EUROPEAN COMMISSION

nuclear science and technology

Improved cancer risk quantification for environmental, medical and occupational exposures to low doses of ionising radiation by mechanistic models

(Low Dose Risk Models)

Contract N° FIGH-CT1999-00005

Final report
(summary)

Work performed as part of the European Atomic Energy Community's research and training programme in the field of nuclear energy 1998-2002 (Fifth Framework Programme)
Generic research in radiological sciences

Directorate-General for Research
Euratom

2008

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Introduction

This project has contributed to the solution of the important open problem of quantification of somatic radiation risks to humans at low doses (below, say, 10 mSv) and low dose rates of ionising radiation of various qualities at least for the organs lung, liver and bone. Since this problem cannot be solved by basic biological investigations or by epidemiological studies alone, the scientific knowledge from both areas has been combined to develop and test quantitative mechanistic models for the dose-time-effect surfaces of radiation carcinogenesis in these organs. Existing data and theories were evaluated and used for the modelling of radiation transport and interaction, for the modelling of subsequent damage and repair of DNA, genes, cells and organs taking normal oxidative damage into account, and for the multi-step modelling of carcinogenesis. Conclusions were drawn for the general radiation protection of the worker and the public.

Objectives

It was the main goal of this project to improve our knowledge on the, perhaps, most important practical and basic problem in radiation protection, namely the risk estimates for somatic health effects in various groups of humans after exposures to low doses of various types of ionising radiation at dose rates that occur in the workplace, in the normal living environment (i.e. from natural radioactivity), around nuclear facilities (e.g. by normal and accidental emissions, leaking waste repositories) and in diagnostic medicine.

Our project aimed to provide a better quantification of the dose-time-effect surfaces for age, sex, sensitivity, etc. dependent radiation risks at doses below, say, 10 mSv by photons, neutrons, by alpha particles and beta particles from incorporated radionuclides, etc. Thus, our project was important for the evaluation of exposures of workers in industry, from nuclear fission and fusion devices, in mines, in water processing facilities, in waste handling facilities, in aeroplanes, in health care, etc., as well as of the general public.

The project was organised in a "bottom-up design" manner (topic of highest applied importance last, necessary supporting studies first) into five work packages (WP1 to WP5); each work package was subdivided into up to five sub-tasks. The objectives in more detail were:

WP1 "Critical processes and data evaluation"
Collect and critically evaluate data on bystander effect (occurrence of damage in non-irradiated cells following irradiation of neighbouring cells) and adaptive response (low-dose-induced resistance to subsequent irradiation with higher doses), with the main aim of quantifying their role – and more generally the role of cellular communication – in modulating the response to low doses. Collate and evaluate appropriate benchmark data-sets to test models against. Collect and critically assess data on the induction of chromosome aberrations by ionising radiation and present the results in a form suitable for modelling. Collect cellular radiobiological data needed for mechanistic cancer modelling specifically at low doses and dose rates and obtain data-sets that may help to develop in WP4 mechanistic models for radiation carcinogenes. Evaluate available data on the number and size of radiation-induced pre-malignant clones.

WP2 "Primary damage models"
The main goals of WP2 were to improve and extend simulation models to additional radiation qualities and to higher-target models of the track structure codes for the induction of primary cell and organ damages. Different topics were then: 1) development of new sets of cross sections, 2) modelling of the molecular geometries of DNA and higher-order structures, 3) calculations of primary physical and chemical interactions of various radiations with these models, 4) adaptation of voxel phantoms and coupling of the transport codes to the geometry codes, 5) improvement of these more complex simulation codes, and 6) assessment of the oxidative damage and its computer representation.

WP3 "Repair-consequences model"
Development of quantitative computer models for the repair/misrepair of radiation-induced DNA damages for low dose and dose rates at both the gene and chromosomal level, identification of critical components of that damage which lead to loss of genetic integrity, such as simple and complex aberrations, mutations and their transmissibility in proliferating cells. Assessment of the long-term consequences of loss of genetic integrity on cell and organ function taking into account the role of inter-cellular communication and its consequences for carcinogenesis.

WP4 "Mechanistic cancer model development"
Determine dose-time effect surfaces for cancer risks following irradiation at low doses and dose rates, based on mechanistic models of radiation-induced carcinogenesis, and compare them with the commonly assumed linear – no threshold (LNT) hypothesis. Develop mechanistic, quantitative mathematical models of radiation oncogenesis, specifically for lung, thyroid, and bone tumours. The ongoing experiment at DKFZ on preneoplastic lesions of the liver suggested also looking at data on liver tumours. Study selected animal data sets for clarification of open questions.

