number of X-ray Depts.	Number of Patients	Number of * Dose measurements
Chest	8	16	211	300
Skull	5	12	117	223
Lumbar Spine	7	14	149	204
Pelvis	6	13	139	134
Urinary Tract	7	15	155	191
Breast	8	15	160	160
Total	10	24	931	1,212

* All projections together.

Table 3 : General statistics of the quality criteria trial.

3.1 The validation of the image criteria.
The percentage of wasted films (declared unacceptable for the diagnosis by the radiologists), the percentage of radiographs for which the radiologists participating in the quality criteria evaluation have answered positively with respect to all diagnostic criteria, and the percentage of hospitals where the average patient dose has met the CEC dose requirements, are shown in Table 4 for

each x-ray projection.

Examination Type	Wasted Film (%)	Film meeting all diagnostic criteria (%)	Hospitals meeting dose requirements (%)
Chest PA	2	79	69
Skull AP	10	74	89
Skull PA	10	65	56
Skull (Lateral)	5	67	75
Lumbar Spine AP	10	69	50
Lumbar Spine (Lateral)	4	77	69
Pelvis AP	6	77	58
Pelvis (Lateral)	5	83	67
Urin. Tract (before inject.)	6	62	87
Breast	5	79	33
Average	5.1	72	65.6

Table 4 : Validation of trial results.

As one may notice the wasted film rate values compare well with those generally found in the literature. It can therefore be deduced, from such a point of view, that there was no methodological bias associated with the trial, either in terms of excessive attention paid by radiographers in performing the examinations, or in terms of particular severity required by radiologists for the quality of radiographic images. Two examination types (skull and lumbar spine) score particularly high (10%), the other ones reach, on average, a figure of 5%. Such an encouraging finding, nevertheless, raises the more general problem of the wasted film level which should always be kept as low as possible in diagnostic radiology to limit unnecessary patient exposures.
Compliance rates with the provisional CEC dose requirements markedly vary (close to a factor of 2) depending on the projection considered.
A tentative attempt was made to establish a relationship between the percentage of hospitals meeting dose requirements and "good radiological practices" which can be defined as a low level of wasted film. Unfortunately no significant correlation was found between those two indicators. For instance, the skull A/P projection (carried out in 89% of x-ray departments under correct irradiation conditions) has the highest reject rate (10%). Conversely, for chest x-rays, which are characterized by the lowest reject rate value, reference dose values are exceeded in 31% of x-ray departments considered.
As regards the percentage of radiographs having positively met all image criteria, the resulting average figure, all examinations together, is relatively high : 72%.
In particular one may notice that, if for each examination one adds the corresponding reject rate figure, the score of 100% is never reached.
This means that some radiographs are judged by the radiologists as acceptable for diagnosis, even if they do not perfectly comply with all diagnostic criteria. In other words, when radiologists make a

judgment on the acceptability of the radiographic image for a particular diagnosis, they would spontaneously be less restrictive than the CEC image criteria.
This result raises the problem of adapting the list of the image criteria to the routine radiological practice.

3.2. The analysis of responses to the questionnaire (Chest PA)

An example of the responses to the questionnaires concerning the image criteria is reported in the following table.

IMAGE CRITERIA CHEST (PA)	YES (%)	NO (%)
- Six anterior costal arches ?	98.6	1.4
- Symmetrical reproduction of the thorax ?	98.6	1.4
- Costopleural boundary from the apex of the lung to the diaphragm ?	94.7	5.3
- The vascular pattern in the lung periphery ?	98.1	1.9
- Sharp reproduction of the peripheral vessels, the border of the heart and the diaphragm ?	96.6	3.4
- Retrocardiac lung and mediastinum ?	88	12
- Is this film acceptable for diagnosis ?	99	1

Table 5 : Image criteria questionnaire results (Chest PA).

As can be seen, 12% of chest x-rays do not suitably show retrocardiac lung and mediastinum. Although such a finding can be attributed to the high kilovoltage technique that was used by the majority (90%) of the x-ray departments in the trial and which does not comfortably allow the radiologist to study the mediastinum, a proper evaluation of such an anatomical region should always be possible on a chest PA film. It is therefore very surprising that only 1% of the radiographs are not accepted for diagnosis.
In the light of these preliminary findings, "hospital by hospital" data analysis was carried out to further evaluate the relevance of the image criteria. A "multi-score system" was then developed for each criterion and implemented for all considered projections.

3.3. The image scoring system

The starting point from which the scoring system was created was the apparent discordance observed in the questionnaires between the different radiologists' answers.
If one refers for example to the chest PA projection, (see Table 8) one may notice that among all considered films, 12% do not allow the retrocardiac lung and the mediastinum to be visualized, 5.3% do not reproduce the costopleural boundary from the apex of the lung to the diaphragm whereas only 1% of them are judged unacceptable for diagnosis.
One may therefore assume that there must be an aggregating process implicitly followed by the radiologist when checking radiographic image quality. Through such a process the radiologist would attribute to each criterion a relative weight according to its relevance to the final result i.e. the

acceptability for the diagnosis. A tentative attempt was made to translate "a posteriori" this process into a numerical scoring system. A group of four experienced radiologists was therefore set up and involved in this phase of the trial evaluation. For each criterion listed in the CEC draft document the group was asked to identify the basic factors making it relevant to the image acceptability. Three different dimensions were deduced from such a practical approach : Medical, Technical and Positioning.
Considering for example the same criterion as above, "retrocardial lung and the mediastinum", the group conclusions were : this part of the body should always be visualized on the film because of its importance for the diagnosis (Medical dimension); such visualization is possible only through a satisfactory contrast level (Technical dimension) and may depend on the positioning of the patient (Positioning dimension). In a more general way, making a radiological film acceptable requires that all potentially visible anatomical structures should be shown (**M** for "medical") and adequately contrasted (**T** for "technical"), and that the part of the body projected on the film should be aligned to the x-ray field size (**P** for "positioning").

IMAGE CRITERIA **CHEST (PA)**	**M***	**T****	**P*****
- Six anterior costal arches.	2	2	2
- Symmetrical reproduction of the thorax.	2	2	2
- Costopleural boundary from the apex of the lung to the diaphragm.	2	3	1
- The vascular pattern in the lung periphery.	2	2	0
- Sharp reproduction of the peripheral vessels, the border of the heart and the diaphragm.	1	2	1
- Retrocardiac lung and mediastinum.	3	3	1
GLOBAL SCORE	12	14	7

Scoring system : 0 = irrelevant ; 1 = minor ; 2 = important ; 3 = fundamental.

M * : Medical Score; T ** : Technical Score; P *** : Positioning Score.

Table 6 : Example of the image criteria scoring system (chest PA).

On such a basis, agreed by all radiologist group members, it was therefore decided to translate this process into a multi-numerical scoring system related to the previously mentioned dimensions. An example of this scoring system for the chest image criteria is given in the Table 6.
As shown on the table, each criterion has three scores corresponding to the basic dimensions mentioned before and each score may range from 0 (irrelevant component) to 3 (fundamental component).
Supposing the M-T-P scoring system reliable, one may see that the same image criterion, for instance "sharp reproduction of the peripheral vessels, the border of heart and the diaphragm" has not the same relevance for the radiologist when medical or technical view point is taken into account.
This **theoretically** implies that, when the M-T-P scores are generally low, the answer to a criterion

may be negative without having any impact on the final result of the film, that is to say, its acceptability for the diagnosis.
Conversely, when a particular criterion scores higher, for instance "visualization of the retrocardial lung and the mediastinum", a negative answer strongly affects the acceptability of the x-ray film.
This scoring system, combined with the questionnaire answers, was then used to find out the possible existing relationships between the dosimetric results and the quality of the radiographic images obtained in the participating x-ray departments.
Due to the absence of any further information regarding the actual x-ray films, the mean global score value, averaged over all the x-ray examinations carried out in each hospital considered, was taken as the most relevant numerical index to proceed towards the practical implementation of this scoring system.

3.4. The image criteria evaluation

A comparison between the dosimetric results and the image quality evaluated using the previously mentioned scoring system, was carried out for all the considered projections. Figure 1 shows the increasing average entrance skin doses measured for the chest PA projection in 16 participating hospitals (vertical bar chart) and illustrates the corresponding mean global score values.

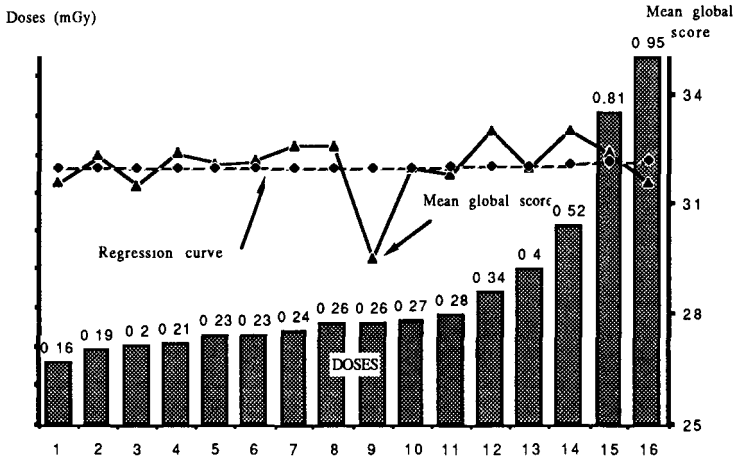

Figure 3 : Image quality score and doses. Chest PA.

Despite the idea that this type of examination would technically be the most "standardized" one, the average doses estimated for each x-ray department markedly vary (factor of six) from a minimum value of 0.16 mGy to a maximum of 0.95 mGy.
Compliance with all the image criteria, identified in figure 1 by the mean global score value, is achieved for all examinations in only two out of sixteen hospitals (number 12 and 14) ; unfortunately the corresponding average entrance skin dose values are above the provisional CEC dose reference value of 0.3 mGy.
These two hospitals, as indicated in the figure, are therefore characterized by x-ray films for which answers to all the image criteria are all positive and by the maximum mean global score value.
Three other hospitals do not comply with the CEC dose requirements but their mean global score values are much lower.
In order to be able to assess the general trend of the image quality as a function of the increasing

doses, a regression of score values on hospitals ranked according to their mean doses is also plotted on the Figure 3 (dotted line).
As clearly shown, the general quality of the radiographic images tends to be comparable between all the considered x-ray departments.
This demonstrates that, without taking any consideration of patient doses, almost the same level of reliability in radiological practice is achieved in different hospitals. Unfortunately, such a "good" medical practice is not often accompanied by a suitable dose level and may result in a wide range of doses as shown on Figure 1.

4. Selecting the "optimal" technique.

In this final step of the evaluation of the image criteria relevance, the scoring system was used to select the "optimal" radiographic technique which would comply with both diagnostic and dosimetric requirements recommended for the trial.
To reflect, as realistically as possible, the type of radiological information to be imaged on the film in order to guarantee its acceptability for the diagnosis, priority was therefore given to the **medical dimension** of the global score.
For each x-ray projection type, a subset of films was created keeping only those films for which the total medical score was within 1 of the maximum value.
To this subset obviously belong all films complying with all image criteria, and some other films for which, from the medical viewpoint, a minor image criterion i.e. one having a medical score of 1 or zero, is not met.
As regards radiolgraphic technique, only two aspects were considered, namely automatic exposure control and the speed of the film-screen combinations, in order to compare results obtained with so many different radiological units.
Basically, discrimination was made between the x-ray tables equipped with an Automatic Exposure Control system (AEC) and those manually operated (NO AEC). Regarding the film-screen combinations, attention was paid to whether the CEC document speed requirements were met or not.

4.1. The selected techniques.

On average, because of their bad medical score, 20% of all "acceptable for diagnosis" films were eliminated through the above selection procedure. Furthermore, if one adds to that figure the compliance with the dose reference values, the percentage of the discarded films reached 50%. Almost all discarded films were undertaken with unsuitable film-screen combinations and dose influencing parameters manually selected (kilovoltage, tube current, exposure time).
Additionally, automatic exposure control systems have completely proved their relevance in assuring lowest patient doses for the same image quality (films having the same total medical score).
Such findings however may only be achieved through an established radiological equipment quality control policy and skilled personnel.
Figure 2 indicates that implementing the image quality selection procedure leads to keep 167 high image quality score chest x-rays out of 208 "acceptable" films, corresponding to three groups of radiographic technique shown in the chest "pie chart".
There are no manual radiographic techniques using a low speed film-screen class (NO AEC < 200), and very few examinations at such speed (AEC <200) are carried out with an automatic exposure control system (1 hospital).
The selected high image quality score films are undertaken either with an automatic exposure control system or with manual equipment but the great majority have speed class above 200 (AEC ≥200 and NO AEC ≥200 respectively).
It should be noticed that a dose hierarchy is clearly respected as a function of the technique considered : the lowest mean dose value, 0.19 mGy, for AEC ≥200 examination category, 0.39 mGy

for NO AEC ≥200 examination category and, finally, the highest figure, 0.81 mGy, for AEC <200 examination category.
In particular, for this latter, all examination doses exceed the CEC dose reference value of 0.3 mGy. This clearly demonstrates the strong impact of the automatic exposure control devices in improving dose reductions when the appropriate film-screen combinations are used.
Finally, if one combines the image quality selection procedure results together with compliance to the CEC dose requirements, only one radiographic technique remains and 86 x-ray films out of 208 (41%) are kept as being comparable from both image quality and dosimetry points of view.

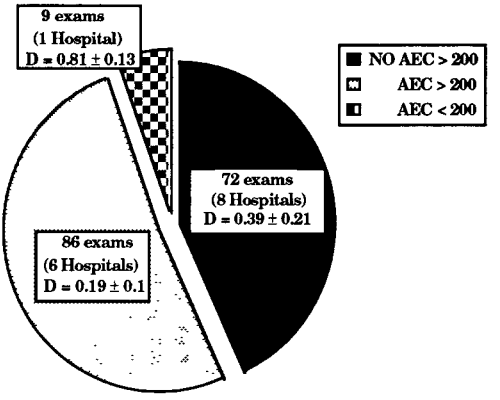

Figure 2 : Selection procedure results for the chest PA.

5. CONCLUSIONS

In view of proceeding toward the harmonization and optimization of radiological techniques commonly used in different European countries, the relevance of quality criteria for radiographic images together with dose requirements were checked on about 900 examined patients.
Due to the type of x-ray projections considered, more than 1,200 questionnaires concerning the x-ray films were collected and evaluated through a scoring system.
This approach has provided information on suitable technique (AEC and fast speeds) for reducing and optimizing patient dose while keeping the essential medical information imaged on the film.
Furthermore, this analysis puts forward two main domains which should be further taken into consideration :
- personnel training in radiation protection (radiologists and radiographers)
- establishment of quality assurance programmes in diagnostic radiology (good usage of radiological equipment and reduction of wasted films).

Through this approach, which is easy to be applied in an x-ray department, a possible modification of the current radiological practices might be envisaged without any interference with the final result of the examination : the diagnosis.
Finally, this first step toward the optimization of the patient radiation protection, emphasises, once more, the necessity of stimulating an active collaboration between radiologists and medical physicists.

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

- National Radiological Protection board (NRPB) Chilton Didcot - Oxforshire OX11 ORQ - U.K

- Ospedale S. Maria della Misericordia (Unità Sanitaria Locale n° 7) Udine, Italy.

- GSF-Institut für Strahlenshutz - Ingolstäter Landstr. 1 - München - Neuherberg. - FRG.

V. Publications:

Articles.

Maccia C.; Wall B.F.; Padovani R.; Shrimpton P.; Husson B. "Results of a trial set up by a study group of the Radiation Protection Programme of the CEC" BIR Report 20 British Institut of Radiology London (1989).

Reports

Husson B. "Contribution à la recherche des critères de qualité d'images d'un cliché radiographique". DEA d'imagerie Médicale, Université de Paris XI, Dec. 1988.

Maccia C. "Les clichés de radiodiagnostic : contribution à la recherche des critères de qualité et optimisation. (résultats d'une enquête multinationale). Rapport INSERM 57. July 1989.

Seminars

Maccia C.; Padovani R.; Contento G. Indagine Europea sulla qualità dell'immagine e la dose al paziente in radiodiagnostica : aspetti dosimetrici, metodologia e risultati preliminari. In Proceedings of "Controlli di qualità ed Ottimizzazione nell'impiego delle radiazioni in medicina" 329-337. Brescia (Italy) 1988.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-F-315-F

Northern Regional Health Authority
Northern R.H.A. Headquarters
Benfield Road, Walkergate
GB - Newcastle upon Tyne NE6 4PY

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

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Telephone number: 091/2738811

Title of the research contract:

Automated quality assurance and patient dosimetry in diagnostic radiology.

List of projects:

Development of a computerized method for automatically monitoring X-ray tube and generator parameters which will be used for performing online quality assurance and patient dosimetry.

Title of the project no.:

AUTOMATED QUALITY ASSURANCE AND PATIENT DOSIMETRY IN DIAGNOSTIC RADIOLOGY

Head(s) of project:

Dr K Faulkner

Scientific staff:

Miss C-L Chapple
Dr R M Harrison
Mr C J Kotre
Mr R A Marsh

I. Objectives of the project:

a) To design and develop a computerised method of automatically monitoring tube and generator parameters to perform online quality assurance and radiation dosimetry.

b) To determine the benefits (quality of diagnostic information and exposure reduction) for the patient due to the introduction of automated quality control.

II. Objectives for the reporting period:

a) To further develop and implement a prototype instrument.

b) To evaluate the potential of the instrument for performing online quality assurance using phantom studies.

c) To perform a preliminary survey using patient equivalent phantoms to assess the potential of the instrument on the reduction of patient doses.

d) To establish links with other research groups, particularly in the fields of paediatric radiology, mammography and digital fluorography.

III. Progress achieved:

Methodology

A Compaq 386 microcomputer has been interfaced to a Picker x-ray unit comprising a Picker vector 70 generator and Dynamax PX401P tube. Nominal values of radiographic factors are inferred from signals produced within the x-ray control console in response to manual selection of factors. The electronic signals controlling the digital displays for the radiographic technique factors are intercepted and decoded using the programmable peripheral interface on the analogue to digital convertor board. X-ray tube voltage is extrapolated from the voltage across a precision potentiometer in the x-ray transformer tank, the tube current being directly measured. A calibrated set of potentiometers attached to the manual light beam diaphragm controls are used to deduce the area of the x-ray beam. A large area ionisation chamber, remotely controlled using the microcomputer, is used to measure the exposure-area product.

Data collection and analysis of the tube potential and tube current waveforms are performed by programs written in PASCAL, whereas the storage of data and patient dosimetry calculations are performed within an integrated software package (SMART). The waveform analysis program determines the mean peak and trough values for each waveform and also detects any transient spikes which may be present. X-ray tube output is specified at 1m and is determined from the exposure-area product and field size. Information is stored within a database containing four files. The first contains nominal values of radiographic parameters, the second the measured values of the parameters, the third the patient dosimetry information for each exposure and the fourth the cumulative dose record for each patient. The four files are automatically updated each time an exposure is made. A permanent record of the results is maintained on the system.

The software has been modified slightly to enable separate calculations of the radiation dose to the patient to be made on a separate computer. Using normalised organ dose data, the program will calculate organ doses, effective dose equivalent, foetal dose (if applicable) and estimate radiation risk. The operator is requested to enter information on technique factors for each radiographic exposure. The operator also decides which dose and risk estimates are to be used.

The accuracy and reproducibility of the automated quality assurance and patient dosimetry system has been investigated in a laboratory study. Quality assurance parameters have been compared with concurrent measurements using standard quality assurance techniques.

In a later series of experiments, doses measured using lithium fluoride thermoluminescent dosemeters loaded into a Rando anthropomorphic phantom were compared with those calculated by the automated system. Assessment of the patient dosimetry calculations was performed over a wide range of exposure factors for a number of radiological examinations.

Results

The mean difference between the tube potential measured using the automated quality assurance and patient dosimetry instrument, over a range of nominal settings from 50-130 kV, and that determined using a non-invasive divider was 2.69 kV. Tube current measured using the automated instrument was compared with that obtained using an oscilloscope at three nominal mA settings. The mean difference between the tube current determined using both techniques was 3.09 mA. A similar comparison was performed for exposure time measurements and the mean difference between the value given by the automated instrument and that derived from the waveform obtained using the non-invasive divider was 1.55 ms. The x-ray tube output measured by the instrument was compared with the readings obtained from a calibrated ionisation chamber. The mean difference between results obtained using the two methods was 0.002 mGy/mAs. Note that in all of the above comparisons there is good agreement between the two methods used, within anticipated uncertainties.

Dosimetry measurements have been performed for a series of radiographic projections and technique factors. It was deduced from the results of these investigations that the agreement between the measured entrance dose and that calculated by the instrument was within 30% for chest examinations and 10% for thoracic spine examinations. Ovary doses were investigated for simulated chest, lumbar spine and pelvis examinations. Agreement between the two methods was within 20% for all examinations except chest x-rays. For chest exposures, the ovaries received a small dose which was comparable with the background reading on the TLD and thus had large uncertainties associated with it.

Conclusion

A series of measurements had been undertaken which indicate that the automated quality assurance and patient dosimetry system can measure a number of radiographic exposure parameters with comparable accuracy to standard techniques.

The results of the dosimetry measurements have several implications. Firstly, they indicate that the automated system provides an accurate estimation of dose to organs lying within the primary beam. However, the dose to organs at the periphery of the primary beam cannot be accurately estimated using calculated organ dose data, which cannot be guaranteed. The calculation of energy imparted is not affected by slight changes in x-ray beam position. An advantage of the automated dosimetry system is that a large quantity of dosimetric data can be collected in a relatively short time. This dosimetry data is automatically entered into a database which facilitates rapid future analysis. A further advantage of the automated system is that low doses may be accurately estimated.

The software for separate calculation of the radiation dose to the patient has been utilised to estimate retrospectively foetal doses for radiographic examinations. Dose estimates made using the computer program were comparable with those using manual calculations.

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

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V. Publications:

1.0 Scientific Journals, Monographs

K Faulkner, C-L Chapple, P Hedley, C J Kotre and R M Harrison. Automated quality assurance and patient dosimetry in diagnostic radiology. Journal of Biomedical Engineering (in press).

C-L Chapple and K Faulkner. Computer curve fitting of normalised organ dose data. Computers in Radiology (in press) (Institute of Physical Sciences in Medicine, York).

C-L Chapple and K Faulkner. Computerised calculation of radiation dose to patients from radiography. British Journal of Radiology, in press.

C-L Chapple, K Faulkner and R M Harrison. An investigation into the performance of an automated quality assurance and dosimetry system in diagnostic radiology. British Journal of Radiology, in press.

2.0 Short Communications, Abstracts, Internal Reports

C-L Chapple, K Faulkner, R M Harrison and C J Kotre. Real time patient dosimetry and quality assurance in diagnostic radiology (abstract). Physics in Medicine and Biology (in press).

K Faulkner, C-L Chapple, C J Kotre, R M Harrison and R A Marsh. 1989 Connection of microcomputer to x-ray generator for purpose of automated quality assurance and patient dosimetry in diagnostic radiology. Regional Medical Physics Department, Internal Report.

C-L Chapple, K Faulkner, R A Marsh and C J Kotre. 1989 Automated Quality Assurance and patient dosimetry: System description, initial results and software documentation. Regional Medical Physics Department, Internal Report.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Ludwig-Maximilian-Universität
Kinderklinik
Röntgenabteilung
Geschwister-Scholl-Platz 1
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Contract no.: BI6-F-211-D

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Telephone number: 089/5160.3102

Title of the research contract:

The principles and the practicability of quality control and quality assurance in paediatric radiology.

List of projects:

1. The principles and the practicability of quality control and quality assurance in paediatric radiology.

Title of the project no.:

The Principles and the Practicability of Quality Control and Quality Assurance in Paediatric Radiology.

Head(s) of project:

Dr.med. Helmut Fendel, Leitender Akademischer Direktor

Scientific Staff:

Dr.med.K.Schneider, Dr.med.C.Bakowski, Dr.med.M.Kellner, Dr.med.S.Burtscher, E.Stein, Dipl.Psych.M.M.Kohn, MTRA K.Schweighofer, cand.med.J.Pehe, cand.med M.Weisbach, cand.med.M.Zeiler, cand.med.M.Hösle, cand.med.R.Pichlmaier, cand. med.H.Maul

I. Objectives of the project:

The project has the objective to screen and assess problems related to radiation protection in paediatric radiology. Optimization, quality control, and quality assurance of radiological imaging studies of newborns, infants, and children are different from those in adults. They are, however, mandatory in terms of radiation protection of the public because they concern the most sensitive part of the general population. The objective of the project is to survey how individual optimization measures can be effective in daily routine and to what extent they are practicable with the final goal of establishing standards for quality control and quality assurance in paediatric radiology.

II. Objectives for the reporting period:

The second part of the survey has been completed. Similar to the first part of the study, surveys and *phantom measurements* have been made for some typical examinations of children, this time in examination centres of clinics and departments which regularly x-ray children but which are *not headed by a full-time paediatric radiologist*. The third part of this study, i.e. surveys and phantom measurements in private pratices, has also been started. Finally, the same surveys and measurements, on patients, have been made in a number of paediatric radiology departments of nearly all member states of the EC and the quality of the examination and image criteria in comparison to the established quality criteria have been evaluated.

III. Progress achieved:

Two work groups have been formed. The first consists of experienced paediatric radiologists of several member states of the EC, who have been chosen with the help of the European Society of Paediatric Radiology. This group has since elaborated a document which is adapted for children and parallels the CEC Working Document "Quality Criteria for Diagnostic Radiographic Images". The second group consists of medical physicists, who have participated on a similar dose measurement trial for adults. Both groups have jointly begun a dose measurement trial for those examinations on those patients for which previously respective phantom measurement have been made. 78 study centres from several member states of the EC have since participated in this trial. The particular x-ray films have been evaluated using the criteria of image and performance quality that have since been established. Comparisons will be made between the measured patient surface entrance dose and both the respective technique and the evaluated quality of the x-ray film. Simultaneously, the usefullness and applicability of these quality criteria are being evaluated.

The results of the surveys and measurements in this year's reporting period again indicate that there are significant differences in patient dose both between and within the individual examination centres. The causes for these major differences also seem evident. The first analysis of the phantom measurements indicate that examination centres which are not headed by an full-time paediatric radiologist generally have higher patient exposures and the technique used is less optimized for the paediatric specialties. Thus far, the measurements, surveys and evaluation of film quality within the EC member states have shown that decisive optimization measures are necessary and possible. The existing "Quality Criteria for Medical Diagnostic Radiographs in Paediatrics" has proved itself to be quite useful.

The results obtained so far need further careful analysis. The dose measurements and film evaluations in all the EC member states must be completed. Further age groups must be included in these studies. Finally, when all surveys will be completed, recommendations for the optimization of x-ray examinations in paediatic radiology will then be compiled.

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

Dr.Drexler, Gesellschaft für Strahlen- und Umweltforschung, Neuherberg/München

V. Publications:

Fendel H, Schneider K, Kohn MM & Bakowski C.
Specific principles for optimization of image quality and patient exposure in paediatric diagnostic imaging.
In: Optimization of Image Quality and Patient Exposure in Diagnostic Radiology. Moores BM, Wall BF, Eriskat H & Schibilla H. (eds)
Britisch Institute of Radiology, Report 20, London 1989

Fendel H, Schneider K, Bakowski C, Burtscher S, Kohn MM, Kellner M, Schweighofer K, Pehe J & Weisbach M.
Resultate der Studie: Geräteoptimierung der konventionellen Röntgenaufnahmeeinrichtungen für Kinder.
In: Pädiatrie Aktuell Band 2: Neue bildgebende Verfahren in der Pädiatrie.
M. Zuckschwerdt Verlag, München (in press)

Fendel H.
Probleme der Qualitätssicherung in der pädiatrischen Röntgendiagnostik.
In: Qualitätssicherung in der Röntgendiagnostik. Flesch U. (Hrsg.)
Konstanz: Schnetztor-Verlag (in press)

RADIATION PROTECTION PROGRAMME

Progress Report

1989

Contractor:

Contract no.: BI6-F-299-P

Laboratorio Nacional de Engenharia
e Tecnologia Industrial (LNETI)
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Telephone number: (1)255.00.21

Title of the research contract:

1. Dose assessment and quality assurance in diagnostic radiology.

List of projects:

1. Dose assessment and quality assurance in diagnostic radiology.

Title of the project no.:

Dose assessment and quality assurance in diagnostic radiology

Head(s) of project:

J.P. Galvão

Scientific staff:

J. Vaz Carreiro, A. Ferro de Carvalho, Rui Serro, Estela Amaral, Paula Rocha, João Alves, Augusto Oliveira.

I. Objectives of the project:

Assessment of patient doses to portuguese population in order to have an overview of the radiological practices and the study of its improvement by application of quality assurance measures to reduce unnecessary doses to patients.

II. Objectives for the reporting period:

- Completion of field work for exposure measurements in mammography and dental radiography.

- Data processing with the NEXT programme.

- Dose assessment.

- Conclusion of dental, mammographic and CT installations surveys.

- Conclusion of the quality control programme for mammography installations.

III. Progress achieved: Dose assessment and quality assurance in mammography, dental radiography and CT equipment.

1. Methodology

In what concerns mammography, the exposure measurements were carried out with LiF thermoluminescent dosemeters (TLD-100) square rods on a perspex phantom with a thickness of 4cm, simulating an average breast. Average glandular dose was calculated taking into account the values of entrance exposure and HVL using the methodology described in NCRP report nº85.

Evaluation of film processing, was carried out through optical sensitometry and pH and temperature control of chemical solutions. Image quality was assessed with a ITO Kodak phantom and resolution patterns.

Concerning the dental survey, two kits with two questionaries were sent by post to each installation to collect data about the radiographic techniques, entrance exposure, X-ray equipment and image quality. Dose measurements were performed with LiF dosemeters.

CT survey was planned to get information on quality and doses. A IMI phantom was used to assess the slice position, accuracy slice thickness, linearity of CT values, high contrast resolution, CT number contrast scale, noise and low control detectability. Dose free-in-air in the isocenter and dose profile are being measured with TLD rods.

2. Results

More detailed information concerning the results of mammography and dental surveys is included in two reports attached to this document.

Regarding the dose assessment in mammography, the mean value obtained for the entrance dose (at surface) was 9.32 mGy, with values ranging from 0.45 - 35.16 mGy and for the glandular dose values range from 0.1 - 4.03 mGy and the mean dose value is 1.45 mGy.

In what respects the image quality, the mean value of the film optical density determined was 1.41 and the mean value for the resolution in sternum-nipple direction was 10.6 lp/mm. Regarding the detectability of details, in 98% of the images it was possible to detect round details of size inferior to 3mm and in 79% of the images, it was possible to detect microcalcifications of 0.2mm.

The results obtained from dental radiography survey show that 50% of X-ray units comply with technical standards and the entrance dose on a molar tooth radiography varies from 0.9 - 40.3 with a mean value of 8.4 mGy.

The data collected in the CT survey is presently under consideration.

3. Discussion

The results of mammographic survey point out, taking into account the results published in other countries, that the general equipment used is up to date, with exception of the viewing boxes. However, defective equipment and large deviation from normal performance were frequently detected. Optimization of mammographic systems can be easily achieved in many centers by improving equipment maintenance and setting up quality control on routine basis.

The results of dental radiography survey, show that a considerable number of X-ray units did not comply with the standards what is attributable to the age of the equipment. Entrance dose values higher than the mean value are related to underdevelopment of films.

This fact was demonstrated in several cases investigated.

Population dose assessment (NEXT Programme)

1. Methodology

The methodology applied is based on the NEXT programme. The exposure measurements are performed with ionization chambers Victoreen 66Ø model on phantoms in the premises. Public hospitals are surveyed considering the frequency of radiological examinations.

2. Results

Field work and data processing was completed. The average values and ranges of variation for the main technical parameters and the weighted average and the ranges of variation for the skin entrance exposures were obtained for the twelve surveyed projections in 65 premises involving 175 X-ray tubesspread all over the country. More detailed information is pres-

ented in attached document.

3. Discussion

A comparison was made with the data already published and the results comply with the mean values and ranges of variation for the more common technical parameters, skin entrance exposure and organ doses.

IV. Objectives for the next reporting period:

- Analysis of CT data and re-evaluation of field dose measurements.
- Statistical analysis of NEXT data.
- Elaboration of final reports.

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

- Direzione Sicurezza Nucleare e Protezione Sanitaria -
  ENEA
  Via Vitaliano Brancati 48
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  Viale Regina Elena 299
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VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1989

Contractor:

Physikalisch-Technische
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Bundesallee 100
D-3300 Braunschweig

Contract no.: BI6-F-316-D

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

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Title of the research contract:

Reduction of dose in X-ray in diagnostics by the choice of the optimal screen-film combination.

List of projects:

Reduction of dose in X-ray diagnostics by the choice of the optimal screen-film combination.

Title of the project no.:

Reduction of dose in X-ray diagnostics by selecting the optimum film-screen combination

Head(s) of project:
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Physikalisch-Technische Bundesanstalt
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Scientific staff:

Dr. Egbert Buhr

Mrs. Su-yue Fan

I. Objectives of the project:

The so called "image quality parameters" of film-screen systems, i.e. the characteristic curve, the modulation transfer function, and the Wiener spectrum, determine the detectable information content of medical X-ray images to a large extent. By using digital image processing methods this influence will be quantitatively investigated. Images are changed with respect to the "image quality parameters" and afterwards presented to medical doctors for evaluation by means of ROC-curves. The results should give hints, how to reduce the dose by the usage of screen-film combinations of higher speed without the loss of information.

II. Objectives for the reporting period:

Interim Report

During the reporting period the filtering operations and the production of noise, similar to the quantum noise, have been tested in the image processing system. The appropriate software for measuring the noise was developed. ROC experiments were done. The "image quality parameters" of the film-screen systems used in the project were measured. Nodules contained in images of the thorax were analyzed with respect to their profile of optical density.

III. Progress achieved:

Interim Report

1. A digital image processing system was used to generate two dimensional filter functions which correspond to the MTF-curves of film-screen combinations used in this project. With the help of these zero-phase-shift filters smoothed images of white noise and other structures like gratings or circles were produced.

2. The software was developed which is necessary to measure the autocorrelation function and the power spectrum, e.g. the noise power spectrum of digital images by using the image processing system.

3. Since the X-ray images shall be evaluated by physicians by means of the ROC-analysis, simple experiments were performed to test the ROC-method. By means of the image processing system noisy images were produced for this purpose whose noise power spectrum approaches the noise power spectrum of high sensitive film-screen combinations. The noisy images were superimposed with sharp edged or smoothed squares and rectangles of different size (from 4.5 mm^2 to 8 mm^2) and of different contrast. In this way 100 different test images consisting of these signals were produced and displayed on the computer screen to seven human observers who had to decide whether there was a "signal" in the noisy images or not. The results were analyzed by means of the ROC-method; two examples of the ROC-curves obtained are shown in Fig. 1 and 2. In Fig. 1 it can be seen that an increase of the contrast of the signal compared to the noisy background from 4 to 5 grey values (total dynamic range: 256 grey values) considerably improves the visibility of the signals. Fig. 2 shows that rectangles which are oriented horizontally can be recognized far better than rectangles oriented vertically. With the help of images scanned on film, it will be investigated whether this result is due to the computer screen or the human eye.

4. In order to measure the MTF and the sensitometric properties of the film-screen systems the combinations were exposed at PTB as well as at the district hospital in Wolfsburg. Square wave gratings of lead were used for the measurement of the MTF. The sensitometric curve was determined by means of aluminium step wedges and with the inverse square law PTB-sensitometer. The experiments were arranged to show the fading of latent image, the effects of different development conditions, and the consequences of the different sensitometric methods.

5. By means of a Joyce-Loebl microdensitometer X-ray images of the human thorax were analyzed to characterize the optical density profile of spherical nodules. With the help of the image processing system it is intended to generate such nodules of different size and variable contrast and insert them into X-ray images of the human thorax.

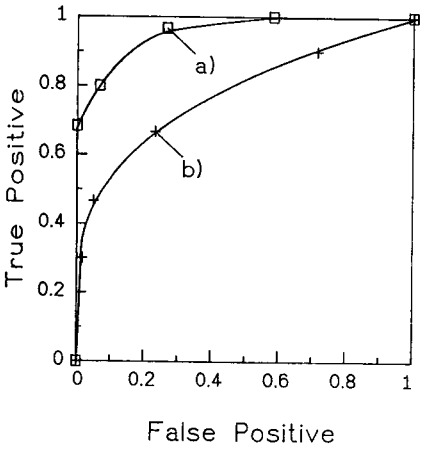

Fig. 1: ROC-curves, mean grey value: 100, signals: sharp edged squares of size 4 mm^2, noise amplitude: +/- 18 grey values; curve a): grey value of the signal is 5 grey values above mean grey value; curve b): grey value of the signal is 4 grey values above mean grey value

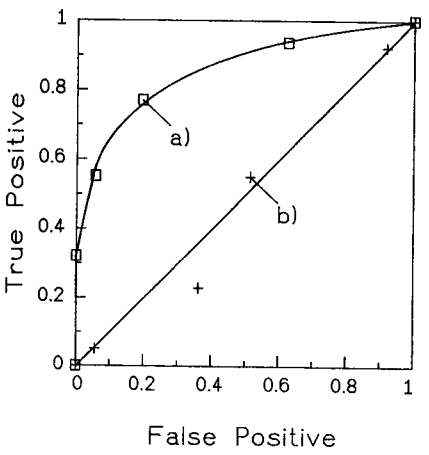

Fig. 2: ROC-curves, mean grey value: 100, signals: smoothed rectangles of size 4 mm * 2 mm, noise amplitude: +/- 18 grey values; curve a): rectangles with the longer legs oriented horizontally; curve b): rectangles oriented vertically

IV. Objectives for the next reporting period:

During the forthcoming reporting period, medical X-ray images should be scanned, manipulated by means of an image processing system, rewritten on film with a laser scanner, and evaluated by medical doctors.

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

medical doctors

VI. Publications:

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-F-133-D

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und Umweltforschung mbH.
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Telephone number: 089/31.87-2241

Title of the research contract:

Analysis of exposure in radiology

List of projects:

1. Analysis of exposure in radiology

Title of the project no.:

BI6-F-133-D

ANALYSIS OF EXPOSURE IN RADIOLOGY

Head(s) of project:

Dr. G. Drexler

Scientific staff:

Dr. Nina Petoussi
Dipl.-Math. Maria Zankl
Dipl.-Phys. W. Panzer
Dipl.-Phys. R. Veit
Dr. D.F. Regulla

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:

III. Progress achieved: Monte Carlo Calculations

In order to estimate the risk to patients from radiological procedures, it is necessary to assess the dose equivalents to single organs. As organ doses cannot be measured, they must be determined by simulating the interactions between radiation and matter by computational methods. The source and the human body and its organs are then described mathematically, and for the simulation of the radiation transport within the phantoms, Monte Carlo methods are employed. Most of the heterogeneous mathematical phantoms in use for dose calculations are based on the ICRP Reference Man data (1). At the GSF, two sex-specific adult phantoms ADAM and EVA were developed (2), based on the design characteristics of the MIRD-5 phantom (3). Although the body characteristics of the MIRD-type phantoms are in good agreement with those of the reference man and woman, they have some disadvantages related to the location and shape of organs and the shape of the body.

1. A new generation of phantoms

In order to overcome these disadvantages and to obtain more realistic phantoms for different ages, a technique was developed based on data from whole body computer tomographic (CT) examinations (9, 13 - 15). This technique allows any physical phantom or real body to be converted into computer files which can be attached to a code for organ dose calculations (15). The single scan picture data consisting originally of matrices of 256 x 256 picture elements (pixels) with CT numbers between 0 and 4095 are renormalised to grey values between 0 and 255 and then manipulated using suitable image analysis software (4). New pictures are created in which each pixel contains an organ identification number instead of the original grey value. Each organ and tissue of this "voxel" phantom then consists of those volume elements (voxels), derived from the CT data, having the respective identification number. Therefore, the location and shape of the organs and tissues is accurately modelled. Special care is given to the modelling of the red bone marrow. The relative amount of bone marrow in each voxel within the skeleton can be estimated directly from the CT grey values of the respective bone pixels. Although it is not possible by this method to model the complicated trabecular bone structure exactly, the spatial distribution of the bone marrow can thus be assessed with a high resolution corresponding to the voxel size. So far, two CT phantoms from data of real persons were constructed, one of an 8 week old baby and one

of a 7 year old child (15). The construction of a third phantom from the data of a 12 year old child is under work. Figure 1 shows a three dimensional reconstruction of the skin and main organs of the phantom of the 7 year old child. To construct a whole family of realistic phantoms of various ages including adults is very cumbersome because there are only very few situations justifying whole body CT scans.

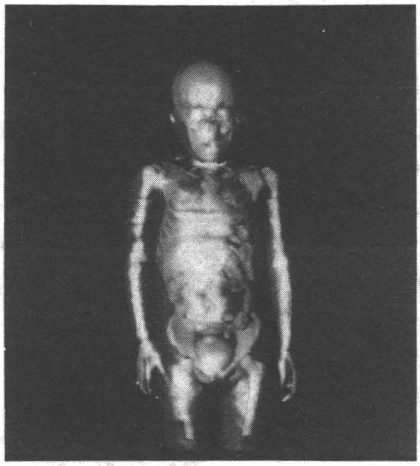

Figure 1:
Three dimensional reconstruction of the skin and main organs of the phantom of a 7 year old child derived from the CT data of a real patient

2. Organ dose calculations

Using these newly constructed phantoms as well as the available MIRD-type phantoms ADAM and EVA, organ dose calculations were performed for diagnostic radiology and radiotherapy situations.

2.1 Diagnostic radiology

2.1.1 Pediatric radiology

Using the tomographic phantoms BABY and CHILD, organ dose calculations for the examinations most common for the respective age class were performed (14, 17). The exposure conditions (field size, tube voltage, filtration, focus-to-film distance and focus-to-skin distance) were simulated as they are typically applied in the Children's Hospital of the University of Munich (5). As an example, organ dose equivalents per entrance air kerma free in air are given in table 1 for some examinations as calculated for the seven year old child (17). Results of these calculations were compared to organ doses compiled by Rosenstein et al. (6) which were calculated using MIRD-type children phantoms. The exposure conditions considered by

Table 1: Organ dose equivalents per entrance air kerma free in air (Sv/Gy) for X-ray examinations of a 7 year old child

	Skull a.p.	Skull lat.	Thorax a.p.	Thorax p.a.	Abdomen a.p.	Pelvis[a] a.p.
Brain	0.285	0.351	0.003	0.003	*	*
Eye lenses	1.299	0.563	0.006	0.002	0.003	*
Lungs	0.047	0.020	0.466	0.479	0.200	0.001
Ovaries	*	*	0.003	0.001	0.569	0.505
Testes	*	*	*	*	0.124	0.184
Thymus	0.173	0.060	0.616	0.238	0.036	*
Thyroid	0.417	0.585	0.799	0.163	0.012	*
Uterus	*	*	0.001	*	0.627	0.636
Skeleton (hard bone)	0.694	0.691	0.351	0.502	0.449	0.309/0.303
Red bone marrow	0.078	0.066	0.042	0.061	0.075	0.057
Total body	0.123	0.122	0.126	0.136	0.235	0.137/0.131

* conversion factor smaller than 0.0005.

[a] The pelvis a.p. examination results, for some organs, in slightly higher doses for girls (1st value) than for boys (2nd value) due to shielding of the testes.

Rosenstein are common in the USA and are somewhat different from those simulated in the above calculations with regard to field size, focus-to-film distance and photon spectra. Doses for organs well inside the beam did not differ greatly in this comparison. As expected, the higher discrepancies occurred for organs near the edges of the fields which were in the beam in one of the calculations and outside in the other. Differences in the red bone marrow doses were expected due to the entirely different modelling of this tissue in the phantoms considered.

2.1.2 Radiology of adults

Using the adult mathematical phantoms ADAM and EVA, the dependence of organ doses on tube voltage and focus-to-film distance was studied. For these parameters the maximum and minimum values as recommended in the CEC Working Document "Quality Criteria for Diagnostic Radiographic Images" were taken. It was found that for single organ doses the differences could be kept within approximately 40-50% (18). Examples for the variation of organ doses depending on the technical parameters are shown in table 2.

Table 2: Organ doses for the female adult phantom normalised to dose at the image receptor (Sv/Sv) for a thorax p.a. examination. Exposure conditions as recommended in the CEC Working Document

Voltage (kVp)	110	110	150	150	125
Focus-film distance (cm)	150	200	150	200	180
Breast	7.87	8.00	7.31	6.75	7.20
Colon asc.+transv.	0.26	0.31	0.30	0.30	0.29
Lungs	32.08	31.89	26.74	23.72	26.60
Red bone marrow	7.22	7.50	6.41	5.99	6.42
Skeleton	17.62	18.40	13.65	12.82	14.49
Thyroid	3.52	3.99	3.71	3.70	3.59
Total body	7.55	7.89	6.21	5.81	6.42
Entrance surface	70.50	66.11	53.29	42.04	49.66

As part of a common study of several CEC contractors on the determination of organ doses caused by CT examinations, Monte Carlo calculations have been performed using all available phantoms. In these calculations the exposure conditions most common in the Federal Republic of Germany were simulated, namely a 360° rotation of the fan beam, a distance of 76 cm from the focus to the axis of rotation, a tube voltage of 125 kVp and filtrations of 2.2 mm Al + 0.2 mm Cu and 2.2 mm Al + 0.4 mm Cu, respectively (16, 19). The calculations were performed as contiguous single slices of 10 mm thickness from the bottom of the trunk up to the top of the head for the adult phantoms and as contiguous single slices of thickness 8 mm and 4 mm from the toes up to the top of the head for the 7 year old child and the 8 week old baby, respectively. The results will be combined with similar calculations performed by the NRPB for differing irradiation conditions to provide a common data basis for all contractors.

2.2 Radiotherapy

2.2.1 Simulation of a leukemia treatment

The whole body irradiation for leukemia treatment of the 7 year old child whose CT data were used to construct the child phantom was simulated. The Co-60 irradiation before bone marrow transplantation aims at the patient receiving a uniform dose to the red bone marrow to kill all leukemia cells - the planned midline dose in the pelvis was 12 Gy. The lung dose, however, should not exceed 9 Gy to prevent complications caused by pneumonia after the radiation treatment. For the calculation, the shielding blocks of different transmission, the blocking lung filter constructed according to

the shape of the lung on the X-ray picture, and various projections and field sizes were simulated as applied for the actual treatment. The resulting doses are shown in table 3.

Table 3: Calculated doses to the red bone marrow, the lungs and the skeleton (hard bone only), resulting from the leukemia irradiation treatment of a seven year old child

Organ	Dose (Gy)	
Red bone marrow:		
Total	11.76	
Arm bones	11.56	
Clavicles	9.59	
Facial skeleton	11.81	
Leg bones	13.48	
Pelvis	11.76	
Ribs	8.91	
Sacrum	11.59	
Scapulae	10.52	Doses aimed to achieve:
Skull	11.27	
Spine	10.59	Lungs 9 Gy
Sternum	10.14	Midline pelvis 12 Gy
Lungs	8.56	
Skeleton	11.44	

2.2.2 Calculations for radiotherapy of cervical cancer

Using the female adult phantom EVA, calculations were performed to estimate the absorbed doses, due to radiotherapy treatment for cervical cancer, to various organs and tissues in the body. This work was part of a follow up study of the International Radiation Study of Cancer of the WHO who provided the records of 161 patients treated for cervical cancer in order to establish 20 or more years later the relationship of recurrencies of secondary cancers to the dose received. Calculations were performed for the simulation of intracavitary sources such as ovoids and applicators filled with radium, Co-60 and Cs-137 as well as for external beam therapy applying various anterior, posterior and lateral fields resulting from megavoltage, Co-60 and Cs-137 therapy machines. Extensive tables were compiled and can be used to estimate the doses for different combinations of internal and external sources (10, 11).

3. Improvements and extensions to the Monte Carlo code

During the reporting period, the following features of the Monte Carlo code were introduced:

3.1 Secondary electron transport

Whereas the energy depositions at each point of interaction previously have been calculated using the kerma approximation, now the energy lost by the photon can be transferred to secondary electrons which are then pursued further using the continuous slowing down approximation. This difference is important mainly at interfaces, such as between cortical bone and bone marrow. In the tomographic phantoms, these skeletal components are clearly separated and, consequently, this method enables a more realistic estimation of red bone marrow doses. In the MIRD-type phantoms, this difference is not important as the skeleton is modelled as a homogeneous mixture of hard bone and bone marrow.

3.2 Geometric dose distribution

In addition to the calculation of organ doses, the possibility of calculating the geometric dose distribution in selected parts of the body was implemented: Isodose contours can be assessed in three dimensional space together with the mean doses to whole organs. This is important mainly for therapy planning considerations. Figure 2 shows an example of a three dimensional visualisation of isodose contours calculated for treatment of a tumour in the head. Selected tissues as the tumour (which should receive the maximum dose) and the eyes (where the dose should be minimum) are shown together with the isodose contours (12). In this way, the dose distribution achieved by a certain treatment can be compared to the intended one.

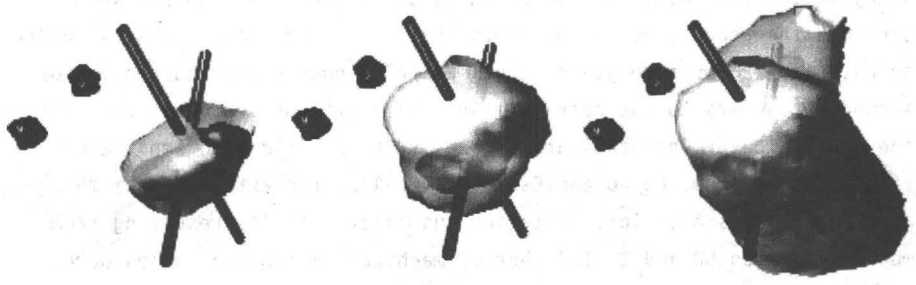

Figure 2:

Three dimensional visualisation of 80%, 50% and 20% isodose curves, respectively, for Co-60 irradiation of a tumour in the head, together with the tumour volume and the eyes. The calculation simulates a combined application of two fields whose incident angles are also shown.

3.3 Non rectangular fields

In former calculations all fields were simulated as being rectangular. In reality, however, there are situations where parts of the irradiating beam are shielded. Examples for such situations are the shielding of the gonads in a.p. X-ray examinations of the pelvic region, the blocking lung filter applied during the irradiation for leukemia treatment and the shaping of the beam according to the shape of well determined tumours in "beam's eye view", a technique applied in radiotherapy in order to reduce the dose to healthy tissues surrounding the target volume. The possibility of considering these non rectangular field shapes has been properly integrated in the Monte Carlo code.

4. Verification of the Monte Carlo method

In order to compare calculated doses with measured ones in a systematic way, a physical Alderson-Rando phantom loaded with TL dosemeters in 28 locations was scanned in a CT machine from head to bottom. From these data a three dimensional mathematical voxel model (figure 3) was constructed (20).

Figure 3:
Three dimensional reconstruction from CT data of the Alderson-Rando phantom

For the TL measurements, dosimetric sets of 2 or 6 LiF dosemeters in PVC cylinders were prepared for every target fitting the holes in the Alderson slices. The TL detectors were individually calibrated by means of ionisation chambers under exposure conditions in the phantom and the dose mean of the TL detectors in each target was taken.

For the conversion of doses measured by TLD into average absorbed doses in the target volumes as well as for the Monte Carlo calculation of target

doses, the chemical composition and density of the various Alderson tissues had to be analysed.

Major problems for the calculation were the air spaces between the physical slices of the Alderson phantom, the air contained in the bone marrow spaces in the skeleton and field inhomogeneities across the target volumes.

The doses from the whole body CT scan (from which the voxel Alderson phantom was constructed) and a Co-60 whole body irradiation (a.p. and p.a.) were compared. The results are shown in table 4. Comparison of doses from some common X-ray examinations is still under work.

Table 4: Differences between measured and calculated absorbed doses in the target volumes of a physical and a voxel Alderson Rando phantom, respectively (all figures are given in %)

	CT*	Co-60 a.p.	Co-60 p.a.
Range of differences	-10 - 15	-15 - 13	-10 - 15
Differences within 10%	93	80	85
Average difference	0.2	-5	1.7
Standard deviation of the differences	5.9	6.3	7.1

* 125 kV, 2 mm Al + 0.4 mm Cu, scan angle 360°

Field survey

A field survey for the evaluation of dose values and exposure parameters occurring in CT examinations in the Federal Republic of Germany was performed, covering 122 facilities in hospitals and medical practises (19, 21). The participants of the study were sent capsules containing TL-dosemeters to be exposed in a single slice free in air on the axis of rotation under conditions as usual for the various examinations at the respective facility. In addition a form was to be completed concerning the physical exposure conditions. The results can be summarised as follows:

- All the institutions were equipped 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 examination (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 (see 2.1) complicates the preparation and presentation of data for this purpose (16).