WP5 "Conclusions for radiation protection"
The objective of this work package was to describe the possible implications of the work in this project for radiation protection.

Results

First, progress in *work package 1 "Critical processes and data evaluation"* is reported: UMIL evaluated available data on bystander effect (BE), i.e. induction of damage in non-irradiated cells, probably due to communication with irradiated cells. Three categories of experiments were identified: 1) broad-beam irradiation with very low doses of alpha particles; 2) microbeam irradiation, which allows targeting of specific cells; 3) treatment of unexposed cells with irradiated conditioned medium (ICM), i.e. medium taken from irradiated cell cultures. All the data indicated higher levels of damage than expected for different end-points. UMIL also evaluated data on adaptive response (AR), i.e. low-dose (between 5 and 20 cGy) induced resistance to subsequent irradiation with higher doses. UMIL reviewed data on chromosome aberration induction by neutrons and their interaction products and calculated the linear and quadratic components of neutron-induced dicentric dose response via integration of experimental data into the FLUKA transport code. While good agreement between model predictions and data was found for the linear component, the data themselves indicated that the neutron quadratic component cannot be obtained by "averaging" the

contributions of nuclear reaction products. This supports the hypothesis that neutron-induced aberrations increase linearly with dose.

GSF and RIVM obtained several crucial data sets needed for further model development. These data were made available by the respective owners at no cost to the project. GSF included the possible effects of low-dose hypersensitivity in the tumour models developed within WP4.

MRC compiled monographs on microdosimetric parameters and frequency of energy depositions by low-energy protons and alpha particles. Furthermore, MRC found that: a) it is the low-energy secondary electrons that are the critical features of low-LET radiation; b) it is the spatial rather than temporal features of radiation tracks which are important in predicting biological response, even for the induction of genomic instability; c) the twofold increase in RBE in cell inactivation above the C_K edge for X-rays is due to preferential localisation of events on DNA; d) results from C_K X-ray experiments provide further support for the lesion-non-lesion theory of chromosome aberration induction; e) charge migration over short distances is one of the major consequences of DNA photo-ionisation; e) modelling of Br/IdU incorporation indicates a shift towards greater complexity of clustered DNA damage; f) the complexity of DNA damage increases with increasing radiation quality and the majority of base damage is formed within clusters by α radiation, which may compromise repair and lead to increased mutability; g) the newly developed mechanistic model of the bystander effect agreed well with published experimental data.

NRPB provided a survey of all published data on the production of dicentrics in human lymphocytes irradiated in G_o, for which it is generally accepted that the relationship between the yield of dicentrics and dose follows the relationship $Y = c + \alpha D + \beta D^2$ (acute exposure). At low LET, β is measured as about 0.06 dicentrics per cell per Gy^2 with an uncertainty of about 10 %. α varies with photon and electron energy: 0.015 dicentrics per cell per Gy for cobalt-60 γ-rays, 0.04 for X-rays of 200-250 kVp, in the range 0.11 to 0.27 for low-energy X-rays (only one laboratory), from 0.6 to 0.9 for fission neutrons. For 14 MeV neutrons, on average the value of β is about 0.06 dicentrics per cell per Gy^2 but the uncertainty is at least 50 %. The value of α shows more consistency and is about 0.2 dicentrics per cell per Gy with an uncertainty of 10 %. At energies below 0.5 MeV, one laboratory claims a steady reduction in dicentric yield while another suggests no reduction.

The excellent agreement found by Salzburg University between predicted and measured transformation frequencies in C3H 10T1/2 cells and between predicted and epidemiologically observed radon-induced lung cancer risk in humans and rats demonstrates the relevance of the input data for the various cellular radiobiological mechanisms used in the state-vector model.

DKFZ analysed liver pre-neoplastic lesions in three groups of BALB/C mice subject to bolus treatment (Thorotrast, 1 MBq and 5 MBq of 213Bi-labelled antibody) by hematoxylin/eosin staining of liver sections and morphometrical evaluation of liver foci. While no liver focal lesions could be distinguished six months after treatment, the second and third sacrifice (12 and 17 months after treatment) showed clear/acidophilic cell foci, on which analysis was concentrated. The focal transection sizes and the liver section area were used to determine each animal's area fraction of foci. The Jonckheere-Terpstra test for the area fraction showed a significant dose response for the second and the third sacrifice time. This points to a relationship between alpha radiation and occurrence of liver focal lesions.

In *work package 2 "Primary damages models"*, new sets of cross sections have been developed for: 1) e- and e+ in the energy range 1eV to 100GeV for elements up to Z = 100, 2) electron impact ionisation cross sections for the constituents of the DNA, 3) low energy protons, neutral hydrogen, alpha particles, and helium ions in water, 4) production of alpha particles following interaction of C and O atoms with different materials, and 5) for low-energy electrons and photons on a bio molecule.

Track structure codes have been developed and/or adapted to more realistic biological end-points with for instance:

- chemical modules and a refined DNA target model were included in PARTRAC,
- FLUKA was coupled with two realistic human body models,
- KURBUC was improved with a chemical part and used to simulate secondaries created by low-energy protons and helium ions in water,
- CPA100 was settled up to simulate the transport in the chromatin fibre and to taken into account the normal oxidative damage,
- New set of cross-sections and new algorithm of the condensed history tracking was implemented in GEANT for biological studies.

These codes have been used to produce data very difficult even impossible to obtain with other methods, for instance:

- Modelling the induction of single and double strand breaks by gamma rays impinging on the SV40 mini chromosome
- Develop a technique for radioprobing DNA structure using Auger electrons,
- quantify the DNA damage induced by incorporated radionuclides
- characterise a radiotherapy proton beam
- modelling the effect of Ultra-soft X rays on DNA and its close vicinity.

In *work package 3 "Repair-consequences model"*, a theoretical model and a Monte Carlo code for chromosome aberration induction in human cells were developed by UMIL. The model, which describes interphase chromosome territories explicitly, assumes that only clustered DNA damage (complex lesions) can lead to aberrations, and that only CL in the same territory or in neighbouring territories can interact and form aberrations. The model provides dose-response curves for the main types of Giemsa- and FISH-stained aberrations (dicentrics, centric rings, fragments, translocations and complex exchanges) induced in human lymphocytes by photons, protons and alpha particles of different energies, both as monochromatic fields and as mixed fields, also taking into account the presence of a background level of aberrations. Very good agreement was found with available experimental data (even at very low doses, of the order of ≈ 0.01 Gy), thus confirming the important role of DNA damage complexity and interphase chromosome localisation.

MRC undertook modelling and calculations of the spectra of the complexity of DNA damage induced by radiations of different quality to provide details and insight into the type, source and complexity of clustered DNA damage. In support of the hypothesis from biophysical modelling, the repair of clustered DNA damage is stalled so that complex damage may be highly mutagenic. The complexity of radiation-induced DNA damage, in contrast to isolated endogenous DNA lesions formed as a by-product of oxygen metabolism in cells, is thought to be a major feature that determines the biological effectiveness of different types of ionising

radiation at environmentally low doses and dose rates. MRC provided the first experimental evidence for the induction of non DSB clustered DNA damage induced in cells by low LET radiation at a level > 4 x that of prompt DSB. The biological consequences of clustered DNA damage may be significant even at low doses, since there is a finite chance of a clustered DNA damage DNA being formed in a cell by a single radiation track. The implications from cellular repair studies are that the majority of non-DSB clustered DNA damage sites are not converted into DSB.

MRC have developed also a quantitative computational models for repair based upon enzymatic approaches relating the kinetics of multi-step processes of the rejoining of DNA double strand breaks (DSB) with the kinetics of formation of simple chromosome exchange aberrations in irradiated mammalian cells, based on benchmark experimental data available in the literature. The first evidence is presented that 'dirty' DSB termini are less readily rejoined than clean DSB, so they have extended lifetimes in the cell and may increase mis-repair pathways and aberrations.

Extension of modelling to the modulation of gene expression involves in part non-linear processes that evolve from the linear dose responses for initial DNA damage induced by ionising radiation.