- Beam profile analysis could be performed for 2 mm slices and 4 mm slices revealing an overlapping of contiguous slices due to special construction of the collimating systems or its misadjustments. This effect leads to a dose enhancement factor which has to be considered when performing patient dose estimations based on single scan dose measurements free in air. The findings in the field study (mean dose enhancement factor ± standard deviation, range):

2 mm sclices	1.49 ± 0.35	1.01 - 2.26
4 mm sclices	1.19 ± 0.32	0.88 - 2.22

- Dose values even for the same exposure conditions (tube voltage, filtration, distance) and normalised to the same product of the tube current and exposure time showed a surprising variation (19, 20). 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. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Dr. v. Haunersches Kinderspital, University of Munich, W-Germany

- National Radiological Protection Board, Chilton, England

V. Publications:

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RADIATION PROTECTION PROGRAMME

Progress Report

1989

Contractor: Contract no.: BI6-F-317-DK

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Title of the research contract.

The impact from quality assurance on dose reduction in computerized tomography in Denmark.

List of projects:

1. The impact from quality assurance on dose reduction in computerized tomography in Denmark.

Title of the project no.:

The impact from quality assurance on dose reduction in computerized tomography in Denmark

Head(s) of project:

Dr. K. A. Jessen

Scientific staff:

K.A.Jessen, J.Juul Christensen, J.Jørgensen and E.W.Sørensen

I. Objectives of the project:

An assessment of the collective effective dose equivalent from computerized tomography (CT) imaging techniques to the whole Danish population investigated by a complete periodically registration of the use of CT examinations.
Evaluating the relation between the usage of dose and the scanner performance as a base for formulating quality criteria for CT images and for optimizing the use of CT.

II. Objectives for the reporting period:

Questinnaires adapted from the running programme are send to all CT installations in Denmark. Data on each examination are collected for a given period in order to get a full documentation of the actual national use of CT. The associated quality assurance performed on each CT system are also registered. Dose measurements are performed for the most commonly used sets of scanning parameters for all systems and a site visit is planned for performing identical quality control measurements of physical image parameters on standard phantoms. All data are placed in a database for further evaluation.

III. Progress achieved:

1. Methodology.

In order to get a full documentation of the actual use of computerized tomography (CT) in diagnostic radiology in the Danish Health Service a questionnaire has been send to all CT installations in use primo 1989. It was 24 scanners for the whole country, all located in diagnostic departments in hospitals. The questionnaire has been used for two periods each of three weeks and similar data to the ongoing investigations in other CEC member states have been collected for each examination (number of scans, thickness of slice, mA/mAs, body area etc.) In the same inquiry questions on the actual performed quality assurance on each CT system have been answered.

Dose measurements with TLD dosimeters have been collected from nearly all CT installations by a postal services. Measurements of doses free-in-air at the axis of rotation for a single slice and for the most commonly used sets of scanning parameters have been performed giving the axial dose profile and the computed tomography dose index,

$$CTDI = \frac{1}{T}\int_{i=1}^{i=n} D_i \cdot t$$

where D_i is the dose in i'th TLD, t the thickness and n the number of the TLD's and T the nominal slice thickness.

A site visit for doing identical quality control measurements of physical image parameters on phantoms for a well defined sets of scanning parameters has been performed so far to 18 of the CT installations. Dose measurements free-in-air and in phantoms have been induced in these tests with TLD and a pencil shaped ionization chamber, which give the multiple scan average dose (MSAD) - the average dose across the central slices from several contiguous slices spaced by the slice thickness.

2. Results.

All data collected have been prepared for a SIR database and the reporting are in progress. When the last data are collected primo 1990 statistical evaluation and dose calculations will initiate. Preliminary results from the dose measurements

show a fairly good agreement between the ionisation chamber measurements and the results for multiple scan (7 contiguous slices) measured as maximum values with TLD (Table).

CT-system	kV/mAs	Slice-thickness mm	Free-in-air CTDI mGy	Phantom surface (220 mm)			
				Ion.chamber mGy	TLD(multi) mGy	TLD(single) mGy	M/S f_e
EMI1010	120/ -	2 x 10	44.7	52.9	52.6	33.8	1.56
GE9800	120/368	10	95.6	56.0	57.7	41.8	1.38
SOMATOM DR	125/520	8	36.9	39.8	41.5	31.5	1.32
SOMATOM DH-G	125/520	8	43.5	41.9	38.4	29.2	1.32
TOMOSCAN LX	120/486	10	105.2	71.5	74.6	57.9	1.29
TOMOSCAN 350 (240 mm)	120/360	9	56.8	51.7	54.6	41.5	1.32
TOMOSCAN 350 (400 mm)	120/530	9	60.8	50.3	51.0	39.9	1.28

The ratio between dose values for multi scan and single scan expressed with the socalled enhancement factor, f_e, is very similar for the different systems. But the ionisation chamber measurements and the CT dose index measured free-in-air (CTDI) show differences up to 70% for some systems indicating the use of shaped filters.
The free-in-air axial dose profiles is a reliable detection of misaligned collimators. Deviations up to 100% from nominal values for very narrow slices have been measured for some systems. Such deviations results in a doubling of patient doses for contiguous slices.
The CT systems suitability for quantitative use of CT numbers have been tested by phantom measurements of the sensitivity to variations in size, shape and position of the phantom in the gantry aperture.

3. Discussion.

The preliminary results from the dose measurements show that it is very important to know the details about the filter located in the X-ray beam. Shaped filters complicate the interpretation of the free-in-air axial dose measurements and

if CT dose index values based on such measurements are used for organ dose calculations information about the filtration has to be integrated into the calculations.
The deviation of the dose profiles from nominal values indicate for narrow slices, that in some CT systems the collimation is performed before the detectors, but after the patient. That was not clearly expressed to the user and therefore in same cases caused unnecessary high doses to the patient.
The conclusion based on the data collected the first year of this contract can be that such information is important for formulating quality criteria for CT images and for optimizing the use of CT and thereby establishing a good practice for the application of this modality in diagnostic radiology on well-advised indications.

IV. Objectives for the next reporting period:

In the next 6 month a final report will be prepared including statistical evaluations of the collected data on CT examinations in Denmark. Calculation of the collective effective dose equivalent from CT in Denmark will be performed. On the basis of the evaluation of the quality control performed quality criteria for CT examinations will be proposed.

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

Barry Wall & Paul Shrimpton, NRPB, Chilton, U.K.

A. Ferro de Carvalho, LNETI, Portugal.

VI. Publications:

K.A.Jessen and J.Jørgensen: Quality control in quantitative computed tomography. BIR Report 18, British Institute of Radiology, London 1989, pp.84.

K.A.Jessen and J.Jørgensen: The influence of patient size and shape on absolute CT numbers for different scanner systems. Br.J.Radiol. (submitted).

K.A.Jessen, J.Juul Christensen, J.Jørgensen and E.W.Sørensen: Dosimetric Quality Control of CT scanners. (in preparation).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no : BI6-F-134-IRL

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Title of the research contract:

Specification of uniformity and noise limited exposure reduction in radiological image intensifier - TV systems as an adjunct to quality control and optimization of exposure.

List of projects:

1. Specification of uniformity and noise limited exposure raduction in radiological image intensifier - TV systems as an adjunct to quality control and optimization of exposure.

Title of the project no.: 1

Specification and Study of Uniformity and Signal to Noise in Image Intensifier - TV Systems.

Head(s) of project: J.F. Malone.

Scientific staff: P. Cooney, K.P. Maher, M.K. O'Connor, W. van der Putten.

I. Objectives of the project:

The project originally included objectives (a) to (c), and (d) was added later in conformity with the broader needs of the programme as a whole.

(a) Development of parameters to be included in methods of specification, acceptance testing and quality control in Radiological Systems based on new technology.

(b) Reduction of the dose required in individual examinations through correct specification of exposure levels required to meet particular imaging needs.

(c) Optimization of the use of medical exposure through a clear understanding and specification of how particular measures of image quality are achieved with digital systems.

(d) Assessment of requirements in practice to allow Image Quality Criteria proposals be implemented in one large department.

II. Objectives for the reporting period:

(a) Selection of a definition of non-uniformity applicable to II-TV Systems.

(b) Survey of Non-Uniformity in a wide range of Hospital based Fluoroscopy Systems.

(c) Study of contribution to overall non uniformity of individual components of Imaging Chain.

(d) Study of technical features of importance to above and optimization process including input conditions, saturation effects, matrix size, influence of videorecording, tolerances and limiting values, linearity of components.

(e) Study, to act as pilot for further work, on the relationship between system SNR at a specified level of performance, the specified SNR of the system, input exposure and image properties for two flurooscopy units.

(f) Study of the influence and behaviour of automatic exposure controls (AEC) and automatic brightness controls (ABC) viz a viz (d) and (e) above.

(g) Establishment of Contact with individuals, groups, professional bodies, supplies and standards bodies with an interest in the field.

III. Progress achieved:

The material presented follows the following sequence:

1. Introduction.
2. Methodology.
3. Results and Discussion:
   - 3.1. Definition of Uniformity.
   - 3.2. Uniformity Measurements.
   - 3.3. Noise, Signal to Noise Ratio, and Automatic Exposure Control Systems.
   - 3.4. Performance of a Digital System for Cardiac Imaging.
   - 3.5. Multiformat Cameras.
   - 3.6. The Image Quality Criteria Proposals and contact with other Bodies/Groups.
4. Conclusions.
5. References.

1. INTRODUCTION.

During the last decade digital systems were introduced into routine use in radiological practice. The area of most widespread application is presently Digital Subtraction Angiography. This relies on the use of traditional fluoroscopic imaging systems and digitization of the output of the TV Camera. With the introduction and establishment of these systems it became clear that there were no protocols or criteria to ensure that they functioned optimally. Furthermore the technical approach to setting them up was almost entirely based on the acquisition of a good image and paid scant attention to optimization criteria.

The study undertaken in this project was designed to investigate a number of criteria likely to be of importance in Digital Fluoroscopic Imaging. Specifically two endpoints Uniformity and Image Noise were selected for investigation. Many others could have been chosen for evaluation but were not as the intention of the work to draw attention to the absence of work in the area, rather than to definitively resolve the problems involved. In addition some work in relation to the ongoing programme on Optimization of Image Quality Criteria was undertaken.

2. METHODOLOGY:

In all cases the methodology for the studies outlined below has been, or will be, fully described in the associated publications. Hence in view of the limited space it will not be detailed here. However it may be useful to note that where well established techniques are employed, such as x-ray beam dosimetry, the methods used are standard uncontentious ones. On the other hand, where techniques not in widespread use are presented sufficient reference is made to the method in the text to allow it be envisaged. However full technical details are not presented, but are available elsewhere.

3. RESULTS AND DISCUSSION.

3.1. Definition of Uniformity: The literature was reviewed for definitions of uniformity applicable to Image Intensifier - TV Fluoroscopy Systems. Those available during the early phase of this study were developed by Bodies such as IEC and were both impractical for field use and unsuitable for the optimization process (IEC, 1977, 1984). In view of this an alternative approach was taken and the U.S. National Electrical Manufacturers Association (NEMA) Definition for uniformity of gamma cameras was adapted for assessing XII-TV System Uniformity (NEMA, 1980). The definition used is that as established by NEMA for Integral Uniformity over both the useful field of view (UFOV) and the central field of view (CFOV), Integral Uniformity is defined as:

$$\text{Integral Uniformity} = \pm \frac{C_{max} - C_{min}}{C_{max} + C_{min}} \times 100\%$$

where C_{max} and C_{min} are the maximum and minimum pixel values respectively. In some circumstances it was necessary to limit measurements to a vertical or horizontal band of the image. If this was the case then uniformity was defined as:

$$\text{"Band Uniformity"} = \pm \frac{C_{max} - C_{min}}{C_{max} + C_{min}} \times 100\%$$

where C_{max} and C_{min} are, respectively, the maximum and minimum pixel values in that band of the image.

3.2. Measurement of Uniformity:

Once the definition of uniformity was established it was necessary to determine the influence of a wide range of variables, and measurement conditions on this property. Those examined included input exposure, size of image acquisition matrix in the measurement system, the presence/absence of noise reduction techniques, non linearities and saturation effects in various components of the system, etc. In addition to facilitate measurements on remote systems use of a video tape recorder was necessary, and its impact on the measurements had to be assessed.

No important change in the measured System Uniformity was observed for changes in the input exposure rate to the II, or when various levels of noise reduction were applied to the images. The measured System Uniformity was seen to improve with reductions in the size of the image acquisition matrix. Thus all System Uniformity measurements were performed using the same image acquisition matrix size. The video cassette recorder was found to have no influence on the measurements.

System Uniformity measurements over the UFOV for each of 10 Image Intensifier TV Systems are shown in Table 1, with and without magnification. A considerable variation between systems for both II field sizes is evident.

TABLE 1. System Uniformity over the UFOV for both Normal and Magnification 1 field sizes.

System No.	System age (years)	Normal field (UFOV)	Mag. 1 field (UFOV)
1	5	± 50.3%	± 33.5%
2	6	± 90.8%	± 57.9%
3	10	± 57.4%	± 34.3%
4	10	± 38.6%	-
5	2	± 55.8%	± 36.6%
6	4	± 46.7%	± 34.2%
7	12	± 44.4%	± 31.0%
8	10	± 37.2%	± 20.0%
9	12	± 48.1%	± 31.3%
10	1	± 56.9%	± 40.9%
11	2	± 53.8%	-
12	1	± 52.5%	-
Mean		± 52.7%	± 35.5%
Coeff.	Variation	26.0%	28.5%

It is seen that XII-TV Systems as a whole exhibit very large non-uniformities and it is felt that there is a lot of room for improvement. A substantial improvement in the measured uniformity is seen in all cases with the introduction of magnification. Similar improvements were observed when measurements were performed over the CFOV. The limiting value for the System Uniformity would presently appear to be in the region of ± 50% to ± 60% over the UFOV and ± 20% to ± 30% over the CFOV.

The contribution of the main subcomponents of the Imaging System to non-uniformity were assessed in detail. For this purpose the System was divided into (i) the X-Ray Beam; (ii) the X-Ray Beam plus the Image Intensifier; and (iii) the TV Camera. This involved developments in methodology which are detailed elsewhere. However in practice it was not possible to assess the non-uniformity throughout the imaging field in all cases, and measurements were limited to a Horizontal Band through the field of view. Table 2 presents the results of Horizontal Band measurements of component non-uniformity.

TABLE 2. Component horizontal band uniformity.

System No.	System Age	System H.Band	Beam H.Band	II+Beam H.Band	TV Camera H.Band
1	5	± 41.2%	± 7.6%	± 21.3%	± 12.7%
2	6	± 86.9%	± 5.0%	± 29.1%	± 23.9%
3	10	± 29.1%	± 10.7%	± 22.8%	-
4	10	± 21.6%	± 6.1%	± 21.1%	-
5	2	± 39.9%	± 5.2%	± 25.6%	± 9.5%
6	4	± 44.8%	± 6.2%	± 25.2%	± 15.5%
7	12	± 43.1%	± 13.0%	± 12.6%	± 9.9%
8	10	± 35.7%	± 7.9%	-	-
9	12	± 27.5%	± 9.4%	± 17.9%	-
10	1	± 49.8%	± 9.7%	± 18.9%	-
MEAN		± 42.0%	± 8.1%	± 21.6%	± 14.3%
COEFF. VAR.:		43.0%	32.2%	22.5%	41.2%

It is clear that there is considerable variation between systems for each component. Furthermore it is reasonably clear that the greatest contribution to non uniformity is the Image Intensifier, followed by the TV Camera and the Beam respectively. Also it can be seen that the Image Intensifier + Beam added to Camera Horizontal Band Uniformities does not combine arithmetically to produce the System Horizontal Band Uniformity. This is perhaps due, in part, to the fact that two different measurement techniques were used with Image Intensifier + Beam measurement having a larger measurement matrix. Nevertheless the data does give a reasonable indication of the level of contribution of the system components to the overall System Uniformity.

3.3. NOISE, SIGNAL TO NOISE RATIO AND AUTOMATIC EXPOSURE CONTROL SYSTEMS (AEC).

In practice the input/patient exposure level in almost all Image Intensifier/TV Fluoroscopy Systems is regulated by an automatic exposure control system. In theory this should minimize patient dose and optimize image quality. Observation during the measurements in 3.2. above and Quality Assurance field survey rendered it clear that there were substantial differences between the performance of such systems. Furthermore review of suppliers specifications for them did not suggest that their performance could easily be assessed in terms of any measure of image quality. In view of this a detailed study was undertaken in respect of input exposure, and a number of quantitative and qualitative indices of image quality. The study involved development of measurement methods which have been applied in a pilot investigation of three different manufacturers' Systems. Quantitative studies were performed by digitising the images using a digital image processor (Quantel, Intellect-100). This was used to measure two objective indices of image quality (i) noise, and (ii) the signal to noise ratio (SNR) for the graded levels of x-ray beam attenuation. Exposure rate measurements were made at the entrance plane of the II using an ion chamber. X-Ray beam attenuation was provided by inserting standard size telephone books into the beam, and attenuation thickness is expressed in the results in mm of paper (163mm paper = 10cm water, approx.). A subjective comparison of image quality between the three systems was also performed using the Leeds Test Objects for noise (T.N3), contrast detail (T.10), and the Huttner resolution Test Pattern.

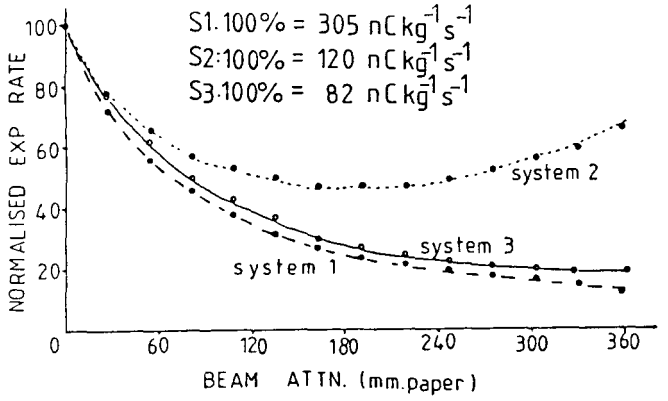

FIG. 1. Relationship between exposure rate and beam attenuation.

Figure 1 plots the normalised exposure rate achieved by the AEC in each system, against increasing amounts of beam attenuation. It is clear that in each case the AEC does not hold the exposure rate at the entrance plane to the II at a constant value and that there is considerable differences in the levels of exposure rate between systems.

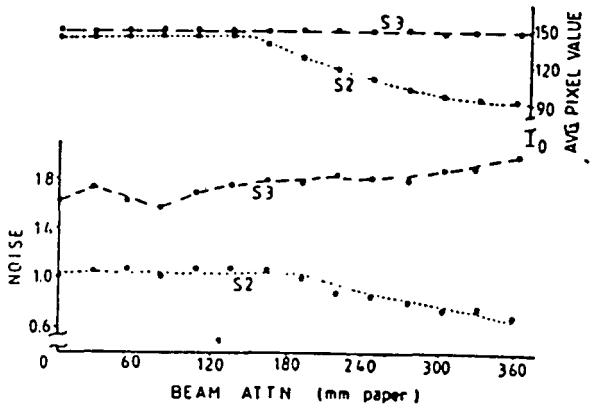

FIG. 2. Relationship between noise, average pixel value and beam attenuation.

With System 1 the measured SNR dropped by a maximum of 4.5 dB for increasing amounts of beam attenuation. This drop was largely dependent on a reduction in the signal level while the measured noise remained relatively constant. With System 2 and 3 the SNR remained relatively constant over the attenuation range examined. This was achieved with each system by different means, see Fig. 2. System 2 exhibited a drop in signal level for increasing attenuation, with a corresponding drop in the measured noise. System 3 held the signal level constant with only a small increase in the measured noise at the higher attenuation levels. Thus one system was unable to maintain a constant SNR while two of the three systems did not maintain a constant image brightness level over the range of exposures examined, indicating the failure of the AEC facilities to operate as one might expect.

Despite the variations observed in the SNR measurements, very little difference was observed in the noise and contrast detail performance of each system, as assessed using the Leeds Test Objects. For each system the drop in the spatial resolution with increasing attenuation was the same.

3.4. Performance of Digital System for Cardiac Imaging:

Cardiac cine angiography is among the procedures giving rise to the highest individual patient doses per investigation, if the doses from interventional radiology are excluded (Faulkner et al. 1986). This technique involves the use of an II/TV Fluoroscopy system which is also optically coupled to a specially adapted 35mm cine camera. There is considerable scope for dose reduction and optimization in this field, particularly,if and when, Digital Technology reaches a stage of development that will leave it comparable with film. This study describes an initial evaluation of one of the most advanced digital systems for cardiac imaging (DCI), which was performed with the above considerations in mind.

Evaluation of the imaging performance was undertaken for resolution under static and dynamic conditions (Huttner Test Pattern), contrast detectability and system noise (standard Leed's Test Objects). The digital system (DCI) resolution was not as good as that for cine. However, contrast detectability with DCI was somewhat better. In addition, DCI performed better than cine when a substantial amount of scatter was present. This was especially clear with the imaging of the noise test phantom.

TABLE 3. Clinical Evaluation: Detection of stenotic lesions, comparison between DCI and cine.

Identical on DCI and cine	49%
Significant lesion missed on DCI (observed on cine)	1.3%
Significant lesion missed on cine (observed on DCI)	1.3%
Minor lesions missed on DCI	17%
Lesion diagnosed more severe on DCI	12%
Lesion diagnosed less severe on DCI	7%
Lesion observed in different location on vessel compared to cine	9%
Lesion observed in different vessel compared to cine	4%

A preliminary clinical evaluation of the system was also performed. This was done by scoring lesions on a standard anatomical diagram. Results were scored directly from the monitor in the case of DCI and subsequently in the normal way from projection of the cine film. The results, for a total of seventeen patients with a total of seventy five stenotic lesions are given in Table 3. These results have to be taken as preliminary as it is only a small series and not a random blind study. It is seen from Table 3 that a significant number of stenotic lesions are missed on DCI. However 90% of these were minor and not of clinical significance and are seen mostly in vessels in which major obstructions already had been identified with both DCI and cine. The one exception was a 50% occlusion of the left main stem which would be regarded as clinically significant. On the other hand there was also a clinically significant lesion which was missed on the initial review of the cine film and was detected with DCI.

TABLE 4. Estimated dosemeter readings (uSv/exam), calculated from personel dosemeter readings, for a pacemaker implantation. (For angiography multiply x 5; angioplasty x 15).

	Conventional	Digital
Body	4.4	2.0
Eyes	7.2	3.2
Hand	20.4	22.1
Neck	6.4	2.3

A comparative study was also performed on the radiation dose received by the DCI operators over the period since its installation, to a similar period when only conventional angiographic units were used. This was performed by summing up all the dose records for the individual users

taking account of dosemeter position and the total number of investigations performed. Results indicate that the DCI system does appear to decrease the radiation dose to staff, see Table 4. However this cannot be contributed to an inherently lower radiation exposure rate of the DCI system, as both the DCI and the conventional systems were seen to give similar input dose rates when measured at clinically relevant attenuation levels. One explanation is that the DCI system can run at a frame rate of 12.5 f/s, in contrast to the 25 f/s. of the conventional system. In addition it is felt that the DCI system facilitates somewhat faster work, though this remains to be confirmed.

3.5. Multiformat Cameras.

Multiformat cameras (MFC) form an important part of the imaging chain in a wide range of modalities in radiology. Although quality assurance of the video-chain in these instruments is important, it is not in itself sufficient. It has been found that the physical condition of the MFC is an equally, if not more, important aspect in any Q.A. programme for such instruments. Large amounts of dust and dirt which accumulate on the optics and CRT of the MFC over a period of time without proper cleaning will affect their performance. The effect of such contamination on the performance of a MFC was studied and some initial results have been obtained. MFC performance was assessed by comparing films of (i) video test pattern generated grey-scale images, (ii) Huttner resolution test pattern images, and (iii) clinical images, taken on the MFC before and after cleaning. Results indicated that image degredation was present prior to cleaning in the form of limited contrast resolvability and decreased resolution. It was also seen that even a MFC which is cleaned every three months has a demonstratable degredation of performance at the end of this period. With this limited investigation, the contribution a contaminated MFC can make to the performance degredation of any Radiological/Digital Imaging System was clearly identified as being very important.

3.6. The Image Quality Criteria Projects and Contact with other Bodies/ Groups.

The Image Quality Criteria Document (CEC, 1989) was taken as the basis for a detailed study on the Implementation of a Quality Assurance Programme in a large new Imaging Department opened at about the same time as the document was issued. In addition detailed consultation with a small number of individuals and organisations was undertaken. Arising from these initiatives a number of conclusions were reached including:

(a) The general approach of providing guidance on Image Quality, Radiographic Technique, and Good Imaging Performance, is a good one.

(b) The actual guidance issued under the three headings needs further refinement if it is to be widely applicable throughout the community in large and small departments. This refinement is in terms of the specific items or values recommended under each heading, and the internal consistency of the recommendations. In particular there is some conflict between the requirements for Radiographic Technique and Good Imaging Performance.

A detailed submission elaborating on the above has been prepared after consultation with individuals in the Community and the USA.

In addition to the above close contact was established with a number of National, European and International Organisations/Groups, and individuals that could have an interest in these tolerances and limiting values that might be developed and applied to fluoroscopic instruments using Image Intensifier-TV Systems with or without digital technology. From this a clear insight into the likely and desirable directions of development was established.

4. CONCLUSIONS.

The work in this project has demonstrated the need for much further study under the two main headings: Image Uniformity and Noise, which were the subject of this investigation. While the study was originally aimed at Digital Systems using Fluoroscopic Technology, it is now clear that the Fluoroscopy units in their own right are far from optimized. Clearly very large amounts of non uniformity are routinely accepted and the performance of automatic exposure control systems is not directly related to simple markers of image quality such as noise or SNR.

Additional studies within the project evaluated the quality of image achievable in Cardiac and Phantom Studies using traditional cine and the most advanced (1989) digital technology. The results demonstrated advantages for both, but as opposed a few years ago, the digital systems are now within reach of those using film. However the optimization process for both is, for practical purposes, seldom a consideration in establishing technique. Likewise the most commonly used systems for hardcopying film from digital equipment (multiformat cameras) are seldom subject to optimization processes.

Given the above the most valuable conclusion reached in this study is that there is a serious absence of optimization criteria, methods, and protocols for the emerging digital systems. At a more basic level it became clear during the project that these features were also, in practice, missing for much conventional fluoroscopic imaging technology and for many of the patient studies that use such systems. In consequence a major proposal to address this gap was developed for the next programme.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]: The following were involved in collaboration at some stage during the project:

Dr. R. Kirkham, Chairman TC-62 Electromedical Equipment - CENELEC.
Dr. M. Moores, Manchester, U.K.
Dr. K. Faulkner, Newcastle, U.K.
Dr. C. Maccia, Paris, France.
Dr. L. Malone, Dublin, Ireland.