Work at NRPB has demonstrated that no one single model can explain all of the data on radiation induced chromosome aberrations in human lymphocytes. It has been shown that to explain the near constancy of the ratio of centric rings to dicentrics the two arms of each chromosome have to lie in separate neighbouring domains. Exchanges between breaks occur soon after irradiation but at early times only one half of the exchange has been observed. The remaining half of the exchange occurs much later. This supports the breakage first model but the reported linearity with dose of the initial exchanges is not obviously explainable. This observation needs to be verified. The observation that the spectrum of the types of complex aberration is dependent on radiation quality is easily explained by the proven assumption that chromosomes exist in domains. A confounding factor has been reported recently. The translocation yield in cells in their first post-irradiation division is dependent on their early or late arrival at metaphase. This is explainable for high LET where some cells receive very much higher dose than others and it is known that dose causes delay in reaching mitosis. An experiment to check this finding at low LET is underway. The indications so far are that the effect, if present, is not very strong in human lymphocytes.

In *work package 4 "Mechanistic cancer model development"*, several mathematical models for radiation carcinogenesis were used in parallel which emphasise different aspects of the multi-step cancer development process. This approach is followed because the precise nature of the carcinogenic process is not known. All models indicate that radiation is an initiating agent, and found additional radiation action necessary. The character of this additional radiation action, however, is not yet agreed on. Tumour incidence data were described with dependence on radiation in the last mutational step ('transformation'), or in clonal expansion ('promotion'). The former version corresponds to the classical view that radiation acts only in a mutagenic way, while the latter allows a promoting effect, similar to that of cytotoxic chemicals. The question whether there is such promoting action of radiation is important, as it would not be directly linked to mutations and thus to damage in the DNA. It requires that not only a cell, but also the tissue of an organ is may respond to the insult. Appropriately this question has been debated at length during the contract.

GSF made a new proposal to possibly explain a promoting action of radiation in the lung: Radiation inactivates cells which are replaced by dividing neighbouring cells. If cells on the way to malignancy can fill the hole faster than healthy cells, their number increases. A moderate increase in the replacement probability is sufficient to achieve agreement between this theory and experimental data. With this scheme, cell inactivation by radiation may indirectly cause a major part of the radon-induced lung cancers in the Colorado miners.

The (not yet publicly available) data from the A-bomb survivors with a longer follow-up became available in Hiroshima. These data showed effects which point in the direction that also acute gamma radiation might act initiating and promoting. This conclusion is supported by work on data from the Janus mice exposed to gamma rays (GSF).

A detailed biophysical model for lung cancer induction by radiation was developed by Salzburg University to understand better the discrepancy between epidemiologically derived (3.88 mSv/WLM) and dosimetry based (15 mSv/WLM) dose-exposure estimates for inhaled radon progeny. The dosimetry model which was finally selected predicted a weighted effective dose of 7.6 mSv/WLM for indoor exposure conditions, compared to 14.5 mSv/WLM based on the ICRP model. Thus, dosimetric estimates do not differ appreciably from the epidemiologically derived dose convention of about 4 mSv/WLM.

In order to utilize experimental data on lung cancer risk in rats, the question arises whether the same exposure conditions in humans and rats produce the same cellular doses. Comparison of predicted hit probabilities for sensitive basal and secretory cell nuclei indicated that the average hit probability of bronchial cells in the rat lung is approximately twice as high as that for the human lung.

Data on mice exposed to Ra-224 at GSF, Institute of Pathology, show a distinct inverse protraction effect at high doses, whereas at lower doses this effect becomes insignificant. Such a behavior is well reproduced in the TSCE model. For exposure rates exceeding about 8 mGy/d, increased exposure times (at a fixed exposure rate) result in higher ERR per exposure unit. At exposure rates below 4 mGy/d longer exposure times lead to reduced ERR per exposure unit due to age-at-exposure effects. Between 4 and 8 mGy/d, the difference in ERR/exposure does not depend strongly on duration.

The TMC modelling results of RIVM of human and beagle data on the different bone seekers Ra-226, Ra-228, Sr-90, and Pu-239 consistently show a linear-quadratic shape of the dose-effect relationships for bone cancer incidence at low doses, due to a linear radiation effect on both mutational steps. This implies that the current risk estimates for bone seekers at low doses, which are based on a linear extrapolation, might be overestimated by an order of magnitude.

Together with consultants from Seattle, GSF showed that there is no strong evidence to support the published claim of Pierce and Mendelsohn that excess cancer risks for the solid tumours depend only upon attained age and not on age at exposure or time since exposure. Although the A-bomb survivors' cohort is the largest epidemiological data set for the study of radiation and cancer it is not large enough to discriminate among various carcinogenic mechanisms. The data appear to be consistent with a number of different interpretations of the role of radiation in carcinogenesis.