V. Publications: IN SCIENTIFIC JOURNALS, MONOGRAPHS.

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P. Cooney, W. van der Putten, P. Crean and J F Malone. (1989) An Evaluation of the Specification and Imaging Performance of a Digital Cardiac Imaging System. In "Computers in Diagnostic Radiology" (in press) (York, Institute of Physical Sciences in Medicine).

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-135-UK

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

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Physics Department
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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:

A number of reports have been published containing data essential for the completion of this project. In addition to a detailed account of the methods and results of our national x-ray patient dose survey (NRPB-R200, 1986) a further NRPB report (NRPB-R201, 1986) contains an update on the frequency of x-ray examinations in Britain and details of the age and sex distributions of x-ray patients. The data on organ doses received by patients and on the frequency of examinations enabled us to calculate the collective effective dose equivalents shown in Table 1 which appeared in an article in the NRPB Radiological Protection Bulletin (No 77, 1986).

Table 1

Annual collective doses to the British population from diagnostic medical radiology

Practice	Collective effective dose equivalent, S_E (man Sv)
Medical x-rays (excl. CT)	15500
Computerised tomography	500*
All medical x-rays	16000
Dental x-rays	200
Nuclear medicine	950
All diagnostic radiology (medical and dental)	17150

*Estimated value

The patient age and sex data for different types of x-ray examination were used to calculate the reduction in opportunity for the expression of long term radiogenic cancers and genetic effects for patients. Log normal distributions for the pattern of cancer incidence with time after exposure were combined with current life expectancy data for people of different ages, to predict reduction factors for somatic effects in patients. Values of about 0.7 for leukaemia and 0.5 for solid cancers were typical for most types of examination. Child expectancy data as a function of age and sex were used to predict similar reduction

factors for genetic effects and a typical value of about 0.2 was found. When combined with appropriate risk factors for the induction of fatal and non-fatal cancers, and genetic effects the average individual risks for x-ray examinations shown in Table 2 were obtained.

Table 2

Average individual risks for nine types of x-ray examination

Examination	Probability of radiation effect occurring x 10^{-6}					
	Somatic				Genetic	
	Fatal		Non-fatal			
	Male	Female	Male	Female	Male	Female
Skull	1.7	1.7	4.7	4.7	-	-
Chest	0.27	0.47	0.11	0.32	-	-
Thoracic spine	7.0	11	13	16	-	-
Lumbar spine	25	26	11	11	0.2	16
Abdomen	9.4	9.5	3.9	4.0	2.0	11
Pelvis	3.9	3.9	2.4	2.4	24	6.3
IVU	26	37	20	30	23	19
Barium meal	26	31	15	21	0.8	9.4
Barium enema	37	38	20	21	5.4	26

A paper was prepared, in collaboration with contractors from Paris and Udine, comparing radiology practice and patient doses in France, Italy and Britain. Some apparent differences in national criteria for the justification and optimisation of x-ray examinations are discussed. The wide distributions in patient doses for nominally the same type of examination have been studied further and results are presented in NRPB-R200. Analyses of selected percentile values of these distributions demonstrates that dramatic reductions in the range of doses observed can be achieved by rejecting only a few percent of the observed values at the extremes of the distributions. Inter-hospital variations in radiographic techniques and patient doses have so far revealed only the use of rare-earth screens as a consistent contributor to lower doses.

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

C Maccia, CEPN, Fontenay-aux-Roses, France
R Padovani, CRAD, Udine, Italy

V. Publications:

Doses to patients from routine diagnostic x-ray examinations in England. P C Shrimpton, B F Wall, D G Jones, E S Fisher, M C Hillier, G M Kendall and R M Harrison. British Journal of Radiology, 59, 749-758 (1986).

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A comparison of diagnostic radiology practice and patient exposure in Britain, France and Italy. G Contento, M R Malisan, R Padovani, C Maccia, B F Wall and P C Shrimpton. Brit. Jour. Radiol., 61, 143-152 (1988).

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:

To complete the national CT survey and report on individual and collective patient doses from current CT practice in the UK.

III. Progress achieved:

The world's first EMI brain scanner was installed in the Atkinson Morley's Hospital in London in 1972. The number of Computed Tomography (CT) scanners in the UK has subsequently increased steadily with as yet no apparent sign of saturation (Figure 1) and currently includes 39 different types of scanner from ten manufacturers. As of June 1989, there were 174 scanners in clinical use in National Health Service (NHS) hospitals, with a further 26 scanners in the private sector, bringing the total number of operational scanners in the UK up to 200.

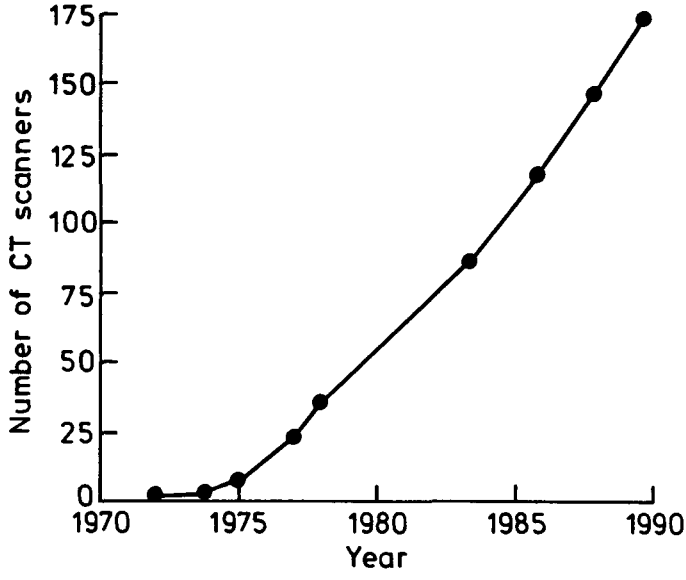

Figure 1 The steady increase in the number of CT scanners installed in UK NHS hospitals

With this continually increasing availability of scanners accompanied by regular reductions in scan times as the equipment has been improved, CT examinations are rapidly becoming more frequent and more extensive and are already making a major contribution to the collective population dose from diagnostic radiology in most European Community countries. To assess the situation in the UK a national survey has been carried out to establish the extent of CT activity and to study the associated levels of patient dose. This survey has been undertaken in conjunction with similar studies by other contractors to the CEC Radiation Protection Programme in Germany, France and Italy. New dosimetry techniques have been jointly developed that for the first time allow reliable estimates of organ doses to patients from CT examinations that are essential for assessing the radiation protection aspects of CT. By adopting a common dosimetry protocol reasonable comparisons can be made between CT practice and patient protection in the different member states.

METHODOLOGY

In collaboration with the UK Institute of Physical Sciences in Medicine (IPSM), a questionnaire on CT practice was distributed in June 1989 through 66 local hospital physicists to each of the 174 CT units in clinical use in NHS hospitals. This sample represents nearly 90% of all operational scanners in the UK. Details of patient workload, scanner servicing, physics support, quality assurance programmes, repeat rates and technique factors for up to 20 specified types of CT examination were requested in the questionnaire.

In order to derive typical levels of patient dose for CT procedures, Monte Carlo computer techniques have been employed to simulate the irradiation of a mathematical representation of a standard patient. Existing computer programs for common x-ray views (Jones and Wall, 1985) have been extended to model the rotational fan-beam geometries characteristic of CT examinations. In CT the x-ray spectrum and the dose rate incident upon the patient vary considerably between scanners, due especially to the use of complex beam shaping filters. Consequently, details of the precise irradiation conditions, and in particular the complete pre-patient beam filtration system, have been sought from CT manufacturers.

Monte Carlo calculations were to be performed by NRPB for each commonly used set of exposure conditions on each of the most popular types of scanner in the UK, Germany, France and Italy. Conversion factors relating organ doses to the free-in-air dose on the axis of rotation of the scanner were to be calculated for each of a series of 200 contiguous 5 mm thick transverse slices of the mathematical phantom. Typical CT examinations were to be simulated by selecting factors for an appropriate combination of the individual slices.

Similar Monte Carlo calculations were carried out at GSF, Munich but only for Siemens CT scanners. A comparison was made of the organ dose conversion factors calculated by the groups at NRPB and at GSF for three simulated CT examinations with the same model of Siemens scanner on the same mathematical phantom.

A programme of measurements of the required free-in-air axial dose profiles was conducted on a sample of about 70 NHS scanners in the UK. This sample was confined to those important types of scanner for which Monte Carlo calculated dose conversion factors would be available and was selected to be representative of the distribution of scanners in the UK by both geographical location and by type of scanner. The dose profile across the width of the x-ray beam was recorded by a series of thermoluminescent dosemeters (TLDs) stacked inside a hollow plastic cylinder, which was aligned along the axis of rotation of the scanner using a simple support jig. Sets of dosemeter capsules and a support jig were posted to local physicists, who carried out up to five dose profile measurements for each scanner, representing the most common combinations of applied potential, physical filter, focus-to-axis distance and nominal slice width in clinical use.

Similar sets of free-in-air axial dose profile measurements were carried out on samples of CT scanners in Germany, France and Italy by the other collaborating contractors.

RESULTS

Questionnaire

Information was received back from 82% of the 174 scanners which had been circulated with questionnaires. Approximately one quarter of the scanners were considered primarily to be for neuroradiological applications and three-quarters for general purpose radiology. A third of all scanners were partially utilised in treatment planning for patients undergoing radiotherapy. The median age of all operational scanners was about 3.4 years, with the oldest being 15.7 years.

Estimates for the total number of patients examined per year per scanner varied from 250 to 8000, with a mean annual workload of 3400 patients. On the basis of this figure, the total number of patients having CT examinations in 1989 in the UK is estimated to be 680,000. This is about three times the previously estimated total for 1983.

Other aspects of CT practice dealt with by the questionnaire included the provision for quality assurance (QA) for each scanner. For 85% of all installations, comprehensive servicing and maintenance cover were provided by the manufacturer, whilst local physicists conducted measurements monthly or more frequently on less than a fifth of scanners. Approximately 40% of scanners carried out some type of QA measurement (apart from calibration) on a daily basis, with a further 20% including some weekly QA procedure.

Monte Carlo Organ Dose Calculations

Sufficient technical information on the precise irradiation conditions for dose modelling purposes was obtained from Siemens (covering 9 models), from General Electric (relating to 9 GE models, 1 model of Picker scanner and 2 models of CGR scanner) and from Philips (representing 6 models). Information regarding the shape and composition of the shaped pre-patient filters was particularly difficult to obtain, but we finally achieved it for all these models. These types of scanner between them account for about 85% of all UK NHS scanners. About 20 different sets of exposure conditions (ie, kV, physical filter, and focus-to-axis distance) were found to cover the common operational modes of all these scanners and the appropriate Monte Carlo calculations are being conducted for these 20 sets of conditions.

The comparison with Monte Carlo calculations from GSF, Munich for a Siemens scanner showed close agreement to within 2.5% for all important organs, in the three CT examinations that were simulated. This served to confirm the reliability of the two sets of Monte Carlo calculations. The GSF results will be used in the German survey where Siemens scanners predominate.

NRPB has supplied CEPN, Paris with a floppy disk containing a complete set of organ dose conversion factors for 200 contiguous 5 mm thick scans covering the head and trunk with a CGR scanner. This should be sufficient to allow them to calculate the patient doses from all types of CT examination in France where the scanners are exclusively made by CGR. Similar data for a Siemens DRH scanner were sent to USL-7, Udine for use in the Italian survey and data for other types of scanner used in Italy will be provided by NRPB when they become available.

Dose Profile Measurements

A sample of the free-in-air axial dose profiles obtained during the UK CT dose measurement programme are shown in Figure 2.

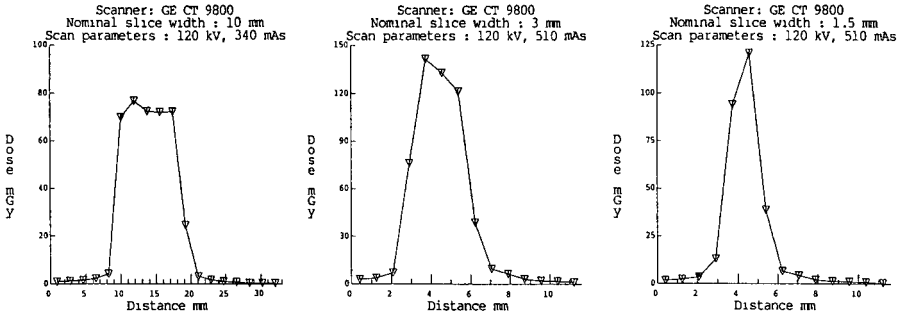

Figure 2 Axial dose profiles recorded for three nominal slice width settings on a GE CT 9800 scanner.

Many of the dose profiles indicated a significant spreading of the x-ray beam beyond the nominal slice width, particularly for narrow slices. For a series of nominally contiguous slices, there will be an overlap of the dose profiles and an enhancement of the dose in adjacent slices. One dose quantity that takes account of this is the Computed Tomography Dose Index (CTDI), which is defined as the linear integral along the dose profile divided by the nominal slice width. The value of CTDI calculated for a profile can thus be considered to represent the equivalent peak dose for a perfectly collimated scanner. Such perfect collimation is assumed in the Monte Carlo calculations modelling patient dose, hence requiring that the calculated dose conversion factors should be used with values of CTDI rather than the observed peak doses, to reflect accurately the effect of the measured beam profiles on patient dose.

Normalised values of CTDI derived from over 350 dose profile measurements range from 0.06 mGy/mAs to 0.47 mGy/mAs. Variations between scanners will be due in part to the different applied potentials, focus-to-axis distances (FAD) and filters used. Normal operating potentials vary from 120 kV (GE and Philips) to 125 kV (Siemens) and 130 kV (CGR and Picker), whilst values of FAD employed range from 487 mm (Philips 305N) up to 780 mm (GE CT 8800/9000). In addition the geometric enlargement (ge) facility on Philips 310/350 scanners allows the FAD to be set at either 487 mm (ge2), 608 mm (ge3) or 770 mm (ge4). There are also several broad categories of filtration option available; filters may be either flat or shaped and the total filtration fixed or variable. Results of CTDI per mAs measurements for wide slice widths for a selection of scanner types illustrating these differences are shown in Table 1. Filtration is fixed for the Siemens DRH (flat filter only) and the GE CT 9800 (shaped filter), whilst it may be varied for the Philips 350 (selectable flat filter) and the Picker 1200SX (selectable shaped filter) scanners.

Table 1
Variation of mean values of CTDI/mAs
observed for a selection of scanner types

Scanner type	Slice width (mm)	No. of scanners	Mean CTDI/mAs (mGy/mAs)	Max / Min
Siemens DRH	8	8	0.097	2.1
Philips 350	9 (ge2:filter)	5	0.171	1.3
GE CT 9800	10	5	0.215	1.6
Picker 1200SX	10 (head filter)	5	0.308	1.3

The normalised values of CTDI shown are the means of observations for a number of each type of scanner. In addition to inter-scanner differences, significant variations are also apparent between individual results for the same type of scanner. Ratios of the maximum to minimum value for each of the four types of scanner are indicated in the last column of the table and range from 1.3 to 2.1. Since all scanners of the same type are operated at the same mAs values for a particular type of examination, such ranges in normalised CTDI will result in similar ranges of patient dose.

For many scanners, the value of CTDI/mAs observed also varies with the slice width setting (typically 1 mm - 10 mm) since the quantity CTDI is sensitive to the collimation of the x-ray beam relative to the nominal slice width. The ratio of CTDI to the peak dose is a measure of dose enhancement for the imaging of contiguous slices and values generally increase with decreasing slice width. Ratios observed vary with the type of scanner and are mostly in the range 0.9 to around 1.5, although much higher values were measured for certain scanners. In particular, ratios of over 2 were consistently observed for Siemens DRH scanners operating at the 1 mm nominal slice width setting, where a 2 mm beam and narrower post-patient collimators are employed. Such enhancement factors have important implications for increased patient dose.

DISCUSSION

Further detailed analysis of the questionnaires on CT practice regarding the technique factors typically employed for common examinations is currently underway, as are Monte Carlo calculations of

organ dose conversion factors for the remaining types of CT scanner. Until these are complete accurate estimation of patient doses and of their variation between different types of examination and scanner, and of the total collective dose from CT examinations in the UK is not possible.

Provisional estimates of the effective dose equivalents from three types of CT examination with a Siemens DRH scanner, for which the results of Monte Carlo calculations are available, are shown in Table 2 below.

Table 2
Effective dose equivalents for a Siemens DRH scanner

Examination	Effective Dose Equiv. (mSv)
Head	4
Lungs	6
Abdomen	8

Table 1 shows that the Siemens DRH scanner has a lower value of CTDI/mAs than most other scanners and since the mAs values typically used by Siemens scanners are not significantly higher than other makes, the above effective dose equivalents might be regarded as underestimating those for other types of scanner. However, Siemens scanners do not have shaped pre-patient filters, which will probably increase the organ dose per axial CTDI compared with scanners that do have them. The above effective dose equivalents may consequently be fairly typical for most types of scanner.

On this assumption, a very provisional estimate of the total annual collective dose to the population of the UK from CT examinations would be about 3400 man Sv. This is based on clear indications from the survey that examinations of the head still predominate in CT. This collective dose for 1989 is nearly seven times higher than the value predicted for 1983, that appears in the report of the earlier project in this CEC contract. Verification of this figure must await the results of the ongoing analyses, but a rough indication of the relative contribution of CT compared with other types of diagnostic medical examination to collective dose in the UK is shown in Table 3.

Table 3
Contributions to collective dose from diagnostic medical examinations

Type of examination	Annual collective effective dose equivalent (man Sv)	
CT	3400	(1989)
Lumbar spine	2900	(1983)
Barium enema	2700	(1983)
Barium meal	2400	(1983)
Intravenous urography	2200	(1983)
Abdomen	1600	(1983)
Pelvis	1250	(1983)
Chest	450	(1983)
Nuclear medicine	1000	(1982)

The figures for conventional x-ray examinations were obtained from the national x-ray dose survey referred to in the other project of this CEC contract. The very major contribution from CT, albeit provisional, underlines the importance of studies of this sort in the CEC Radiation Protection Programme. They bring to the attention of the medical profession the large collective dose implications of modern methods of medical imaging and help to identify differences in procedures or equipment that exist both within and between member states of the European Community.

REFERENCE

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

D Drexler, GSF, Munich, FRG

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R Padovani, CRAD, Udine, Italy

V. Publications:

None.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.. BI6-F-136-I

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Title of the research contract:

Refinement of methods for the assessment of organ doses, and possible reduction of patient exposure.

List of projects:

1. Refinement of methods for the assessment of organ doses, and possible reduction of patient exposure.

Title of the project no.:

Refinement of methods for the assessment of organ doses, and possible reduction of patient exposure

Head(s) of project:

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USL 7, Udine, Italy

Scientific staff:

Gilberto Contento
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 patient and collective doses from different types of examination.

III. Progress achieved:

ANALYSIS OF DOSIMETRIC SURVEY DATA

1. Methodology

Data concerning doses of 1600 patients undergoing 14 different types of examinations have been collected in the dosimetric survey concluded at the beginning of 1985. By combining the mean organ doses for each examination and the frequencies of examinations the collective doses to the population, namely Genetic Significant Dose (GDS) and the Effective Dose Equivalent (EDE), have been evaluated. From the genetic and somatic doses per caput for each examination it is possible to recognise the radiological procedures that are the major contributors to the risk to the population.

A collaborative study with the National Radiological Protection Board (NRPB, Great Britain) and the Centre d'Etude sur l'Evaluation de la Protection dans le Domain Nucleaire (CEPN, France) concerning the comparison of radiological practice and patient exposure in Britain, France and Italy has been conducted. Aspects of diagnostic radiology practice have been studied that provide an indication of the emphasis placed on justification and optimization of the X-ray examinations in these three countries. Data concerning the levels of provision of radiology staff and facilities, the relative frequencies of different types of X-ray examination and the age/sex distribution of patients were obtained by the national or regional surveys. Five common types of examination have been selected for detailed study: pelvis, abdomen, lumbo-sacral spine, intravenous urography and barium meal.

This is believed to be the first time that surveys of this type have been conducted in different countries using identical measurement and analysis techniques. The study thus enables comparisons to be made between national strategies for providing radiology services and for perfoming certain types of examination, and the resulting doses to patients.

2. Results

Table I shows the relative contribution of the most relevant examinations to the collective doses in the NE-Italy. Barium meal is the most important contributor to the EDE; other major contributors are: lumbo-sacral spine, intravenous urography (IVU), barium enema and pelvis. Pelvis is the most important contributor to GSD to males; other major contributors are: hip and femur and IVU. Lumbo-sacral spine is the most important contributor to GSD to females; other major contributors are barium enema, IVU and full spine.

Table II shows the first result of the comparison of radiological practice and patient doses in Italy, France and Britain. It is clear that, per head of population, staff and facilities in Britain are rather less than those available in France and Italy.

The frequencies of X-ray examinations in the three countries are shown in Table III. In terms of the total numbers of medical examinations per 1000 inhabitans per year(excluding

dental and mass chest screening), France performs approximately twice as many as Britain and Italy about 1.5 times as many. These figures, when combined with the data of table II, also indicate that each radiologist was responsible for approximately 16000 examinations per year in Britain, 10000 per year in France and 9000 per year in Italy. Radiographers workload would appear to be more evenly matched, British radiographers each handling 3200 examinations per year, French 2900 per year and Italian 2300 per year.

The mean doses to organs for five different types of complete X-ray examination are shown in Table IV and V. Similar number of film are seen for all three countries for abdominal and pelvic examinations, with some differences evident for lumbosacral spines. British and Italian doses to organs are similar and French doses to organs are a factor of up to three times higher per examination, in line with the higher observed entrance surface doses. Table V contains results for 2 more complex examinations of the urinary system and the gastrointestinal tract that involve the use of contrast media, larger number of films and often fluoroscopy. Mean values for the dose-area product (Gy.cm2) derived from Diamentor measurements and for doses to four different organs are shown. Details of examination technique included in the table indicate that the French use considerably more films than the British and Italians during IVU, but Italians have a more marked preference for tomography. The French are also seen to utilize fluoroscopy for the majority of IVU's, while the Italians use it in only one-quarter of cases and the British hardly at all.

3. Discussion

The observations on national radiology practice provide only a limited insight into the attitudes adopted by the different countries towards the justification and optimization of medical radiology. Criteria for selecting both patients and examination techniques will be influenced by the different rates at with developments in diagnostic imaging and in the clinical management of patients have been introduced and accepted by the radiology professions in the countries concerned, and possibly by different national patterns in the prevalence of disease.

It has not been possible in these surveys to obtain sufficient information to clarify the reasons for the divergences observed in staffing levels, and in the frequency and choice of examinations. Some of the main differences in radiology practice, however, occur for procedures whose value has been reduced by the availability of alternative, less hazardous techniques or a decline in the diseases for which they were initially indicated. The introduction of quality assurance programmes in diagnostic radiology, which is being actively encouraged in Europe by CEC, will help to establish and mantain optimal procedures in this field. The surveys reported have provided a baseline of data concerning practices and doses delivered in diagnostic radiology against which the effects of future quality assurance programmes and reassessments of the need for certain procedures may be judged.

Table I. Percentage contribution of X-ray examinations to GSD and per caput-EDE by type of examination.

Examination	GSD (%) male	GSD (%) female	EDE (%)
Head	<0.1	<0.1	1.1
Chest, Chest photofl.	<0.3	<0.3	7.1
Adbomen	9.5	8.7	4.8
Pelvis	28.1	10.9	6.6
Hip, femur	18.2	0.9	1.7
Full spine	9.9	12.2	3.8
Thoracic spine	<0.1	<0.1	2.0
Lumbosacral spine	11.3	28.3	10.8
Intravenous urography	16.8	12.2	10.7
Barium meal	<0.1	6.1	26.2
Barium enema	1.8	13.1	10.7
Others	3.	7.6	14.5
Total (uSv/year)	253 (sd=17)		848 (sd=18)

Table II. Level of provision of medical radiology staff and facilities in Britain, France and NE Italy.

Staff and facilities	Number per million of inhabitants Britain (1983)	France (1982)	NE Italy (1983)
Radiologists	28	91	84
Radiographers	143	340	330
X-ray tubes	198	244	310
CT scanners *	1.7 (3)	1 (4)	4 (4)

(*) numbers in parentheses refer to 1986

Table III. Frequencies of X-ray examinations in the three countries.

Examination category	Annual number of examinations/1000 inhabitans Britain (1983)	France (1982)	NE Italy (1983)
Medical	444	825	665
Dental	165	545	118
Mass chest screening	12	166	81
Total	621	1536	864

Table IV. Film usage and mean organ doses for three types of examination.

	Lumbosacral spine UK	F	I	Abdomen UK	F	I	Pelvis UK	F	I
Mean no. of films/exam:	3.5	4.2	3.2	1.4	1.4	1.4	1.1	1.3	1.3
Mean dose to organs (mGy):									
Breast	.07	.24	.07	.03	.06	.02	*	*	*
Lung	.29	.99	.31	.08	.18	.06	*	*	*
Red marrow	1.4	2.0	1.0	.40	1.0	.44	.18	.36	.27
Bone	1.6	2.9	1.3	.54	1.3	.57	.32	.52	.50
Ovaries	4.3	3.6	3.6	2.2	4.2	1.6	1.2	2.3	1.9
Testes	.06	.06	.06	.40	.83	.29	4.6	9.2	8.4

Table V. Film usage, fluoroscopy time and doses for IVU and barium meals.

	Intravenous urography UK	F	I	Barium meals UK	F	I
Mean no. of films/exam:	8.2	19.3	10.0	7.8	16	14
Percentage of tomograms	6	9	16			
Percentage of fluorographic examinations	2.3	9	25	100	100	100
Mean fluoroscopy time (s)	150	55	78	193	247	337
Mean dose*area (Gy cm2)	30	90	-	19	20	38
Mean dose to organs (mGy):						
Breast	3.9	13	8.7	2.3	7.8	1.5
Red marrow	1.9	3.0	1.4	2.6	1.9	3.8
Bone	2.6	4.4	4.4	3.7	2.9	5.8
Testes	4.3	23	23	0.3	1.7	0.1

OPTIMIZATION OF INTRAVENOUS UROGRAPHY EXAMINATION

1. Methodology

Intravenous urography (IVU) is one of the radiological procedures which mostly contribute to collective doses. In the survey conducted in NE-Italy in 1983-84 this examination accounted for 11.8% of the total per caput-EDE from radiological examinations. Since 1984 significant variations in the methods of conducting IVU have occurred so that a reappraisal of doses and frequencies was thought to be worth.

The study was carried out in the major radiological institute of the region where 283 IVU examinations were analysed to assess which films are essential for reporting normal or pathological findings. Moreover, entrance skin doses for each exposure and breast and testis doses for the whole examination were evaluated by using NRPB Montecarlo conversion factors. As a cumulative index EDE was used.

2. Results and discussion

Table VI reports the effect of a region-wide adoption of the optimized methodology in use at the University Hospital of Trieste. The mean number of exposures per examination has changed, from 1983 to 1988, from 11.5 to 9 and entrance skin dose for a full lenght view from 6.9 to 4.8 mGy. Progress in film-screen systems accounts for lower entrance skin dose while the increased use of non-ionizing techniques such as ultrasound is the preminent reason for reduced frequencies.

Table VI. Trends of frequencies and doses for IVU.

Year	Frequencies exam/1000	EDE/examination mSv	per caput-EDE mSv/year	Reason of changes
1978	16.8	not available		
1983	13	7.07	0.091	
1988	10.5	4.83	0.051	(*)
future	6.5	3.5	0.023	(**)

(*) Lower frequency due to ultrasound; higher sensitivity of film-screen systems; less films/examination.
(**) Lower frequency due to ultrasounds; less nephrotomograms.

PATIENT DOSES IN COMPUTED TOMOGRAPHY IN ITALY

1. Methodology

In medical diagnostic radiology collective doses and associated risks can be estimated from frequencies and types of examinations and related patient organ doses.
A nationwide survey has been conducted in Italy to establish frequency and technical parameters adopted in each type of CT examination. Questionnaires were sent to all the facilities (293) to collect relevant information including kilovoltage, filtration, mAs, number of slices, slice thickness, couch increment, scan angle, number of examinations per week. A tomogram typical of each type of examination was requested as a supplemental source of information to help identify the anatomical landmarks of the irradiated part of the body. The examination types selected were the 10 most frequently performed and precisely: head,chest,abdomen,cervical spine,

lumbar spine, pelvis, petrous bone, hypophysis, orbits, skeletal segments. Finally, a section of the questionnaire was devoted to collect modalities of execution of QA Programmes and frequencies of QC tests. Organ doses to patients who undergo CT examinations will be estimated using Monte Carlo (MC) simulations techniques. Conversion factors relating mean organ doses to the free-in-air dose at the centre of rotation are being calculated by NRPB (UK) for a variety of make and type of CT scanners. To allow for variations in X-ray spectrum, beam collimation and scan geometry encountered in practice on different machines and facilities, a dosimetric survey has been carried out on a sample of scanners randomly covering the whole Italy. On each scanner the free-in-air axial dose profile for a single slice at the centre of rotation has been measured by exposing a row of TLDs suitably spaced inside a small plastic cylinder. Dose profiles have to be measured for each selection of the scanning parameters commonly used for the different examinations. Typical dose profiles measured on a single scanner for different slice thicknesses are shown in fig. 1. Since patient doses depend not only on peak dose but also on the width and shape of the dose profile, the CT Dose Index (CTDI) has been used as the normalising factor for organ doses. The CTDI per unit tube workload is defined as

$$CTDIN = \frac{CTDI}{mAs} = \frac{\int D(z)\, dz}{mAs\ T}$$

where D(z) is the measured dose along the axial direction z in a slice of thickness T with a tube workload of mAs.
The dose D to a certain organ for a complete examination can be evaluated as follows

$$D = \Sigma_i\ f_i\ CTDIN_i\ mAs_i\ p$$

where the sum is extended over all the slices carried out for that examination, f_i is the MC conversion factor for that organ and that slice, mAs_i the workload for the slice i and p, the packing factor, is the ratio between the nominal scan thickness and the couch increment.

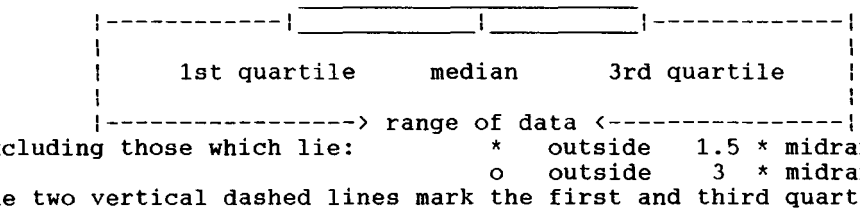
The methodology here described has been adopted by other research groups in England, France and Germany so as to allow a proper comparison of results.

2. Results

So far, 88 questionnaires out of 293 have been returned with a provisional reply rate of 30%, but the reply rate is expected to improve. The mean numbers of CT examinations of all types per week are shown in fig. 2, where it appears that most CT scanners perform 40-140 examinations per week. QC tests are usually done by technicians of the manufacturer on a monthly basis (65%). For each of the 10 types of selected examinations histograms of technical parameters and frequencies have been obtained. As an example, fig. 3, 4, 5 and 6 show some data for the examinations of the head (mAs, number of slices per examination, thickness of slices, frequency).Table VIIreports

how many examinations of each type are performed weekly on average.

Dose profiles for a single slice at the centre of rotation have been measured at 36 sites. We totalled 160 profiles obtained in different conditions of irradiation on a variety of make and type of CT scanners. In fig. 7 the distribution of measured CTDI per unit workload is shown. All profiles are included in the figure irrespective of type, slice thickness, kV or filter used. The gaussian distribution with the same mean (171 microGy/mAs) and S.D. (83 microGy/mAs) as the actual distribution is superimposed for comparison. In the schematic plot of fig. 8 the same data are disassembled by manufacturer. The key to the distributions is the following:



The two vertical dashed lines mark the first and third quartile of the distribution of all the CTDIs measured (see fig. 7). Whereas the distributions of Toshiba and Pfizer scanners are not reliable because there are few data, there is a significant difference between CTDIs of Siemens and Ansaldo scanners and those of G.E. and Philips scanners which may be attributed to the concurrence of many factors such as filter thickness, focus-axis distance, dose enhancement factor (the ratio between peak dose and CTDI). Fig. 9 shows a 3D-plot of CTDI as a

Fig. 1. Axial dose profiles.

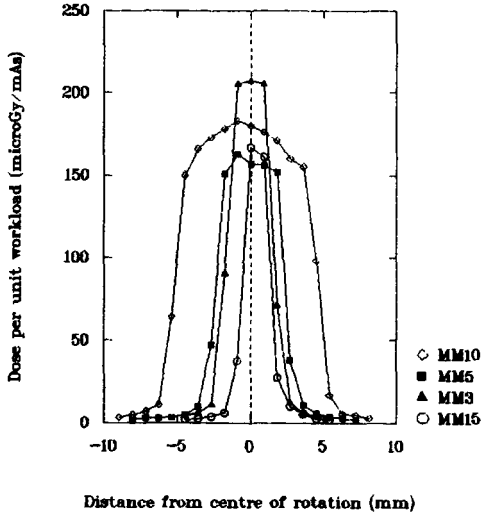


Fig. 2. Italian CT survey

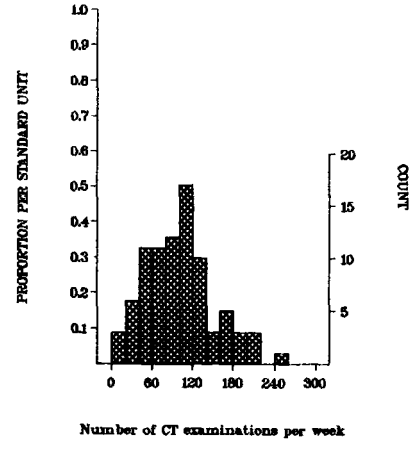


Fig. 3. mAS

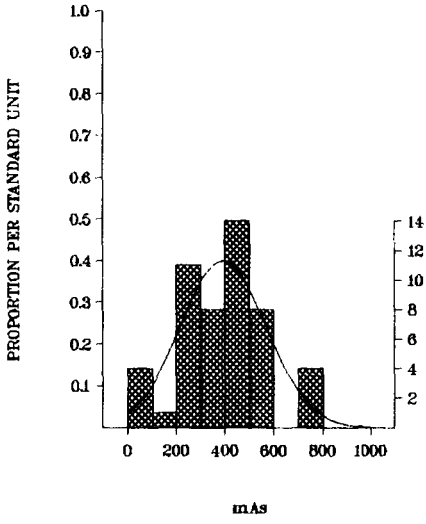


Fig. 4. Number of slices

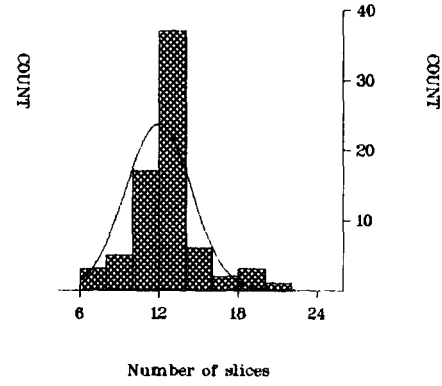


Fig. 5. Slice thickness

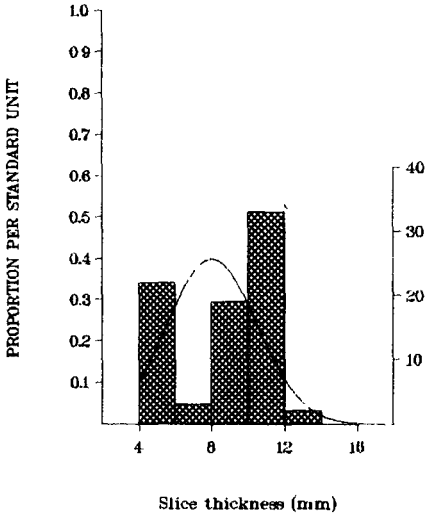
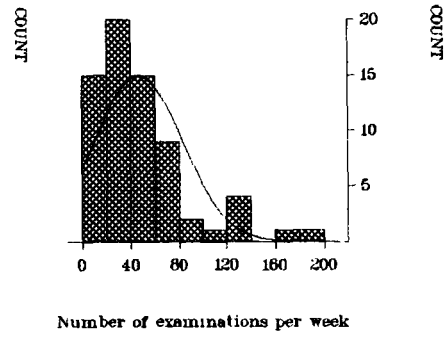


Fig. 6. Frequencies



function of slice thickness and kVp. Only the profiles of General Electric (G.E.) scanners (the most numerous in our sample) are included. The plotted surface is a distance weighted least squares fit through the measured points. This plot generally shows an increase of CTDI with decreasing slice thickness. This is due to the fact that the enhancement factors are generally larger for the smaller slice widths, where precise collimation is obviously more difficult.

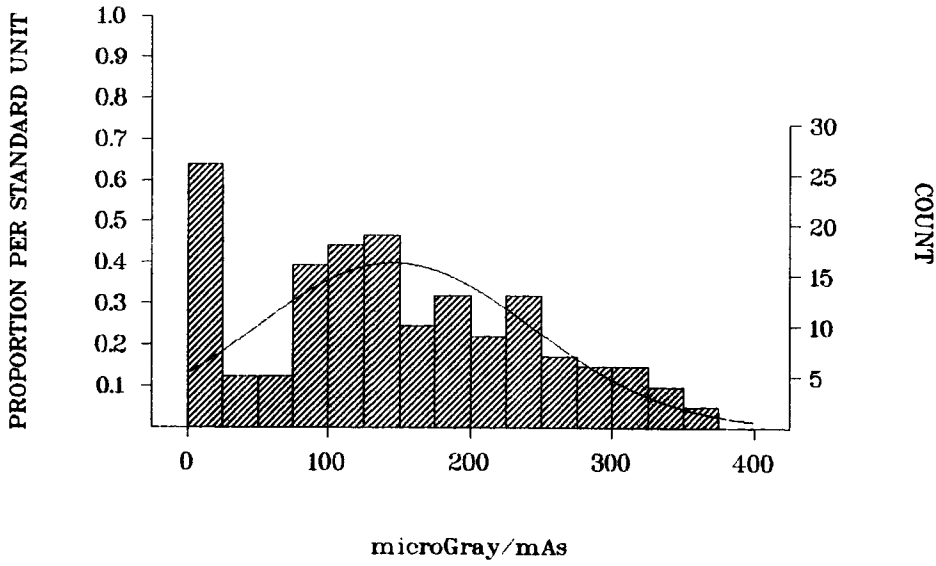
As MC simulations are not yet completed, final evaluation of patient doses has been delayed.Table VIIIreports a tentative estimate of the average patient doses for the examination of the head. The estimation is based on the following assumptions:

- the examination is performed with contiguous, not overlapping scans across the whole brain;
- for each scan CTDI is given by the average value of the measured sample for slices wider than 5 mm (150 microGy/mAs);
- tube workload is equal to the average value of the distribution shown in fig. 3 (405 mAs);
- geometry of irradiation and beam quality are those of a Siemens Somatom DRH scanner for which at this time the MC conversion factors are available.

3. Discussion

As happens for conventional X-ray diagnostic radiology, also for CT the spread of examination techniques between installations is very large. The distributions of technical parameters (see fig. 3, 4 and 5) are characterised by high coefficients of variation and positive asimmetry. As a

Fig. 7. CT Dose Index measured at 36 Italian scanners



consequence for any given examination the complete range of patient doses received in practice will extend as widely as one or more orders of magnitude.

Differences between CTDI's per unit tube workload may or may not affect patient doses. Where these differences are due to different levels of peak dose in the profile, it can be reliably supposed that a number of mAs inversely proportional to peak dose is needed to obtain images at the same noise level. On the contrary, dose enhancement factors greater than one adversely affect patient dose without improving image quality.

Since on the average 94 examinations per scanner are carried out weekly in Italy, the total annual number of CT examinations can be estimated in 1.5 million, corresponding to 2.5% of all types of X-ray examinations (Padovani et al.,1987). Patient doses are much higher than those delivered by conventional radiography concerning the same anatomical compartment. For instance, the tentative estimate of the Effective Dose Equivalent for a CT examination of the head - 2.95 mSv, should be compared with that for the conventional examination of the head - 0.224 mSv (Padovani et al.,1987). These first data confirm that CT procedures are assuming an increasing importance as contributor to population doses in medical diagnostic radiology, although the most valuable information obtained by CT examinations has to be accounted for in any risk-benefit analysis.

Fig. 8. CTDI by manufacturer

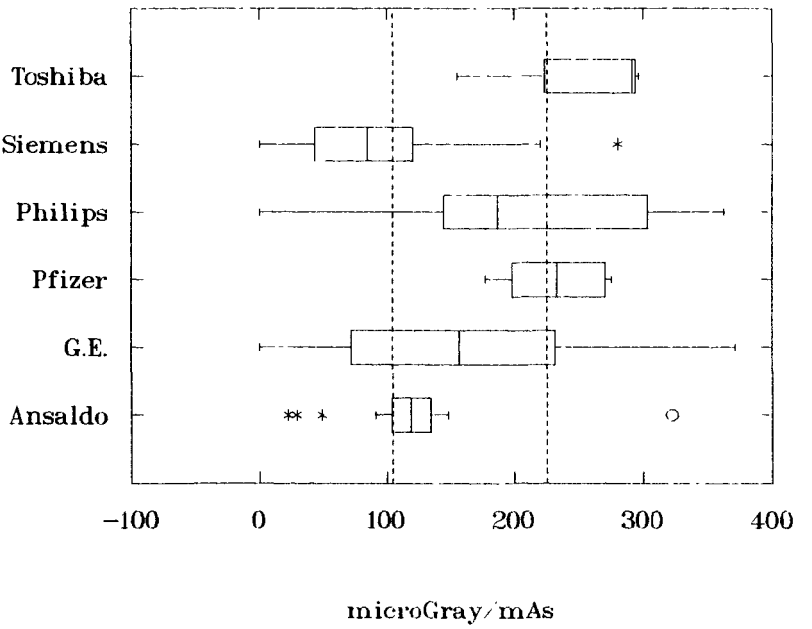


Fig. 9. CTDI(slice thickness,kVp) for G.E. scanners

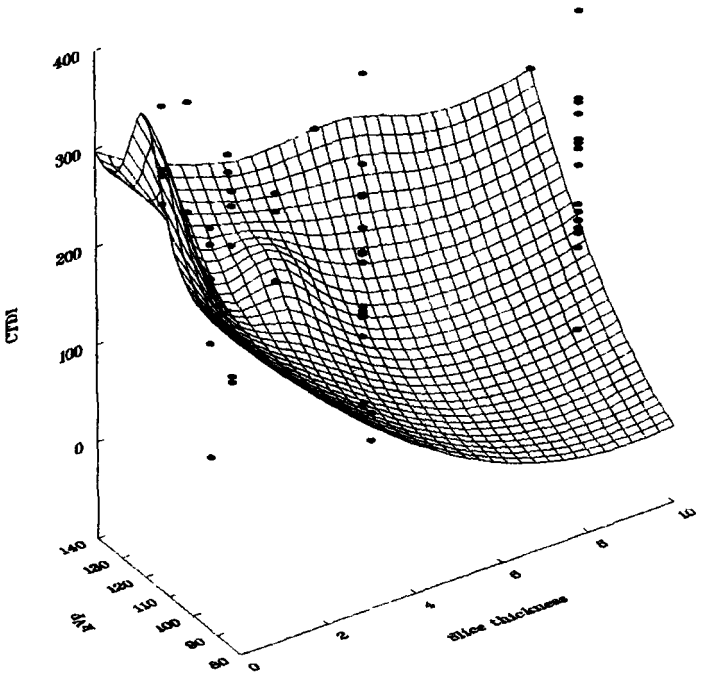


Table VII. Weekly average numbers of examinations.

Examination type	Weekly number of examinations	Percentage
Head	37.2	39.7
Abdomen	17.4	18.6
Lumbar spine	12.8	13.6
Chest	9.7	10.3
Pelvis	7.4	7.9
Cervical spine	2.3	2.4
Petrous bone	1.8	1.9
Hypophysis	1.7	1.8
Skeletal segments	1.6	1.7
Orbits	1.3	1.4
Others	0.6	0.6
All examinations	93.8	100

Table VIII. Average patient doses for the examination of the head.

Tissue	ICRP weighting factor	Dose (mGy)
Red bone marrow	0.12	2.53
Lung	0.12	0.09
Thyroid	0.03	1.66
Bone surface	0.03	10.70
Brain	0.06	37.70
Skin	--	2.92
Lens	--	48.70
He (mSv)		2.95

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V. Publications:

R Padovani, G Contento, M Fabretto, M R Malisan, V Barbina, G Gozzi, 'Patient doses and risks from diagnostic radiology in North-east Italy, Br. J. Rad., 60,155-165, 1987.

G Contento, M R Malisan, R Padovani, C Maccia, B Wall, P Shrimpton, 'A comparison of diagnostic radiology practice and patient exposure in Britain, France and Italy', Br. J. Rad., 61,143-152,1988.

L Dalla Palma, F Stacul, F Pozzi-Mucelli, G Contento, R Padovani, 'Urography: Optimization of the technique to lower patient exposure', Workshop:'Optimization of image and patient exposure in diagnostic radiology', Oxford, sept 1988.

C Maccia, B Wall, R Padovani, P Shrimpton, B Hussen, 'Results of a trial set up by a study group of the radiation protection programme of the CEC', Workshop:'Optimization of image and patient exposure in diagnostic radiology', Oxford, sept 1988.

G Contento, G Fabris, M Leonardi, 'Dose al paziente nelle procedure neuroradiologiche per il trattamento dell'ernia discale lombare', Rivista di neuroradiologia, 2:55-59, 1989.

R Padovani et al., 'An estimation of the genetic and somatic risk from diagnostic radiology in Friuli Venezia Giulia, Italy', Proc. of the VII Int. Conf. on Med. Physics, Helsinki, Aug 1985.

G Contento, MR. R Malisan, R Padovani, C Maccia, B Wall, P Shrimpton, 'A comparison of diagnostic radiology practice and patient exposure in Britain, France and Italy, Symposium: Radiation protection advances in Yugoslavia and Italy, June 1988, Udine, Italy.

C Maccia, R Padovani, 'Indagine europea sulla qualita' dell'immagine e la dose al paziente in radiodiagnostica: aspetti dosimetrici, metodologia e risultati preliminari', Convegno nazionale AIFB: Controlli di qualita' ed ottimizzazione nell'impiego delle radiazioni in medicina', Dic 1988, Italy.

R Padovani, G Contento, 'La dose al paziente in TC', XXVI Congresso dell'Associazione Italiana di Radioprotezione, Sept 89, Verona, Italy.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-F-137-D

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Title of the research contract:

The effective dose equivalent due to X-ray diagnostic examinations and the impact of quality control on medical exposure.

List of projects:

1. The effective dose equivalent due to X-ray diagnostic examinations and the impact of quality control on medical exposure.

Title of the project no.: BI6-F-137-D

The effective dose equivalent due to X-ray diagnostic examinations and the impact of quality control on medical exposure

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Introduction

In recent years investigations about radiation exposure of X-ray diagnostic examinations have been carried out all over the world. The results of these investigations are more or less all the same: Even similar diagnostic questions lead to a variation in patient exposure by a factor of 10 to 30. There are two possible reasons to explain this behaviour: In the first place there are different ways of performing an examination and different conditions of using the X-ray equipment. In the second place there are unrecognized faults (deviations from predefined conditions) and misadjustment of the equipment.

In different countries as well as in the European Community standards have been developed and published to optimize and to unify the conditions of exposure. In the framework of this project a comparison of different standards has been realized.

While at the beginning of the project (1985) the necessity and procedure of constancy tests have still been under consideration, these methods have been established in 1989 - at least in the FRG. Within the scope of our project different methods of constancy tests have been compared and long term analyses have been accomplished.

The aim of recommendations and prescriptions in X-ray diagnostics is the reduction of radiation exposure without lack of diagnostic information. The success of this effort is stated in comparison to the effective dose per examination or per exposure. The direct determination of the effective dose is difficult and expensive. Therefore we used special methods of evaluation and as an example we compared the results with direct measurements. In many cases the radiation exposure has been expressed as absorbed energy instead of effective dose. The absorbed energy has been deduced from the exposure-area-product after Shrimpton and Wall (Chilton, Didcot, GB).

Special attention has been drawn on new methods of examination like Digital Subtraction Angiography (DSA) and Percutaneous Transluminal Angiography (PTA).

Comparison of recommended conditions of exposure

In cooperation with G.-A. Brandt (GDR, Berlin-Buch) and A. Gurvich (USSR, Moscow) the "Quality Criteria for Radiographic Images", developed in 1985/1989 Radiation Protection Programme, have been compared with examination standards of a number of European countries. The resulting variation of parameters is listed in table 1 with respect to different groups of organs.

Table 1: Variation of recommendations in technical parameters for production of radiographic images for different organ groups in European countries.

Parameter of image production	examined organ group**) I	II	III_1	III_2	IV
tube voltage (kV)	40-55	55-75	60-80	120-150	75-100
generator and tube power (kW)	1*)	30*)			
anti-scatter grid, grid ratio	-	r=7; 30/cm ... r=8; 40/cm		r=7; 30/cm ... r=15; 70/cm	
focus-film distance (cm)	100-140	*)	100-120	150-200	*)
speed of film/ screen combin.	25-50	50-100	100-200	100-200	200-400

*) Identical recommendations in almost all countries.

**) organ group I: limbs distal of hand and foot joints including these joints

organ group II: limbs excluding group I + skull

organ group III_1: gall-bladder, kidneys, lumbal a.p., thoracic and cervical spine

organ group III_2: chest

organ group IV: lumbal spine frontal, stomach, colon, pelvis

To our suprise the conformity is reasonably good. However, going into detail, there are certain deviations from dates given in the working document "Quality Criteria for Diagnostic Radiographic Images (Feb89)":

- The recommended range of X-ray tube voltages is larger in eastern countries like USSR and CSR than in countries of the European Community.
- The recommended screen-grid ratio is smaller in the USSR, CSR and Hungary.
- The proposed speed classes are considerably lower in all countries (except pelvis exposure) than fixed in the working document of the EC.
- An additional copper filter is only requested by the FRG for the exposure of children.

Constancy tests

a) In general different constancy tests have been established for film development and for X-ray units. For film development the so called conventional sensitometric/densitometric methods and the visual (alternative) methods are in use. The conventional methods all work on the same principle, but differ considerably in their effort of electronical analysis and equipment with respect to comfort and cost. However, all of the methods and devices are suitable when used carefully and critically. Table 2 shows some of the features investigated, comparing conventional and visual methods of film development testing.

Table 2: Comparison of conventional and visual methods for film development tests.

Parameter	conventional method	visual method
construction prescribed by	DIN 6868 part 2	---
cost	considerable to high	low
comfort	good to very good	simple
delicacy	depending on system	small
advantages/ disadvantages	assessment of trends possible, causal failure advice, but possible misinterpretations	---

A comparison of different tools for X-ray unit constancy tests has been carried out, too. The results are compiled in table 3.

b) Results of the constancy tests.

The long term execution of constancy tests shows surprising results in part.

The constancy of film development has been tested daily for 20 different equipments. Within an arbitrary period of 2 months (43 workdays or 860 tests) 12 serious malfunctions have been detected (1.5% of all tests).

In detail the deviations from tolerances have been

- 5 cases due to equipment maintenance,
- 2 cases due to defect temperature control,
- 3 cases due to variation of developing bath concentration,
- 1 case due to contamination of developing bath,
- 1 case due to mistakes when replacing the developing bath.

Table 3: Comparison of tools for monthly constancy tests of X-ray units

aspects	RöVi (Wellhöfer)	NORMI (PTW)	Bronder (PTW)	QC-system (Rego)
construction rule	DIN 6868, parts 3 and 4			
method	digital	analog	analog	analog
cost	comparable			
comfort	comparable			
advantages	fast and reproducible positioning of attenuation filter at gantry, light-weight test tool			
disadvantages	electronic dose-integrator, instability possible	heavy-duty test tool, because of gantry distant attenuation filter		

Moreover we registered, when and if at all the staff reported the alterations (without knowledge of the test results) to us. In many cases the changes have not been reported for days, in some cases they have not been recognized at all.

The long term experience with constancy tests of X-ray units is - with one exception - completely different. Serious variations of
- optical density
- contrast
- resolution (fluoroscopy)
- relative dose and
- radiation beam field

have not been observed for the X-ray units investigated.

However, serious variations have been found at the so called acceptance test, when comparing our results to those indicated by the manufacturer. This final technical check of the equipment before routine use is extremely important, in order to put the equipment into a well defined condition.

Exposure at special examinations

Special X-ray examinations gain more and more in significance and contribute to the total population exposure because of either high specific radiation exposure or increasing frequency of the examination.

a) DSA

The **D**igital **S**ubtraction **A**ngiography has rapidly developed in the past few years. The radiation exposure drastically depends upon concentration of contrast medium, type of injection and type of equipment (pulse mode or fluoroscopic mode). Compared to conventional angiography the radiation dose level increases by a factor of about 2 using DSA. The average absorbed energy has been determined to be 100 mJ per examination.

b) PTA

Interventional radiology, i.e. **P**ercutaneous **T**ransluminal **A**ngiography, is becoming more and more used, too. Independent of technique (balloon, laser, rotor, lysis), all of the examinations are performed under X-ray control. The average absorbed energy for balloon-dilation has been determined to be 75 mJ per examination.

Investigations about the frequency of the examinations are not available, yet.

c) Digital mammography

The advantages of digital luminescence mammography are believed to be lower patient exposure and the possibility of digital image processing. Basic requirement for the introduction of this method, however, is the visualization of objects of 0.2 mm in diameter, as fixed in the working document (Feb89) of the EC and in the recommendation of the Federal Republic of Germany.

The dose-dependence of resolution for high resolution (HR) and standard (ST) digital luminescence radiography is listed in table 4 with respect to rows and columns of the digital image.
The dose is normalized to 30 uGy (D_{rel}=1) in the cassette plane. (At this dose a resolution of 10 lp/mm with optimum densitiy is achieved using Microvision film (Du Pont) and Orthex Mamma-foil, speed class 15.)

Table 4: Resolution in lp/mm dependent on relative exposure for high resolution (HR) and standard (ST) digital luminescence foils.

D_{rel}	HR row	HR column	HR 45°	ST row	ST column
0.125	3.5	3.5	-	4	4
0.25	4	4	-	-	-
0.5	4.5	4.5	-	4.5	4.5
1	4.5	4.5	5.6	5	4.5
2	5	5	-	5	4.5

A comparison of 50 digital and conventional mammographic examinations using ROC-analysis is in progress.

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1989

RADIATION PROTECTION PROGRAMME
Final Report

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)]:

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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.:

BI6-0214-E(A)

OPTIMIZATION OF PROTECTION IN MEDICAL DIAGNOSTIC RADIOLOGY

Head(s) of project:

Prof. E. Vañó and Prof. L. González.

Scientific staff:

Prof. A. Calzado, Prof. V. Delgado, Prof. P. Morán, Prof. M. Chevalier, Prof. E. Guibelalde, P. Ortiz, M. Marín and M. J. Ruíz.

I. Objectives of the project:

1. Analysis of risks to operating staff and population in diagnostic radiology installations by area and personal dosimetry and its correlation with equipment features and conditions, room workloads and training of operating staff.

2. Analysis of radiological risk to patient from direct dose measurement and numerical estimates and its correlation with equipment features, procedures of radiological examinations and training level of operating staff.

3. Designing and implementing of a pilot programme of Quality Assurance in Diagnostic Radiology and setting up the bases for a nation-wide programme of patient dosimetry and Quality Assurance in Diagnostic Radiology.

II. Objectives for the reporting period:

1. Drawing conclusions from the analysis of area and personal dosimetry, considering room and equipment characteristics, workloads and operating procedures.

2. Attaining results for patient organ doses and effective, collective and genetically significant dose equivalents for different examination types. Doses have been obtained by means of measurements in patients and measurements in anthropomorphic phantoms or numerical estimates using Monte Carlo factors.

3. Designing and implementing of a pilot quality control programme. Assessment of its effectiveness in several rooms and certain types of radiological examinations and setting up the bases for a nation-wide future programme.

III. Progress achieved:

1. METHODOLOGY.

The project has been carried out in four large hospitals of Madrid. A number of results have been also obtained from several outpatient and private centres.

Three hospitals involved in the project belong to the Instituto Nacional de la Salud (National Institute of Health); they count with 4 000 beds and serve to 36% of the population of the Community of Madrid. The fourth hospital is a Central Military Hospital, recently built, which has modern installations and equipment.

Outpatient centres serve patients in a first stage, carrying out basic and general radiology examinations (though one of them are already incorporating computed tomography scanners as standard equipment). The outpatient centres included in the project serve a population between 150 000 and 300 000 inhabitants.

The total number of radiological examinations carried out in 1988 in these four hospitals has been approximately 600 000 (about 1 800 000 radiographs).

It must also be pointed out that during the three years in which the project has been developed the number of radiological examinations has significantly increased, since in 1987 the increase has been of 11% respect to 1986; and in 1988 the increase has been of 7%. These data have been obtained from one of the hospitals which can be considered as the pattern, since its equipment, staff and patients have not suffered any modification during this period.

Along with the project, a radiation protection and control quality training pilot programme was implemented in order to verify the effectiveness of this kind of activity. It was followed by more than 60 radiologists and 120 radiographers in the centres.

Operating Staff Dosimetry.

A careful analysis on the operating staff dosimetry has been performed in the Diagnostic Radiology Services, doubling, on certain occasions, personal dosimeters for comparison purposes, apart from using additional dosimeters to estimate the doses in hands, lenses and/or thyroid when the irradiation risk was located.

Personal dosimetry results have been partly stored in a computer in order to correlate the anomalous values with workloads and room design, operating staff skills and equipment characteristics and conditions under which examinations are carried out.

Personal dosimeters used have been photographic or thermoluminescent (lithium fluoride generally) and when dosimetry has been doubled for intercomparison purposes, several Lecture Centres duly authorized and controlled by the Consejo de Seguridad Nuclear (Nuclear Safety Council) have been employed.

Area Dosimetry.

Area dosimetry main goal has been to verify the radiological protection conditions of the rooms and the suitability of installation barriers. Some dosimeters have also been placed near the equipment as witnesses to rooms workloads.

Such a dosimetry has been carried out with FLi thermoluminescent crystals. Between 5 and 10 positions have been selected in every room, recording the measurements monthly and during periods between 2 and 8 months depending on the data uncertainities.

These results have also been stored on a computer in order to analyze them in similar way that operating personal dosimetry.

Patient Dosimetry.

Dosimetric evaluations to patients were preceded by a careful obtention of general data concerning these centres (number of patients served on a regular basis, emergency cases and other health delivery

care parameters) and also of the examination parameters carried out in each of the rooms: kVp, mAs, number of exposures, screening time, age and sex of the patient, etc.

Patiens dosimetry has been performed at three levels:

a) Measuring entrance dose with ionization chambers and simulating typical conditions of each examination in each room, previously determined over a sufficiently significant patient sample. This level was occasionally applied in simple examinations such as chest, spine, etc., and on samples showing low dispersion in dose values at the entrance.

b) Measuring individual entrance dose (and some organ doses when possible) on a sample of patients with thermoluminescent dosimeters or transmission chambers.

c) Measuring organ doses in REMAB and RANDO anthropomorphic phantoms (Alderson, U.S.A.) in complex examinations (GI tract, urography, CT, angiography, etc.). Between 70 and 100 TL dosimeters per examinations have been used (FLi crystals, Harshaw TLD-100). Contrast media were employed when necessary (REMAB phantom allows this possibility).

During the measurement with phantoms the values of the (dose x area) product have been registered. These results were used along with some organ TLD readings, to estimate organ doses to patients.

Organ doses in the case of simple examinations were mainly derived, whenever possible, using the Monte Carlo coefficients calculated by Jones and Wall. In complex examinations mixed approximations have been used, taking into account the experimental measurements obtained by the anthropomorphic phantoms together with the numerical coefficients of Jones and Wall, and Rosenstein.

For collective dose estimates, we have used data concerning population served in each centre, weighting up later, doses applied in the different rooms and centres, according to the number and type of radiological examinations performed.

At the same time, it has been calculated child expectancy in the area of Madrid, with data from the National Institute of Statistics publications, with the aim of obtaining genetically significant dose values.

Nevertheless it must be pointed out that some of these parameters are being dramatically changed in Spain due to the reorganization that the Health System is undergoing. These changes include the transfer of responsibilities in the field of Health to Autonomous Regions and the change in the health areas assigned to the different public hospitals; such modifications could mean important changes concerning the number of patients in each centre.

From 1986 to 1989, public health attention has increased in Spain from 94% to 99% approximately of the population. At the same time, big groups of population are also obtaining health attention through private assistance, which makes it even more difficult to determine precise figures in the number of radiological examinations per inhabitant.

Quality Control Pilot Programme:

Quality control pilot programme has been planned by analyzing generator equipment, image elements and examinations procedures, with the aim of justifying dosimetric and image quality abnormal findings.

Patient dose values obtained in each of the rooms of the different centres have been compared with reference values set up for certain radiological examinations by a group of experts from the European Community, or with standard values obtained in other countries (especially in the United Kingdom), in order to assess the doses obtained in a specific room (average or high dose), so we will be able to recommend quality control urgent measures which will correct anomalies that can cause high dose values.

After performing quality control and implementing corrective measures, a new evaluation of doses to patients has made possible in some rooms and in some types of examination to verify their effectiveness.

A great effort has been made to computerize the analyzed parameters, in order to design an expert system which could be applied in a future national plan of dose estimation and quality assurance in Diagnostic Radiology .

The following data have been computerized: equipment features, area dosimetry results, operating staff doses, examination parameters and patient data, together with organ doses, when available. The aim is to interrelate data-bases so that abnormal values of doses to patients and unsuitable image quality could be detected in a semi-automatic way.

At the same time, it has been drafted a national programme for dose estimation to patients and quality assurance in Diagnostic Radiology, taking into account that most Spanish Diagnostic Radiology services have not previously made this type of evaluation. Data acquisition forms have been worked out, according to the computerized treatment of this first stage of the project. The operating process has been set up so that the different participating centres can know their dosimetric values and can set operating priorities when abnormal values are detected.

2. RESULTS AND DISCUSSION:

Personal Dosimetry:

More than 5 000 data corresponding to 300 members of staff working with X-ray equipment have been analyzed. For almost 5% of the staff effective dose equivalent value range between 1/10 and 3/10 of the annual limit; about 2% show a dose higher than 3/10; about 5% show certain anomalous dosimetric values along the year -a detailed analysis was performed only in a centre- which can be caused by incorrect operating procedures or, on certain occasions, by ill functioning equipment.

About 52% of the staff exposed to X-ray equipment ionizing radiations, directly belong to Diagnostic Radiology services in hospitals; the rest belong to operating theatres, cardiopulmonary examination services, etc., for which it should be considered the inclusion of this type of services in the control and quality assurance programmes.

One of the conclusions it must be pointed out in this analysis is the convenience that the detail of the rooms in which staff members have been working, should be stored along with the dosimetric records. Thus, records, workloads and characteristics of the rooms will help later to justify anomalies.

For operating staff, a value of 0.5 mSv per month in general radiology rooms has been considered as "investigation level". The specific risk in hands and lenses should be estimated (with additional dosimetry during some months) in the staff who usually works near the patient during the X-ray emission.

Area Dosimetry.

In area dosimetry, 2 670 data of doses corresponding to 655 different positions have been obtained, evaluated upon a total number of 104 Diagnostic Radiological rooms and some operating theatres.

Anomalies in protection barriers have been found out in 16 rooms (11 of which belong to the same centre, what means that nearly half of its rooms have shielding defects).

As a conclusion, area dosimetry should be applied at least once upon installations, especially on those which are older, and after possible modifications that a room might undergo due to barrier or equipment changes.

Patient Dosimetry:

Concerning patient dosimetry, data from approximately 60 000 examinations have been obtained, and in some 3 000 cases it has been possible to calculate organ and effective doses.

Table 1 shows examination frequencies and averaged values of effective dose equivalent in each of them (estimated values before quality control, which is now being implemented in some rooms, was applied).

Figures 1, 2 and 3 show, as examples, age histograms in some of the most significant examinations (paediatrics contribution not included).

The results of our estimations show that in the Community of Madrid (4 800 000 inhabitants), about 680 X-ray examinations per 1 000 inhabitants have been performed in 1989 (considering a yearly mean increase of 8% in the available figures over the last years), without taking into account neither military

or massive labour check-ups nor dental radiological examinations. This would mean values of 1.21 mSv for the effective dose equivalent per inhabitant and year in the Community of Madrid and 5 830 pers.Sv. for the collective dose.

Table 2 shows results obtained for genetically significant doses (estimations of paediatrics examination contribution included).

Table 3 shows results of organ doses and effective dose equivalent for certain standard examinations.

When an analysis of patient doses is carried out in different X-ray examinations, comparing them with the values taken as reference; it is evidenced that the values of dose appear to be too high for given X-ray examinations in 36% of 45 evaluated rooms, This points out the urgency and need for implementing control and quality assurance programmes in these rooms.

In many cases it has been stressed the importance of operating staff training in radiation protection, in order to lower staff radiation risk and, above all, in order to lower patient doses.

Quality Control.

Concerning X-ray generator equipment, the following results have been obtained: 36% out of 53 analyzed equipment could be considered to be in good condition, while 33% would need urgent and immediate attention and the other 33% would require checking, though their working order is acceptable.

In order to assess the equipment, the following data were taken into account: results obtained from kVp control, mA or mAs linearity (depending on the type of generator), exposure time, filtration, half value layer and waveform.

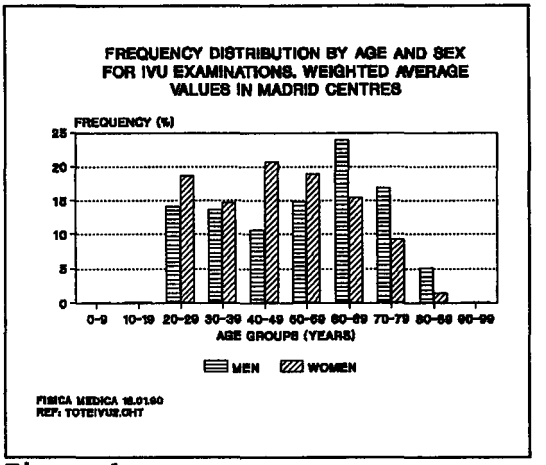

Figure 1

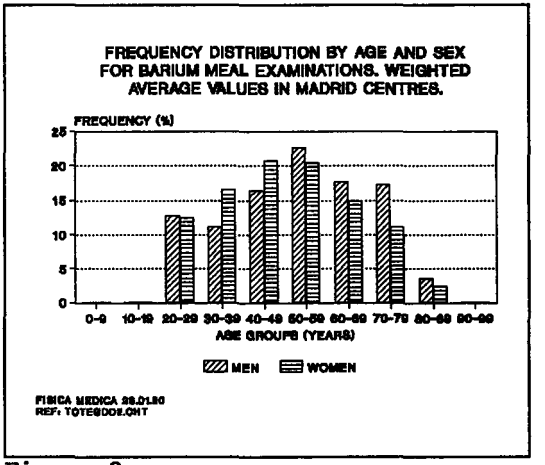

Figure 2

Concerning intensifiers and TV monitors, after studying 18 equipments, the results were the following ones: 9 needed urgent attention, 2 were in reasonable conditions, while 7 were in good conditions. For this assessment, contrast, resolution, dynamic range and distortion were taken into account. There are still some image intensifiers without TV monitors.

With regard to other image elements, 707 viewing boxes, 43 automatic film processors, 30 dark rooms, 6 film storage rooms and 270 cassettes and intensifying screens have been analyzed. The results were as follows: 40% of the viewing boxes were in bad condition, 42% of the processors showed instability in their functioning, 33% of the dark rooms were in bad condition, one of the film storage rooms did not fulfil basic requirements and 3 of them showed reasonable conditions, 30% of the cassettes and screens presented light leaks, marks and serious damages concerning the film/screen contact.

In this case, quality control was carried out by measuring viewing boxes brightness and uniformity; performing daily sensitometric control in processors; controlling temperature and humidity, radiation

level, placement, expiration date and blurring in film storage rooms; light leaks, ventilation and filters and safelights in dark rooms; light leaks, cleanliness and contact in cassettes.

Image quality is considered to be acceptable generally by radiologists. Nevertheless, image tests are showing that it can be significantly improved in many cases, both in fluoroscopy and radiography, since many machines are far away from optimum working, as mentioned in previous paragraphs.

The adjustment of X-ray generator and tube working conditions together with image devices have given rise to patient dose savings of up to 40%. An improvement in the operating staff training, especially in radiation protection subject, has allowed reductions of 10% in rejects, of 20% in patient effective dose equivalent (in certain examinations), of 25% in the screening time and of 70% in gonadal dose.

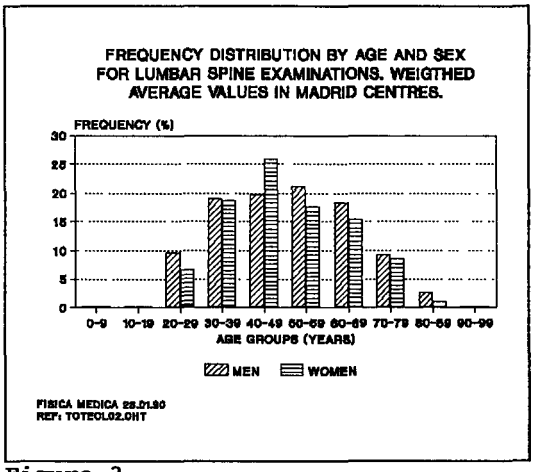

Figure 3

Throughout the project, collaboration in the programme on "Quality Criteria for Diagnostic Radiographic Images" has been carried out, likewise different frequent contacts with other groups which develop similar projects in the United Kingdom, France and Italy.

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TABLE 1

ESTIMATION OF YEARLY EFFECTIVE DOSE PER INHABITANT AND COLLECTIVE DOSE IN THE AREA OF MADRID (1989)

EXAMINATION	EXAMIN./1000 INH. PER YEAR	mSv/EXAMIN.	CONTRIBUTION (%) TO COLLECTIVE DOSE
CHEST	178	0.29	4.30
SKULL	21	0.14	0.24
SPINE	138	2.07	23.50
HIP AND PELVIS	21	2.02	3.50
ABDOMEN	61	1.17	5.90
IVU	17	6.62	9.30
GI TRACT	52	9.70	41.50
EXTREMITIES	104	0.10	0.86
MAMMOGRAPHY	25	1.54	3.17
OTHERS	63	1.50	7.73
TOTAL	680		100.00

Effective dose per inhabitant and year (1989):

1.21 mSv

Yearly collective dose in the Community of Madrid (4 800 000 inhabitants):

5 830 pers.Sv

TABLE 2

ESTIMATION OF GENETICALLY SIGNIFICANT DOSE (PER YEAR)

EXAMINATION TYPE	CONTRIBUTION MEN (μGy)	CONTRIBUTION WOMEN (μGy)	TOTAL (μGy)
Abdomen	11.5	32.0	43.5 (7.2)
Lumbar spine	4.4	34.7	39.1 (3.3)
Pelvis and hips	31.6	12.5	44.0 (5.8)
Urography	48.5	18.8	67.3 (17.4)
Cystography	6.1	5.2	11.3 (4.0)
Chest	0.10	0.18	0.28 (0.04)
Barium enema	1.8	18.3	20.1 (6.0)
Barium meal	9.6	8.9	18.5 (4.6)
CT abdomen	2.7	5.1	7.8 (0.7)
TOTAL	116.3	135.7	252.0 (21.7)

Figures in brackets show statistical errors.

TABLE 3

DETAIL OF ORGAN DOSE (mGy) AND EFFECTIVE DOSE EQUIVALENT (mSv) IN TYPICAL RADIOLOGICAL EXAMINATIONS

EXAMINATION	HOSP./ROOM	NUM. TOTAL SHOTS	D x AREA (cGy x cm²)	DOSE BREAST	DOSE TESTES	DOSE OVARY	DOSE LUNG	DOSE THYRO.	DOSE BONES	DOSE MARROW	DOSE(1) OTHER	EFFECTIVE DOSE EQ.
WEIGHTING ICRP FACTORS:				0.15	0.25	0.25	0.12	0.03	0.03	0.12	0.06	
Abdomen	PO/H1	1	810	0.020	-----	1.494	0.042	0.000	0.351	0.222	15.664	1.358
Dorsal spine	HC/HU1	2	2315	0.078	-----	3.764	0.356	0.000	1.581	1.146	27.047	2.803
Skull	PO/CR2	2	287	0.001	-----	0.000	0.008	0.172	0.636	0.122	1.526	0.131
Hip and pelvis	PO/H1	2	1813	0.007	-----	3.005	0.014	0.000	0.867	0.649	18.994	1.997
Urography	HC/UR1	8	3445	0.078	-----	7.595	0.165	0.000	1.531	0.974	70.398	6.317
Chest	HC/UR3	2	152	0.467	0.00	-----	0.578	0.115	0.314	0.173	1.294	0.251
Mammography	HC/MA1	4	----	10.00(2)	---	----	----	----	----	----	-----	1.500
Barium meal	HM/D5	15	4151	3.09	0.51	----	14.920	2.070	3.455	2.584	82.88	7.830
Barium enema	HC/D4	14	5158	0.58	29.81	----	1.89	0.33	6.34	4.92	69.25	12.74
CT whole body (3)	GU/CT1	47 slices		16.7	0.28	12.5	11.6	1.2	12.00	6.1	60.8	10.3
Angiography digital aorta abdom. and lower extremit. (3)	GU/DG	111		0.06	0.66	2.01	0.10	0.01	----	2.58	13.73	1.58
Angiography conventional aorta abdom. and lower extremit. (3)	GU/DG	32		0.70	1.21	1.59	0.27	0.03	----	4.04	20.70	2.22

(1) Addition of the five higher organ dose values not included in the table.
(2) Glandular average dose.
(3) Estimated dose in REMAB phantom. Dose in breast measured on surface.

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

**Hospitals: San Carlos, Doce de Octubre, Gómez Ulla and La Princesa, together with the outpatient centres Hermanos Miralles and Avda. de Portugal (Diagnostic Radiological Services and Radiological Protection Units of these centres). Madrid.
**Dosimetry Units of the Spanish Ministerio de Sanidad y Consumo. Madrid.
**Instituto de Estudios de la Energía of CIEMAT. Madrid.
**B.F. Wall, NRPB, United Kingdom.
**C. Maccia, CEPN, France.

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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 1989 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 1989

Study Group on "RADE AID: Decision aiding system for use in emergencies", post-Chernobyl action

Brussels (B), 9-11 January 1989
12 participants from 4 countries and the Commission

Principal subjects:
- Specification of the decision logic
- Overall structure of the system
- Radiological models to be included
- Interface with expert system being developed to aid decision on foodstuff intervention

Study Group on "Co-ordination of the MARIA research programme"

Brussels (B), 11-13 January 1989
10 participants from 2 countries and the Commission

Principal subjects:
- Structure of the accident consequence code system, COSYMA
- Data libraries to be included
- Modelling of off-site economic consequences of accidents

Study Group on "Improvement of reliable long distance atmospheric transport models", post-Chernobyl action

Fontenay-aux-Roses (F), 17-18 January 1989
11 participants from 4 countries and the Commission

Principal subjects:
- Analysis of Italian data on the REM (Radioactivity Environmental Monitoring) data bank
- Source term estimation from environmental measurements
- Atmospheric dispersion model suitable for emergency response
- Atmospheric dispersion in complex terrain

Study Group on "Radiological aspects of nuclear accident scenarios: real time approaches", post-Chernobyl action

Fontenay-aux-Roses (F), 19-20 January 1989
10 participants from 4 countries and the Commission

Principal subjects:
- Short, medium and long range atmospheric dispersion models to be included in the system
- Feedback of environmental monitoring results on the model predictions
- Environmental transfer of radionuclides and dose calculations

Study Group on "Evaluation of data on the transfer of radionuclides in the foodchain", post-Chernobyl action

Lisbon (P), 24-25 january 1989
7 participants from 3 countries and the Commission

Principal subjects:
- Hydrological conditions of lake systems
- Distribution coefficients between sediments and water
- Aquatic trophic chains

Study Group on "Radiological aspects of nuclear accident scenarios: real time approaches", post-Chernobyl action

London (GB), 24 February 1989
5 participants from 3 countries and the Commission

Principal subjects:
- Interface of the short, medium and long range atmospheric dispersion models
- Overall system specification

Study Group on "Feasibility of studies on health effects in Western Europe due to the reactor accident at Chernobyl", post-Chernobyl action

Brussels (B), 27-28 February 1989
10 participants from 6 countries and the Commission

Principal subjects:
- Review of the document "Feasibility of studies on health effects in Western Europe due to the reactor accident at Chernobyl"
- Preparation of recommendations for future actions

Study Group on "RADE-AID: Decision aiding system for use in emergencies", post-Chernobyl action

London (GB), 6 March 1989
8 participants from 3 countries and the Commission

Principal subjects:
- Demonstration of commercially available decision analysis software, HICHVIEW
- The possible use of the software in RADE-AID

Study Group on "Quality criteria for radiographic images in paediatric radiology"

Niederpöcking (D), 8-10 March 1989
18 participants form 6 countries and the Commission

Principal subjects:
- Preparation of first trial on quality criteria for radiographic images in paediatric radiology
- Selection of age groups and examinations
- Dosimetry for first trial
- Establishment of quality criteria for the most frequent conventional paediatric radiological examinations: skull, chest, pelvis, spine, urinary tract

Study Group on "Evaluation of data on the transfer of radionuclides in the foodchain", post-Chernobyl action

London (GB), 14-16 March 1989
14 participants from 6 countries and the Commission

Principal subjects:
- Speciation of radionuclides after an accident
- Validation of soil-plant transfer data
- Transfer of radionuclides in semi-natural ecosystems
- Transfer of radionuclides in aquatic ecosytems
- Transfer factors in animal systems

Study Group on "Comparative risk assessment"

Paris (F), 6-7 April 1989
15 participants from 4 countries and the Commission

Principal subjects:
- Energy production and utilization
- Indoor chemical and radioactive pollution
- European data banks
- Discussion of the radiological detriment

Study Group on "Radiological aspects of nuclear accident scenarios: real time approaches", post-Chernobyl action

Rome (I), 20-21 April 1989
7 participants from 3 countries and the Commission

Principal subjects:
- Outline specification of the generic emergency response system
- Interface between the different atmospheric dispersion models

Study Group on "Revision of technical recommendations on dosimetry"

Luxembourg (L), 20-21 April 1989
6 participants from 3 countries and the Commission

Principal subjects:
- Progress report on the revision of technical recommendations for monitoring the exposure of individuals to external radiation

Study Group on "Underlying data for derived emergency levels", post-Chernobyl action

Chilton (GB), 24-25 April 1989
13 participants from 4 countries and the Commission

Principal subjects:
- Radioecological models for the assessment of internal doses
- Distribution and consumption of food
- Metabolic and dosimetric models for the assessment of internal doses
- Measurement of the internal contamination of man
- Emergency management

Study Group on "Job related doses in nuclear power plants"

Luxembourg (L), 17 May 1989
6 participants from 3 countries and the Commission

Principal subjects:
- Analysis of the questionnaires on job related doses
- Preparation of the annual meeting on "Radiation protection in nuclear power plants"

Study Group on "Improvement of reliable long-distance atmospheric transport models", post-Chernobyl action

Culcheth (GB), 25-26 May 1989
11 participants from 4 countries and the Commission

Principal subjects:
- Analysis of Italian data on the REM data bank
- Source term estimation from environmental measurements
- Atmospheric dispersion model suitable for emergency response
- Atmospheric dispersion in complex terrain

Study Group on "Improvement of practical countermeasures against nuclear contamination in the urban environment"

Risø (DK), 14-15 June 1989
5 participants from 4 countries and the Commission

Principal subjects:
- Caesium retention behaviour in natural construction materials
- Caesium retention behaviour in man-made construction materials
- Absorption and displacement of caesium from airborne particulates
- Full scale decontamination experiments

Study Group on "Technical experts on Radiation Protection Dosimetry

Luxembourg (L), 19 June 1989
31 participants from 11 countries and the Commission

Principal subjects:
- Account of work on radiation protection dosimetry
- Progress report on the revision of the technical recommendations on dosimetry
- Exchange of information on the latest dosimetry techniques
- Proposals for future actions

Study Group on "Second European intercomparison of measurement devices and techniques of Radon 222 and of its decay products in an underground mine atmosphere"

La Crouzille (F), 29 June - 2 July 1989
27 participants from 12 countries

Principal subjects:
- Intercomparison in a wetmine atmosphere of measurement devices and techniques of radon 222, of its decay products and of sampling volumes and/or air flow.
  This intercomparison falls within the framework of the International Intercomparison and Intercalibration Programme (IAEA/CEC). It followed the one which took place at Twilight Mines (Colorado) organised by the Bureau of Mines, Denver (USA), in September 1988

Study Group on "Underlying data for derived emergency levels"

Bilthoven (NL), 30-31 August 1989
10 participants from 4 countries and the Commission

Principal subjects:
- Radioecological models for the assessment of internal doses
- Distribution and consumption of food
- Metabolic and dosimetric models for the assessment of internal doses
- Measurement of the internal contamination of man
- Emergency management

Study Group "Scientific Advisory Committee of the European RESSAC programme"

Cadarache (F), 22 September 1989
13 participants from 8 countries and the Commission

Principal subjects:
- Soil maps around nuclear sites in France
- Land use procedures
- Data bases of land use
- Strategies for radiological protection
- Choice of representative European soils

Study Group on "Intercomparison programme of dosemeters used for quality control in diagnostic radiology"

Luxembourg (L), 26-27 September 1989
21 participants from 14 countries and the Commission

Principal subjects:
- Results of the first restricted intercomparison programme of instruments used in radiodiagnostic
- Preparation of an extended intercomparison programme

Study Group on "Intercomparison programme on environmental dosemeters"

Luxembourg (L), 16-17 October 1989
6 participants from 3 countries and the Commission

Principal subjects:
- Finalisation of part II of the report on the intercomparison programme of environmental gamma dose-rate meters
- Future work

Study Group on "Radiation protection in nuclear power plants"

Luxembourg (L), 19-20 October 1989
35 participants from 9 countries and the Commission

Principal subjects:
- Analysis of the data provided by the questionnaires on job related doses
- Exchange of experiences and information on radiation protection during steam generator replacements
- Dosimetry and PH chemistry
- Dose control management
- Dose reduction with remote equipment

Study Group on "Improvement of countermeasures in the rural environment", post-Chernobyl action

Bilthoven (NL), 23-24 October 1989
7 participants from 5 countries and the Commission

Principal subjects:
- Evaluation of countermeasure scenarios
- Study of dependence of soil-plant transfer on plant species
- Effectiveness of application of fertilisers as a countermeasure
- Food processing and reduction of contamination

Study Group on "Evaluation of data on the transfer of radionuclides in the foodchain", post-Chernobyl action

Jülich (D), 14-16 November 1989
14 participants from 6 countries and the Commission

Principal subjects:
- Speciation of radionuclides after an accident
- Validation of soil-plant transfer data
- Transfer of radionuclides in semi-natural ecosystems
- Transfer of radionuclides in aquatic ecosytems
- Transfer factors in animal systems

Study Group on "Improvement of reliable long distance atmospheric transport models", post-Chernobyl action

Ispra (I), 28-30 November 1989
13 participants from 4 countries and the Commission

Principal subjects:
- The potential use of fractal models for interpreting monitoring data and interfacing them with predictions of dispersion models
- Analysis and fitting of selected French data contained in the REM data bank
- Source term estimation from environmental measurements
- The limitations of a two dimensional atmospheric dispersion model for emergency response
- The extent to which simple models, suitably modified, can represent dispersion in complex terrain

Study Group on "Quality criteria for diagnostic radiographic images"

Brussels, 13-14 December 1989
8 participants from 5 countries and the Commission

Principal subjects:
- Analysis of answers to the enquiry on the Working Document "Quality criteria for diagnostic radiographic images"
- Continuation of the Community research action on "Quality criteria for diagnostic radiographic images"
- Project of improvement of the Working Document

Study Group on "Quality criteria for diagnostic radiographic images in paediatric radiology"

Brussels, 14-15 December 1989
15 participants from 6 countries and the Commission

Principal subjects:
- Evaluation of X-ray films, obtained during the pilot trial
- Relation between dose and image quality
- Preamble to guidelines on image quality and patient exposure in paediatric radiology
- Results of the status survey of the European Society of Paediatric Radiology

Study Croup on "Improvement of practical countermeasures", post-Chernobyl action

Leuven (B), 19-21 December 1989
5 participants from 4 countries and the Commission

Principal subjects:
- Caesium retention behaviour in natural construction materials
- Caesium retention behaviour in man-made construction materials
- Absorption and displacement of caesium from airborne particulates
- Full scale decontamination experiments

B

MEETINGS ORGANIZED OR CO-ORGANIZED BY

THE COMMISSION OF THE EUROPEAN COMMUNITIES IN 1989

5th Meeting on "Evaluation of the Radiation Protection Research Programmes 1980-1984 and 1985-1989, by a panel of independent experts"

Brussels (B), 22-23 January 1989
6 participants from 6 countries and the Commission

Principal subjects:
- Interview of programme managers
- Evaluation of scientific programme sectors
- Elaboration of recommendations
- Draft of evaluation report

6th Meeting on "Evaluation of the Radiation Protection Research Programmes 1980-1984 and 1985-1989, by a panel of independent experts"

Brussels (B), 12-14 February 1989
6 participants from 6 countries and the Commission

Principal subjects:
- Finalisation of recommendations and evaluation report

EURADOS-CENDOS Council Meeting

München (D), 8 March 1989
14 participants from 5 countries and the Commission

Principal subjects:
- Project of establishing working party on criticality accident dosimetry
- Discussion on future EURADOS-CENDOS general meetings

EURADOS-CENDOS Meeting on "Research and development needs in radiation protection dosimetry"

München (D), 8-10 March 1989
64 participants from 6 countries and the Commission

Principal subjects:
- Discussion and identification of research needs and priorities in the following areas of radiation protection dosimetry
  - Quantities and units
  - Standards and data for dosimetry
  - Survey instruments for external radiation
  - Skin dosimetry and surface contamination
  - Individual dosimetry
  - Numerical and computational dosimetry
  - Internal dosimetry

International Seminar on "Radiation protection and quality assurance in diagnostic radiology"
co-organised by the Complutense University and CIEMAT (Centro de Investigaciones Energeticas Medioambientales y Technologicas), Madrid and the CEPN (Centre d'étude sur l'évaluation de la protection dans le domaine nucléaire), Fontenay-aux-Roses (F) under the patronage of the Radiation Protection Programme of the CEC

Madrid (E), 13-17 March 1989
40 participants from 4 countries and the Commission

Principal subject:
- Advanced training of medical physicists, radiologists and radiation protection specialists working at or preparing their co-operation in diagnostic radiology departments at hospitals

2nd International Workshop on "Real-time computing of environmental consequences of an accidental release to the atmosphere from a nuclear installation - decision aids to off-site emergency management"

Luxembourg (L), 16-19 May 1989
100 participants from 18 countries plus international organizations and the Commission

Principal subjects:
- Meteorological aspects
- Radiological measurements
- Operational aspects, systems and subsystems
- Demonstrations of software

Tenth Symposium on Microdosimetry
Co-organised with US Department of Energy (DOE), Comitato Nazionale per la Ricerca e lo Sviluppo dell'Energia Nucleare e delle Energie Alternative (ENEA), Italy and Forschungszentrum (KFA), Jülich, (D)

Rome (I), 21-26 May 1989
175 participants from 23 countries and the Commission

Principal subjects:
- Microdosimetry and radiation quality
- Molecular changes and radiation quality
- Radiation quality, concepts and issues
- Energy deposition patterns: experiments and calculations
- Particle tracks and microdosimetric distributions
- Experimental techniques and measurements of microdosimetric quantities
- Fundamental physical and chemical aspects in microdosimetry
- Microdosimetry and effects from charged particles and incorporated radionuclides
- Effects of Auger-electron cascades
- Primary radiation interactions and detector responses
- Understanding of strand breaks
- Models based on track structure
- Microdosimetric applications in radiation therapy

Symposium on "Radiation Protection and the maintenance of nuclear power stations in the context of 1992
Co-organized with the Belgian Radiation Protection Association and the French Radiation Protection Society

Brussels (B), 24-26 May 1989
200 participants from 12 countries and the Commission

Principal subjects:
- Current organisation of radiation protection for the maintenance of large work areas
- Changes in "technical intervention"
- The opening up of the Single European Market and its practical consequences for the radiation protection of workers

Workshop on "Long-term research needs and priorities in microdosimetry for radiation protection
co-organised with US Department of Energy (DOE)

Rome (I), 26-27 May 1989
25 participants from 9 countries and the Commission

Principal subjects:
- Review of long-term research needs in microdosimetry with regard to fundamental processes, radiobiological research and practical applications
- Discussion of relative priorities of research needs and related recommendations

Workshop on "The radiological exposure of the population of the European Community from radioactivity in North European marine waters - Project MARINA"

Bruges (B), 14-16 June 1989
66 participants from 14 countries plus international organisations and the Commission

Principal subjects:
- Source of radioactivity in the N.E. Atlantic
- Environmental measurements and individual doses from exposure to radionuclides in the N.E. Atlantic
- Survey of quantities and utilisation of marine produce
- Collective doses

EURADOS-CENDOS Working Group 5, Sub-Committee on the Applications of Track Detectors to Neutron Dosimetry

Bologna (I), 20-22 June 1989
13 participants from 8 countries

Principal subjects:
- Reports on experimental work in progress
- Description of neutron radiation fields at PTB
- Results of a survey of background determination of track etch dosemeters (CR39), (report published)
- Results of 1988/1989 joint irradiations
- Preparation of programme of 1990 joint irradiations of CR39 dosemeters
- Electronic personal dosemeters

Special session on "Quality criteria for diagnostic radiographic images" at the 17th International Congress of Radiology"

Paris (F), 6 July 1989
40 participants from 10 countries and the Commission

Principal subjects:
- European Standards in Diagnostic Radiology - " The Euro-X-ray?"
- Radiological requirements for the specification of image quality criteria
- Results of a trial with a first list of quality criteria for radiographic images
- Patient exposure criteria

Workshop on "Residential radon epidemiology"
Co-organised with the Office for Health and Environmental Research of the US Department of Energy (DOE)

Alexandria (USA), 24-26 July 1989
18 participants and 17 observers from 10 countries, 4 international organisations and the Commission

Principal subjects:
- Procedures for radon measurements
- Design of epidemiological studies
- Preparation of a standardised protocol
- Feasibility of data pooling

EURADOS-CENDOS Working Committee 2 "Skin dosimetry and surface contamination monitoring

Roskilde (DK), 9-10 August 1989
4 participants from 4 countries

Principal subjects:
- Joint experiments on measurement of dose-rates from promethium-147 beta radiation sources by means of extrapolation chambers
- Evaluation of correction factors for extrapolation chambers for use for measurement of dose-rates from low-energy beta radiations

EULEP Task Group on "Interspecies comparison of lung clearance"

Schliersee (D), 15-17 September 1989
9 participants from 4 countries

Principal subjects:
- Second interspecies clearance study with "solid" cobalt oxide particles
- Interspecies clearance studies of ionic cobalt instilled into the lung
- Interphagolysosomal pH in alveolar macrophages
- Dissolution of cobalt oxide particles in macrophages of different species
- Phagocytosis and associated phenomena affecting particle clearance
- Cytotoxicity of new industrial compounds in alveolar macrophages

Seminar on "The medical physicist as a qualified expert in radiodiagnostic"
Co-organised with the European Federation of Organizations for Medical Physics (EFOMP)

Luxembourg (L), 18-19 September 1989
40 participants from 15 countries and the Commission

Principal subjects:
- The status of medical physics: education and training
- The qualification of the experts in the different Member States
- The recognition of experts by the competent authorities

Workshop on "Radioactivity transfer during food processing and culinary preparation

Cadarache (F), 18-21 September 1989
67 participants from 15 countries and the Commission

Principal subjects:
- Drinks
- Dairy produce
- Fruit, vegetables and cereals
- Meat and fish
- Emergency food decontamination

EURADOS-CENDOS Working Committee 1: "Development and implementation of microdosimetry instruments and methods for radiation protection"

Stockholm (S), 22-23 September 1989
15 participants from 4 countries and the Commission

Principal subjects:
- Discussion of application of tissue equivalent proportional counters (TEPC) as
  - area monitor
  - personal monitor
  - transfer instrument
  - space dosimetry
- Discussion on significance of diagnostic information provided by TEPC for radiation protection practice
- Presentation of results of calculated energy deposition spectra for photons

EULEP Task Group on "Effects of irradiation on pre-implantation mouse embryos and on the development of the central nervous system"

Freiburg (D), 2-3 October 1989
17 participants from 4 countries

Principal subjects:
- Malformation and lethal risk after X-ray exposure
- Chromosomal aberrations after exposure to tritiated thymidine or arginine
- Mitotic block in different mouse strains
- Analysis of interaction of radiation and caffeine
- Effects of low doses on development of cortical neuronal branching
- Lectin-binding sites on the surface of neuroblast
- Structural effects of very low doses on rat cerebral cortex
- Effects on spatial memory in mice
- Cytological changes in the developing ependyma

EURADOS-CENDOS Working Committee 4: "Dissemination and development of computer programmes for dosimetric problems (Numerical dosimetry)"

Teddington (GB), 2-4 October 1989
17 participants from 4 countries

Principal subjects:
- Report on californium-252 benchmark calculations
- Intercomparison of Bonner sphere response function calculations
- Programmes for analysis and interpretation of TEPC data
- Standardisation of phantoms (internal and external dosimetry)

Annual Meeting CEC-US DOE

Louvain (B), 8-11 October 1989
20 participants from the USA and the Commission

Principal subjects:
- Accident consequence assessment
- Radiation biology
- Global warming
- Strategy and plans for future co-operation

EULEP Task Group on "Cell biology of haemopoietic tissues"

Brussels (B), 27 October 1989
18 participants from 3 countries and the Commission

Principal subjects:
- Growth factors in the development of myeloid leukaemia (ML) and thymic lymphoma; role of interleukin-1-beta in ML
- Molecular control of blood cell development and leukaemia: role of interleukin-6 and possible reversal of malignancy
- Gene rearrangement of overexpression in myeloid cell lines

EULEP Slide Seminar on "Neoplastic and non-neoplastic lesions of the bone marrow"
organised by the EULEP Committee of Pathology

München (D), 27 October 1989
20 participants from 6 countries

Principal subjects:
- Histology of normal bone marrow
- Classification and diagnosis of myelodysplasia
- Stromal changes in bone marrow during graft-versus-host reaction in dogs
- Dose-response relationships for myeloid leukaemia
- Haemopoietic pathology in the mouse and rat
- Beagle pathology atlas (of the US DOE Biological Effects Task Group)

EULEP Task Group Meeting on "Reduction of risk of late effects from incorporated radionuclides"

Cadarache (F), 6 November 1989
8 participants from 4 countries and the Commission

Principal subjects:
- Decorporation studies with different trihydrobenzyl compounds
- Decorporation of thorium with diethylenetriaminepentaacetic acid (DTPA)
- Planned studies on the new chelating agent 3,4,3-LIHOPO (Linear hydroxy pyridonate)
- Planned studies on diphosphonates for decorporation of uranium

CEC-AECL (Atomic Energy of Canada Ltd) - Co-ordination Meeting

Brussels (B), 6-7 November 1989
12 participants from Canada and the Commission

The Atomic Energy of Canada Ltd., The Atomic Energy Control Board and the Radiation Protection Bureau, National Health and Welfare from Canada were represented

Principal subjects:
- Epidemiological studies
- Radon in homes and the workplace
- Dosimetry-limits and concepts
- Radioactivity in the environment and foodchain
- Dose rate effectiveness factor
- Practical implementation of ALARA
- DG XI activities in radiation protection
- Public education and expert training in radiation protection

EULEP Task Group on "Stem cells, bone-seeking radionuclides and fetal dosimetry"

Cadarache (F), 6-8 November 1989
25 participants from 6 countries

Principal subjects:
- Radiation injury to bone marrow stromal cells
- Radioresistance of osteogenic capacity of bone marrow
- Response of mouse bone marrow stem cells to plutonium-239, X-rays and fission neutrons
- Leukaemia induction in mice from low levels of radium-224
- Distribution of alpha-activity in children's teeth
- Follow-up of radium-244 patients
- Osteosarcoma risk after administration of AC-227 and Th-227 together
- Comparative distribution and dosimetry of various alpha-emitters in bone
- Protracted gamma irradiation in utero; early and late effects
- Transfer of actinides, polonium and iodine to the foetus and newborn animal; resulting cancer incidence

CEC/US DOE Workshop on "Uncertainty analysis in the context of accident consequence assessment"

Santa Fe (USA), 13-16 November 1989
46 participants from 7 countries and the Commission

Principal subjects:
- Methods and concepts for uncertainty analysis
- The use of expert judgement
- Uncertainty analysis in risk calculations
- Uncertainty analysis in emergency response
- Uncertainty analysis in dose reconstruction

EURADOS-CENDOS Council Meeting

Rijswijk (NL), 17 November 1989
14 participants from 7 countries and the Commission

Principal subjects:
- Relations with EULEP and EUROMET
- Preparation of 9th General Assembly in Lisbon, May 1990
- Discussion and definition of objectives of new and old working groups
- Preparation for election of a new council

EURADOS-CENDOS Working Committee 6 "Assessment of internal dose"

Chilton (GB), 28-29 November 1989
10 participants from 5 countries and the Commission

Principal subjects:
- Results of intercomparison of internal dose assessment cases
- Discussion on project to establish registries of internal dose assessments, of mathematical models used for assessments and of autopsy data
- Stable isotope studies
- Report on the activities of ICRP Lung Model Group

Standing Conference "Health and safety in the nuclear age"

Brussels (B), 5-6 December 1989
100 participants from 15 countries and the Commission

Principal subjects:
- Informing the public on improvements in emergency planning and nuclear accident management
- Detection, evaluation and reporting of a nuclear accident
- Possible intervention measures, rational decision-making for each specific measure, evaluation of the effectiveness of measures adopted
- Public information: national and Community instruments, international conventions

EULEP Task Group on "Cellular basis of late vascular changes in irradiated brain"

Mol (B), 7-8 December 1989
7 participants from 4 countries

Principal subjects:
- Role of microthrombi in late changes in the irradiated brain
- Early oedematous changes preceding structural damage
- Strain differences in damage to irradiated rat brain

C

MEETINGS OF EXPERTS IN 1989

Meeting of the ad hoc Committee on Minor Foodstuffs

Luxembourg (L), 3 February 1989
24 participants from 10 countries and the Commission

Luxembourg (L), 27 February 1989
22 participants from 12 countries and the Commission

Subject :

Draft Commission Regulation laying down maximum permitted levels of radioactive contamination in minor foodstuffs following a nuclear accident or any other case of radiological emergency.

Group of Experts referred to in Article 37 of the Euratom Treaty

Luxembourg (L), 7 March 1989
31 participants from 12 Member States and the Commission

Subject : GOLFECH (F) Nuclear Power Plant

Group of Experts referred to in Article 37 of the Euratom Treaty

Cherbourg (F), 8-9 June 1989
40 participants from 12 Member States and the Commission

Principal subjects :

- *La Hague (F) Fuel Reprocessing Plant* UP_3A + UP_2800

- *Penly (F) Nuclear Power Plant*

Group of Experts referred to in Article 31 of the Euratom Treaty

Brussels (B), 21-22 June 1989
29 participants from 12 countries and the Commission

Principal subjects :

- *Information on the recent activities undertaken by the Commission in the field of radiation protection, in particular in relation to the Chernobyl accident*

- *Information and discussion on the activities within other international organizations concerned with radiation protection (UNSCEAR, IAEA, NEA, etc...)*

- *Status and trends concerning the dose limits for exposed workers and for members of the public*

- *Determination of annual limits of intake for Plutonium and Transplutonium isotopes*

- *Revision of the basic safety standards Directive (80/836/Euratom and 84/466/Euratom)*

Meeting with national representatives : "Training and information in radiation protection in primary and secondary education"

Luxembourg (L), 20 July 1989
9 participants from 8 countries and the Commission

Principal subjects :

- *Actual state of education in radiation protection in primary and secondary education in the Member States*

- *Development of a European manual on radioactivity and radiation protection for teachers*

Group of Experts referred to in Article 31 of the Euratom Treaty

Luxembourg (L), 26-27 October 1989
29 participants from 12 countries and the Commission

Principal subjects :

- *Research and training programme in the field of radiation protection*

- *Completion of Council Regulation (Euratom) n° 3954/87 of 22 december 1987 laying down maximum permitted levels of radioactive contamination of foodstuffs and of feedingstuffs following a nuclear accident or any other case of radiological emergency, as regards feedingstuffs*

- *Council directive informing the population about health protection measures to be applied and steps to be taken in the event of a radiological emergency. Preparation of an explanatory communication on the interpretation of the annexes to the Directive*

- *Initiation to radiation protection in schools*

- *Dosimetry in radiation protection : information on current and planned initiatives in DG XI*

Meeting of the ad hoc Committee on Feedingstuffs

Luxembourg (L), 23 October 1989
29 participants from 12 countries, a Commission expert and the Commission

Principal subject :

Draft Commission Regulation laying down maximum permitted levels of radioactive contamination of feedingstuffs following a nuclear accident or any other case of radiological emergency.

Member States' Representatives

Luxembourg (L), 30 November - 1 December 1989
25 participants from 12 countries, a WHO representative and the Commission

Principal subject :

Development of the early radiological information exchange system set up pursuant to Council Decision 87/600 Euratom

Meeting of the ad hoc Committee on Feedingstuffs

Luxembourg (L), 15 Décember 1989
24 participants from 12 countries, a Commission expert and the Commission

Principal subject :

Draft Commission Regulation laying down maximum permitted levels of radioactive contamination of feedingstuffs following a nuclear accident or any other case of radiological emergency.

V

AUSWAHL EINIGER AUF VERANLASSUNG DER KOMMISSION ERSCHIENENER VERÖFFENTLICHUNGEN

SELECTION OF PUBLICATIONS ISSUED ON THE INITIATIVE OF THE COMMISSION

CHOIX DE PUBLICATIONS EDITEES A L'INITIATIVE DE LA COMMISSION

V. Publications 1989

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 or proceedings. Short descriptions of those publications, published or prepared in 1989, are given on the following pages.

Critical review of animal carcinogenesis by nickel and its organic compounds

Edited by R. MAXIMILIEN

In the framework of its research programme, the Euratom-CEA association is interested in comparison of risk assessment procedures. From a methodological standpoint, the problems of evaluating the carcinogenic risk to human from chemicals and from radioactivity is quite different. The aim of this study was to make a state of knowledge about one well documented group of chemical substances as a preliminary step of comparison with radiological detriment.

Nickel animal carcinogenic biassays relative to 9 inorganic nickel substances (nickel metal, nickel oxide(s), nickel subsulfide, nickel sulfide(s), nickel carbonate(s), nickel hydroxide(s), nickel sulfate(s), nickel chloride and nickel carbonyl) and to nickel mixtures (mixtures of nickel substances, complex nickel substances, nickel alloys...) are reviewed. Critical evaluation of literature data on carcinogenicity has been performed by making reference to CEC guidelines of good laboratory practice (part 1). Experimental results are reported in data sheets (part 2). As pointed out in conclusions, there are few experiments on routes relevant for human risk assessment: no carcinogenic effects have been demonstrated following oral administration for the nickel inorganic compounds under study; results on inhalation route are inconclusive except for nickel oxide(s) for which they could be negative but nickel subsulfide appears highly carcinogenic in one experiment. For routes, which are of less or no relevance for human risk assessment, some results are clearly positive, but methodologically acceptable negative studies are in limited number. Whatever the route of administration is, no primary malignant tumours seemed to occur in remote organs. In most cases, carcinogenic effects have been demonstrated for only one species.

Report EUR 12456 EN, 1989, 2 volumes, 80 and 283 pages

To be ordered through:

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of the European Communities
B.P. No 1003
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Price: ECU 24

Intercomparison of environmental gamma dose rate meters

A comprehensive study of calibration methods and fiels measurements.

The measurements of ambient radiation in the neighbourhood of a nuclear installation is essential to demonstrate that members of the public are not exposed to unacceptable levels of radiation arising from the operation of the installation.

The Commission of European Communities decided during 1983 to help Member States to check the performance of their monitors at background radiation levels and to investigate different calibration techniques. This report describes the results of the intercomparison programme of environmental gamma dose rate meters organized during 1984 and 1985.

Details are also given of the evaluation of ambient background radiation at locations where the relative contributions to the air kerma rates from cosmic and terrestrial radiations were significantly different.

Radiation Protection Series N° 41

Report EUR 11665 EN, 1989, 115 pages

To be ordered through:

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of the European Communities
B.P. N° 1003
L-2985 Luxembourg

Price: ECU 10

Feasibility of studies on health effects in Western Europe due to the reactor accident at Chernobyl

Report of a task group for the CEC by J. Breckow, A.M. Kellerer, E.G. Knox and S. Richardson

Recommendations for research of an international panel of independent experts - R. Doll, J.D. Boice, J. Estève, G. Silini and J.W. Thiessen

The Chernobyl reactor accident caused widespread, low level, radioactive contamination throughout western Europe and there was concern that health effects might occur in the general public. In response to that concern, and to provide guidance on the further research steps to be taken, this report has considered the levels of radioactive contamination in different areas of western Europe, the dose levels to the public as a consequence of the contamination, the potential health effects of low level radiation, the probable numbers of any health effects resulting in different sectors of the public as a result of the Chernobyl accident and the means available to monitor the possible health effects. The report concludes that the incidence of childhood leukaemia would represent the most sensitive health effect and that the availability of statistics on this health effect from childhood cancer registries in different countries would offer the opportunity to follow the incidence of the disease over the coming years. The report has been reviewed by an international panel of independent experts which recommends that although it is unlikely that any effect on childhood leukaemia incidence would be observable at the dose levels caused by the Chernobyl accident, it would be prudent to study the statistics of childhood leukaemia incidence in different parts of western Europe with higher and lower levels of radioactive contamination with reference to the time of the Chernobyl accident.

Report EUR 12551 EN, published in the Radiation Protection series of the Commission of the European Communities, 1990, 93 pages

To be ordered through:

Office for Official Publications
of the European Communities
B.P. No. 1003
L-2985 Luxembourg

Price: ECU 8.75

Technical and Physical Parameters for Quality Assurance in Medical Diagnostic Radiology : Tolerances, Limiting Values and Appropriate Measuring Methods

Proceedings of a Workshop organized by the Commission of the European Communities, Brussels (B), 23-25 February 1988.

Edited by B.M. MOORES, F.E. STIEVE, H. ERISKAT, H. SCHIBILLA

Within the framework of its Radiation Protection Programme, the Commission of the European Communities participates in research which is aimed at the reduction of patient exposure during medical diagnostic radiology. This Community action is directly related to the Council Directive of 3 September 1984 laying down basic measures for the radiation protection of persons undergoing medical examination or treatment. Article 3 of this Directive stipulates that :
"... All installations in use must be kept under strict surveillance with regard to radiological protection and the quality control of appliances."
As a consequence of this, numerous quality assurance measures have been initiated by many radiological departments to cover the quality control of all those parameters which are important for equipment functioning, imaging methods and image interpretation.

In order to compare and evaluate the results of these individual efforts, radiologists, radiographers, research workers in radiation protection and medical physics, representatives from related industries, public health authorities and regulating bodies discussed the requirements governing measuring methods, instrumentation and measuring techniques used for the quality control of those parameters which have the greatest impact on the quality of the radiographic images and patient exposure.

The 51 papers in the Proceedings deal with a variety of subject areas such as : organizational aspects of quality control, parameters of the image producing system using conventional x-ray equipment or special equipment and techniques, image recording systems - system analysis as well as image performance, retrieval - viewing conditions, and dosimetry. Increasing attention was given to special techniques like mammography, computed tomography and digitized radiography.
Efforts were made to find commonly acceptable tolerances for different measuring methods and instruments and to define or to fix limiting values for the most significant parameters. The possibilities were discussed to standardizing those tolerances and limiting values in order to maintain consistently high performance of radiological equipment and image processing. The need for intercomparison of the various quality test methods was stressed so that the prerequisites for the production of comparable radiographs without unnecessary exposure of the patient will result.

Report EUR 11620, 1989, British Inst. of Radiology Report 18, 165 pages

To be ordered through:

British Institute of Radiology
36 Portland Place
GB-London W1N 4AT
Price: £ 28.50

Biological assessment of occupational exposure to actinides

Proceedings of a workshop jointly organized by the Commission of the European Communities and the Institut de Protection Nucléaire, C.E.A. Fontenay-aux-Roses (F), Versailles (F), 30 May - 2 June 1988

Edited by: G.B. GERBER, H. METIVIER, J.W. STATHER, R.G. THOMAS

The problems related to setting annual levels of intake and to monitor exposure from actinide material used in the nuclear industry formed the subject of the workshop. The methods of determining exposure from excretion values, the modelisation of actinide behaviour in the organism, the biological consequences of exposure to uranium, plutonium and other actinides as well as the possible means to deal with over-exposure were reviewed. A round table discussion, which is also summarised in the proceedings, reviewed the problems involved in the radiation protection of persons exposed to such compounds.

Report EUR 12087 EN, 1989, Rad. Prot. Dosimetry, Vol. 26, Nos 1-4, 397 pages

To be ordered through:

Radiation Protection Dosimetry
Nuclear Technology Publishing
P.O. Box N° 7
Ashford
GB - Kent TN25 4NW

Price: £ 65

Implementation of dose-equivalent operational quantities into radiation protection practice

Proceedings of a seminar jointly organized by the Commission of the European Communities and the Physikalisch Technische Bundesanstalt (PTB) and co-sponsored by ICRU (International Commission on Radiation Units and Measuremnts), ICRP (International Commission on Radiological Protection), the US-Department of Energy and EURADOS (European Radiation Dosimetry Group), Braunschweig (D), 7-9 June 1988

Edited by J. BOOZ and G. DIETZE

In 1985, the International Commission on Radiation Units and Measurements (ICRU) proposed in its Report 39 a new system of dose-equivalent operational quantities for external exposure to be used in area and individual monitoring of any ionising radiation.

In 1985, the basic considerations for the selection of these operational quantities and their properties were discussed at a seminar on "Radiation Protection Quantities for External Exposure" in Braunschweig (Proceedings published in Radiat. Prot. Dosimetry 12,1, (1985)) and in 1988 the ICRU published Report 43 with data and arguments underlining the selection of these quantities.

Meanwhile, in many countries, investigations have been performed to study the applicability of the new quantities and the consequences for instrumental requirements and calibration procedures, and the necessary changes in the procedure used to date, and hence another seminar on "Implementation of Dose-Equivalent Operational Quantities into Radiation Protection Practice" (Braunschweig, 7-9 June 1988) was organised by the Commission of the European Communities and the Physikalisch Technische Bundesanstalt (PTB). The objective of the seminar was to discuss the problems of implementing the quantities into practice, especially in the field of individual monitoring.

The proceedings of the seminar start with some basic considerations followed by such topics as conversion factors, the measurement of ambient dose equivalent, instrumentation and calibration procedures in individual monitoring, problems with weakly penetrating radiation and the status of legal implementation into radiation protection practice. The proceedings contain 29 papers and will make a helpful contribution to the further development of the implementation of the new operational quantities into radiation protection practice.

Report EUR 12182, published in Radiation Protection Dosimetry, Vol. 28, Nos. 1-2, 1989, 167 pages

To be ordered through:

Nuclear Technology Publishing
P.O. Box No. 7
Ashford
GB - Kent TN25 4NW

Price: £ 35

Transfer of radionuclides to livestock

Proceedings of a workshop co-organised by the Commission of the European Communities and the National Radiological Protection Board (GB), Oxford, 5 - 8 September 1988

Edited by R. BULMAN

An understanding of the transfer of radionuclides to animals and animal products is essential for evaluating the impact of both routine and accidental discharges to the terrestrial environment. The workshop aimed at reviewing the current information from both field observations and experimental studies on how these and other factors can eventually influence the mechanisms of transfer of radionuclides to human foodstuff. The scope of the workshop was to deal with the effect of a number of parameters such as source and composition of the diet; chemical form of the radionuclides; season of the year; influence of farming practices on the transfer; remedial actions after an accidental release. All these topics are reflected in the papers of the proceedings.

Report EUR 12128, EN, published in Science of the Total Environment, Vol. 85, 1989, 558 pages

To be ordered through:

Elsevier Science Publishers B.V.
Journals Department
P.O. Box 211
NL - 1000 AE Amsterdam

Price: Dfl. 217

Radiation protection optimization "Advances in practical implementation"

Proceedings of the third European scientific seminar organized by the Commission of the European Communities
Madrid (E), 12-14 September 1988

Within the Community, protection against the dangers of ionizing radiation is regulated in conformity with the provisions of two Council Directives. One is of general application for all activities involving a hazard arising from ionizing radiation and lays down the basic safety standards for the health protection of the general public and workers against the dangers of ionizing radiation. The other is derived from the abovementioned one and lays down the basic measures for the radiation protection of persons undergoing medical examination or treatment.

The Commission, in collaboration with the Spanish Ministerio de Sanidad y Consumo, the Consejo de Seguridad Nuclear and the Centro de Investigaciones Energeticas, Medioambientales y Tecnologicas, organized on 12, 13 and 14 September 1988 in Madrid, the third scientific seminar on the optimization principle (Alara) which is a key element of the two abovementioned Council Directives.

The seminar allowed an analysis of the progress made since the previous seminars of 1979 and 1983, in the practical implementation of the optimization principle, in relation to the design and operation of nuclear and industrial installations, natural radioactivity, medical practices and countermeasures.

The report contains the 20 original contributions presented and some general considerations on the results of the seminar.

Radiation protection series; n° 44
Report EUR 12469 EN; 1989; 491 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 40

Seminar on "Application, perspectives and limitations of comparative risk assessment and risk management"

Proceedings of a seminar organised by the Commission of the European Communities in collaboration with the Centre de Developpement des Etudes et Applications en Hygiène et Securité, Paris (F), Nice, 26-30 September 1988

Edited by G. BRESSON, M. OLAST and J.SINNAEVE

The experiences and knowledge as well as methodologies developed and acquired in radiation protection research for assessing risks to man and his environment are now known to be applied in a broader context to prevent, reduce and manage risks linked to numerous human industrial activities. The apparent need to compare such risks and the increasing concern of the authorities responsible for the environment and for the safety and well-being of the population and the workers urged the scientific community to develop methods for risk management based upon a comparative approach. The purpose of this seminar was to come to a multi-disciplinary discussion of the progress made in this field in view of defining the applications, perspectives and limitations of risk assessment and risk management (31 papers).

Report EUR 11465 EN, 1989, 363 pages

To be ordered through:

Office for Official Publications
of the European Communities
B.P. No 1003
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Price: ECU 32.50

Optimization of Image Quality and Patient Exposure in Diagnostic Radiology

Proceedings of a Workshop organized jointly by the Commission of the European Communities and the National Radiological Protection Board - Chilton, Oxford, 27 - 29 September 1988

Edited by B.M. MOORES, B. WALL, H. ERISKAT, H. SCHIBILLA

The purpose of the Workshop was to formulate criteria for optimizing diagnostic radiology from the standpoint of image quality and patient exposure and to weigh these factors against each other. Some of the 77 papers deal with problems of image perception and interpretation and the quantification of image quality. A remarkably wide range of test objects for the evaluation of image quality is described.

From the standpoint of optimization, the requirements for a series of clinical situations are considered in detail. Considerable attention is paid to mammography and paediatric radiology.

Other areas such as chest examinations, urography and dental radiology provide evidence of similar avoidable variations in patient exposure and image quality.

Surveys of trends in diagnostic radiology over considerable time periods in Canada, Sweden and southern Germany have proved that regular quality checking contributes in a large measure to restricting dose ranges and reducing both the mean patient dose and the number of unacceptable radiographs.

The urgency with which quality criteria must be established is also stressed with reference to examinations conducted by means of computed tomography and digital radiography.

A CEC Study Group composed of specialists in radiology, medicine, medical physics and equipment technology has drawn up a list of quality criteria for six common diagnostic examinations : chest, skull, lumbar spine, pelvis and sacrum, urinary tract and breast. These criteria were checked on approximately 1000 patients in 10 European countries and the results are presented and compared with those of other approaches aimed at better image quality and lower patient exposure.

This should constitute a scientific contribution to a conceivable standardization of quality criteria and to the production of comparable radiographs, perhaps even of the "Euro X-ray". The revised list of quality criteria is appended to the Proceedings.

Report EUR 11842, 1989, British Inst. of Radiology Report 20, 288 pages

To be ordered through:

British Institute of Radiology
36 Portland Place
GB-London W1N EAT

Price: £ 30

Risks from Radium and Thorotrast

Proceedings of a workshop jointly organized by the Commission of the European Communities, the U.S. National Cancer Institute and the U.S. Department of Energy, Office of Health and Environmental Research
Bethesda (USA), 3-5 October 1988

Edited by: D.M. TAYLOR, C.W. MAYS, G.B. GERBER, R.G. THOMAS

The workshop considered the epidemiological and experimental data dealing with the effects of radium and thorotrast. Risk assessment for cancer and non-stochastic damage for internally deposited radionuclides rests primarily on information obtained from these two alpha emitters. The papers presented updates of the epidemiological studies in different countries (ie Denmark, Germany, Japan, UK and USA), new information on dosimetry of cells at risk and on ongoing animal studies, and attempts to extrapolate the data to other exposure situations. Two round table discussions, published also in summary, dealt with the respective risks of radium and thorotrast.

Report EUR 12088 EN, 1989, British Inst. of Radiology Report 21, 188 pages

To be ordered through

The British Institute of Radiology
36 Portland Place
GB-London W1N 4AT

Price: £ 40.00

Implementation of dose equivalent meters based on microdosimetric techniques in radiation protection

Proceedings of a Workshop jointly organized by the Commission of the European Communities and the GSF (Gesellschaft für Strahlen- und Umweltforschung), Neuherberg, in co-operation with EURADOS (European Dosimetry Group), Schloss Elmau (D), 18-20 October 1988

Edited by H.G. MENZEL, J. BOOZ and H.G. PARETZKE

Low pressure tissue equivalent proportional counters (TEPC) have been an important laboratory tool in experimental microdosimetry for many years. The recent availability of microelectronics and progress in digital electronics enabled the development of portable TEPC working area monitors and thus the application of this microdosimetric method in practical radiation protection.
In view of the substantial progress achieved in this, a second workshop was organised on "Implementations of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection" at Schloss Elmau (FRG) (18-20 October 1988). The Workshop was attended by scientists involved in the development and application of TEPC instruments as well as scientists working in different fields of radiation protection including health physics.
The proceedings of the Workshop contain papers on new microdosimetric instruments and basic physical aspects of proportional counter measurements. The presentation and discussion of the results of the TEPC intercomparison were central topics of the Workshop as well as their interpretation in terms of the neutron energy dependence of the dose equivalent response and the achievable sensitivity and the influence of parameters such as counter geometry and evaluation procedures on the results. TEPC measurements were reported for photon radiation fields and for radiation fields at various working places. Plans to use TEPC instruments in space radiation dosimetry were also presented. The potential improvement of TEPC response to neutrons was discussed on the basis of the theoretical and experimental studies. Finally, the topics of specification of radiation quality in radiation protection and requirements for the performance of dose equivalent meters in area and individual monitoring with regards to practical and principal considerations were extensively discussed.
The proceedings contain most of the papers presented and two summaries of extended discussions. They provide relevant arguments for the discussion of the potential implementation of these instruments in practical radiation protection.

Report EUR 12188, published in Radiation Protection Dosimetry, Vol. 29, Nos 1-2, 1989, 153 pages

To be ordered through:

Nuclear Technology Publishing
P.O. Box No. 7
Ashford
GB - Kent TN25 4NW

Price: £ 25

Radiation protection training and information for workers

Proceedings of a seminar organized by the Commission of the European Communities
Luxembourg (L), 28-30 November 1988

The meeting reported in these proceedings was organized to discuss the specific problems of providing information and training on radiation protection to workers exposed to radiation, intervention staff and workers likely to be affected by an activity involving ionizing radiation.

Particular emphasis was placed on the need to harmonize basic training on radiation protection in the context of 1992. It seemed advisable for technical training on radiation protection to be introduced into secondary education. To this end, the Commission was asked to draw up a guide for apprentices and students.

In view of the growing diversification of activities involving the use of radioactive substances, the Commission was called upon to intensify its efforts in order to ensure that relevant information and training was provided in all firms to workers exposed to ionizing radiation, and to produce guides for specific categories of workers, such as those responsible for the transport of radioactive materials or those likely to be involved in organizing measures in the event of a radiological emergency.

Radiation protection series; n°45
Report EUR 12177 EN,FR; 1989; 297 pp

To be ordered through :

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of the European Communities
Boîte Postale 1003
L - 2985 LUXEMBOURG

Price (excluding VAT) in Luxembourg ECU 22.50

Selection of radiobiological data for biophysical modelling

Conclusions of a panel of scientists organised by the Commission of the European Communities and the US-DOE (Department of Energy), Dublin, 3 April 1989.

The report presents the conclusions of a panel of scientists which met to discuss the selection of radiobiological data considered most suitable for a benchmark test in biophysical modelling. At a previous workshop held in June 1988 in Oak Ridge a report (US-DOE CONF-8806237) had made a critical evaluation of radiobiological data and had presented an extensive bibliography of data covering basic radiation physics and chemistry, cellular radiobiology and animal radiobiology. The panel in Dublin selected specific topics and data sets in cellular radiobiology to be addressed by the biophysical modellers.

Document Nr XII/644/89, 1989,

Available on request from:

K.H. CHADWICK
DG XII/D/3
CEC
200 Rue de la Loi
B-1049 Brussels

Cell transformation and radiation-induced cancer

Proceedings of a Workshop organised by the Commission of the European Communities, the US-DOE (Department of Energy) and the Nuclear Energy Board of Ireland, Dublin, 4-7 April 1989

Edited by K.H. CHADWICK, S. SEYMOUR and B. BARNHART

The proceedings present an overview of the most recent developments in research of cell transformation and the application of cell transformation systems to the study of radiation effects. Special emphasis is given to the work on the establishment of human epithelial cell transformation systems likely to be more relevant to human cancer. The influence of radiation quality and dose rate on cell transformation in established rodent cell lines is also reported.

The 49 papers cover the following topics;

- the molecular genetic basis of cancer
- pathology, invasiveness, apoptosis
- immortalisation and transformation of human and mammalian cells
- in-vivo/in-vitro transformation systems
- chromosomal rearrangements and mutations in cancer
- oncogenes and retroviruses
- initiation, promotion and interaction
- molecular lesions and cell transformation
- dose-effect relationship
- cell transformation, cancer and risk
- final commentary

Report EUR 12248, published under the Adam Hilger imprint by IOP Publishing Ltd., 1989, 414 pages

To be ordered through:

IOP Publishing Ltd.
Techno House
Redcliff Way
GB - Bristol BSI 6NX

Price: £ 37.50

Second International Workshop on "Real-time computing of the environmental consequences of an accidental release to the atmosphere from a nuclear installation - Decision aids to off-site emergency management"

Proceedings of a workshop organised by the Commission of the European Communities, Luxembourg (L), 16-19 May 1989

Since the first such workshop in 1985, the Chernobyl accident had greatly sharpened interest worldwide in the uses of real-time computer techniques for interpolating and extrapolating in both time and space whatever data is initially available following an accident with a view to providing a stronger basis for emergency response decisions by the responsible authorities.

The proceedings contain some 40 papers involving authors from 15 different countries or international organisations, ranging in subject matter from meteorological input data for atmospheric dispersion modelling through subsequent environmental transfer via the foodchain and data-handling requirements to emergency organisational, decision-making and training aspects.

Report EUR 12320 EN/1 and EN/2 (two volumes), in press

To be ordered through:

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of the European Communities
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L-2985 Luxembourg

Tenth Symposium on Microdosimetry

Proceedings of the Symposium jointly organised by the Commission of the European Communities, the US Department of Energy, the Comitato Nazionale della Ricerca e lo Sviluppo dell'Energia Nucleare e delle Energie Alternative, and Forschungszentrum Jülich, Rome (I), 21-26 May 1989

Edited by J. BOOZ, J. DENNIS, H.G. MENZEL

This Symposium was the tenth in a series organised by the Commission since 1967. It was attended by 175 scientists and more than 90 papers were presented in oral and poster sessions. The research in microdosimetry includes physical, chemical and biological aspects and is aimed at improved understanding of physico-chemical, biochemical and biological effects and mechanisms induced by radiation of different qualities. Papers on physical aspects included experimental and calculational results for descriptions of the energy deposition pattern on molecular and cellular level, on primary radiation interaction and detector responses, on effects of Auger-Electron cascades and on experimental techniques. Based on physical information the topics of molecular changes and radiation quality, of mechanisms of DNA strand breaks and of initial radiation effects in general were discussed. Several contributions focused on the central problem of microdosimetry, the understanding of the dependence of radiation effects on radiation quality and of the problem of low doses and low dose rates discussing biophysical models and radiation quality concepts. These aspects are of immediate relevance to radiological protection. Applications of microdosimetry in radiation therapy was another topic of practical importance. The role of microdosimetry in assessments of radiation risks for both external irradiation and incorporated radionuclides was documented. Of particular interest were papers on the microdosimetry of radon decay products in the respiratory tract.

Report EUR 12864, in press

To be published by:

Radiation Protection Dosimetry
Nuclear Technology Publishing
P.O. Box No. 7
Ashford
GB - Kent TN25 4NW

The radiological exposure of the population of the European Community from radioactivity in North European marine waters - Projects "MARINA"

Proceedings of a seminar organised by the Commission of the European Communities, Bruges (B), 14-16 June 1989

The principal purpose of the seminar was to present in a series of presentations the results of an expert study on the impact on the marine environment and on Community citizens of radioactive materials present in and discharged into the Northeast Atlantic waters. These presentations reflect the various aspects studied:

- the disposal of liquid and solid radioactive wastes in the North European marine waters, including the Irish Sea, the North Sea, the English Channel and the Baltic;
- existing marine dispersion models with a view to agreeing a valid comprehensive model;
- quantities, distribution and end-uses of marine produce (especially fish for human consumption);
- exposure of the public.

Sources of radioactivity taken into account included discharges from all relevant civil nuclear sites (both coastal and riverain) up to the end of 1984, weapons fallout, solid waste dumping from 1949 to 1982, Chernobyl deposition and natural radionuclides. Environmental measurement data and estimates of individual dose were used in the comparison with existing models and resulted in the construction of special model which takes account of the physical, chemical and biological processes and provides better agreement with the experimental data than was previously attained. Dose estimates include both doses to individual members of critical groups and collective dose. (The complete reports of the expert group on the various aspects of the study are in press).

In addition to the presentations arising directly from Project MARINA, the proceedings include a number of papers describing other work in related fields.

Report Nr XI/4669/89-EN

To be ordered through:

Commission of the
European Communities
DG XI/A/1
L-2920 Luxembourg

Individual copies will be supplied free of charge.

The transfer of radionuclides in natural and semi-natural environments

Proceedings of a workshop co-organised by the Commission of the European Communities, the Italian Directorate for Nuclear Safety and Health Protection (ENEA-DISP) and the Regional Centre for Agricultural Experimentation of Friuli-Venezia – Giulia Region (CRSA), Udine (I), 11-15 September 1989

Edited by G. DESMET, M. BELLI, U. SANSONE, P. NASSIMBENI

After the Chernobyl accident, studies on the natural and semi-natural ecosystems have pinpointed a number of pathways that lead directly to man, primarily by way of meat and dairy products. Relatively little was known regarding the cycling of radionuclides of and their bioavailability, their migration in undisturbed natural profiles and transfer of contamination to adjacent areas under such conditions. Due to the heterogeneity of these systems problems arise when transfer factors need to be determined. The usual radioecological definition needed to be correspondingly adapted and the possibility of extrapolation of results obtained in the laboratory and in agricultural fields investigated.

In order to do this, 5 sessions were scheduled.
1. Definition of natural and semi-natural ecosystems
2. Definition of transfer phenomena
3. Natural and semi-natural ecosystems as collections and conveyors of radioactivity
4. Countermeasures in these special areas
5. Contamination of wildlife

In the general discussion, an attempt was made to evaluate the relative contribution of such ecosystems to the contamination of the environment.

Report EUR 12448 EN, in press

To be published by:

Elsevier Applied Science Publishers
Crown House
Linton Road
Barking
GB - Essex IG11 8JU

EULEP Symposium on skin - its relevance in radiological protection and radiation accidents

Proceedings of a symposium organised by EULEP and the European Society of Radiation Biology.
Brussels (B), 11 September 1989.

This EULEP symposium was devoted to two important aspects of the radiation response of the skin, that relating to high dose accidental over-exposure and the other to that of radiological protection and the skin. There were presentations by a number of invited speakers including two from the USA and two from the USSR. A review of the latter topic was timely in that the International Commission of Radiological Protection (ICRP) currently had a Task Group reassess the problem of safer dose limits for the skin.

An introductory review of the biology of skin and of its responses to radiation was presented by Dr. J. Hopewell (Oxford) and this set the scene for the remainder of the meeting. A highlight of the meeting was a major review by Dr. A. Barabonova (Moscow) of the skin lesion in the victims of the Chernobyl nuclear power plant accident. This was undoubtedly the world's largest series of cases of accidental overexposure of the skin to radiation. The discussion included the problems of the physical and biological dosimetric aspects of the re-construction of radiation accidents and an account of the treatment of high dose skin lesions. Dr. M. Fry (Chairman of the ICRP Skin Task Group, Oakridge) introduced the requirement associated with the setting of safe dose limits for skin exposure. There were then detailed talks relating to dosimetry aspects of deterministic effects induced in skin by B-irradiation (Dr. A. Osanov, Moscow), studies of radiation carcinogens in the skin of man and experimental animals and finally an overview of the draft recommendations of the ICRP Task Group were presented by Dr. M. Charles.

To be published in the International Journal of Radiation Biology in May 1990.

To be ordered through:

Dr. J. Hendry
Paterson Institute for Cancer Research
Christie Hospital and Holt Radium Institute
Wilmslow Road
GB - Manchester M20 9BX

OTHER PUBLICATIONS

Catalogue of contracts of the Commission's Radiation Protection Programme 1985-1989

This catalogue has been published in two volumes: Volume I containing information on the management data such as contractor, subject of the research projects, duration, budget, etc... and Volume II containing the scientific description of each project. The first edition (July 1987) has been extended by an addendum covering the research contracts of the Radiation Protection Programme 1985-1989 concluded since then. In total some 338 contracts covering about 440 research projects are summarised. 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.

To be obtained from:

CEC
DG XII/D/3
Rue de la Loi, 200
B - 1049 Brussels

VI

VERZEICHNIS DER FORSCHUNGSGRUPPENLEITER

LIST OF RESEARCH GROUP LEADERS

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RESEARCH GROUP LEADERS

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EUR 13268 — Tätigkeitsbericht — Programm Strahlenschutz — 1985—1989
Progress report — Radiation protection programme — 1985-89
Rapport d'activité — Programme « radioprotection » — 1985-1989
Volume 3

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Radiation protection series

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The final report of the 1985-89 radiation protection programme outlines the research work carried out during the whole contractual period under all contracts between the Commission of the European Communities and research groups in the Member States. More than 700 scientists collaborated on this programme.

Results of more than 440 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 safe production of energy, i.e.:

(i) to improve methods necessary to protect workers and the population by updating the scientific basis for appropriate standards;
(ii) to prevent and counteract harmful effects of radiation;
(iii) to assess radiation risks and provide methods to cope with the consequences of radiation accidents.

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- ☐ 1 Radioactive contamination of the environment
- ☐ 2 Hereditary effects of radiation
- ☐ 3 Short-term effects of radiation, acute irradiation syndrome and its treatment
- ☐ 4 Long-term effects of radiation and toxicology of ingested radionuclides
- ☐ 5 Measurement of radiation and its interpretation, dosimetry
- ☐ 6 Assessment of radiation risks

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- ☐ 1 Contamination radioactive du milieu
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- ☐ 3 Effets à court terme des rayonnements, syndrome aigu d'irradiation et son traitement
- ☐ 4 Effets à long terme des rayonnements et toxicologie des radionuclides ingérés
- ☐ 5 Mesures des rayonnements et leur interprétation, dosimétrie
- ☐ 6 Évaluation des risques d'irradiation

COMMISSION OF THE EUROPEAN COMMUNITIES

Directorate-General
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Radiation Protection Programme

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COMMISSION DES COMMUNAUTÉS EUROPÉENNES

Direction générale
Science, recherche et développement

Programme Radioprotection

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