The model-based analysis of experimental alpha-radiation-induced liver preneoplasia data showed that alpha radiation increases formation rate of preneoplasia as well as their growth (DKFZ).

Two groups worked with data on liver cancer among Thorotrast patients: RIVM found among the Danish data a dependence of the first mutation rate on radiation of the TMC model, which is higher for females than for males. The second mutation rate does not significantly depend on dose. The lifetime radiation risks for the liver calculated on the basis of the model parameters are about the same for males and females, and between a factor of 2 and 10 higher than current estimates.

GSF studied the Swedish data on Thorotrast. Effects of radiation on the initiating mutation and on the clonal expansion rate explained the observed patterns well. The estimated kinetic parameters mean that dose rates of 5 mGy/y double the spontaneous initiation rate. The clonal expansion rate is doubled by 80 mGy/y, and for females it levels at rates beyond 240 mGy/y. The magnitude of the estimated promoting effect of radiation can be explained with a moderate increase in the cell replacement probability of intermediate cells in the liver, strikingly similar to the situation in lung tumourigenesis. The estimated lifetime risk as low doses is substantially smaller than in the model favoured by RIVM.

In *work package 5 "Conclusions for radiation protection"*, initial consideration was given by MRC to possible risk implications of emerging results from some current experimental research. Results for microbeam single-particle irradiation of human lymphocytes with helium-3 ions yielding a significantly increased probability of producing chromosomal changes *de novo* in subsequent cell generations, and results on genetic instability induced in mouse haemopoietic stem cells after 3 Gy X-rays could have implications for radiation induced cancer. In that case extrapolations of risk to low doses and dose rates may be substantially different from the conventional paradigm of radiation acting as a single-stage mutagen in the leukaemogenic process.

RIVM has successfully fitted a two-mutation clonal expansion carcinogenesis model to a number of animal and epidemiological data on radiation-induced cancer. The possible implications of this modelling work for radiation protection concern these *low-dose risk estimations*. For radiation protection the following conclusions are particularly important and might have practical implications:

- the model is a biologically motivated cancer model useful for the analysis of epidemiological data, the application of which leads to a better scientific insight into the dose, age and time relationships of radiation-induced cancer;
- although dose-effect relationships for radiation-induced cancer are not always strictly proportional to dose, in general the model indicates that the radiation effect increases linearly from zero dose without a threshold; the model thus supports the currently used LNT concept for radiation protection;
- for several cancers the model indicates a strong effect of age at exposure, i.e. for exposure at young ages the effect is much higher than for exposures at older ages; it is worth considering to take this into account for practical radiation risk assessments;
- the model indicates a much lower risk for bone-seeking alpha emitters for low intakes than current estimates due to a supralinear curvature for the dose-effect at low doses, the implication of which for radiation protection is recommended;

– the model provides a scientific basis for the reduction of the radiation risk for low dose rates and low doses, which deserves further investigation.

Implications for radiation protection

Many aspects of the implications of our project work for radiation protection have been discussed in the section above.

MRC found that the spatial clustering of energy deposition on the nanometre (DNA) scale is the critical feature of radiation tracks that determine a variety of biological effects. Even a single electron track is capable of producing clustered DNA damage, involving multiple damage sites within a few base pairs. Contrary to classical theories of formation of chromosome aberrations there is now strong supporting evidence for the ability of radiation-damaged DNA to interact with undamaged DNA in forming a simple chromosome aberration. Since many such aberrations are non-lethal to the cell, these aberrations represent a genetic risk that may contribute to cancer in somatic cells or to heritable risk in germ cells. These results would imply that even a single electron such as from environmentally low levels of radiation has a finite, albeit very small, probability of producing genetic rearrangement.

The highly nonlinear features of the state-vector model developed by Salzburg University point to a nonlinear dose-effect relationship at low doses and dose rates, which has to be considered when extrapolating biological effects from moderate and high to low doses.

A benefit for the community is that now a set of computer codes (mainly PARTRAC, FLUKA, KURBUC, CPA100 and GEANT) with improved cross sections are available to simulate the transport of nearly all radiation qualities and to calculate primary physical and chemical damages not only in liquid water but also in complex DNA targets and its higher-order structures. Now that internal radiotherapy and photo activation have an increasing use for the precision of targeting and the comfort of the patient, the use of these simulation codes will be of great help.

Besides helping in elucidating the underlying mechanisms, the model of chromosome aberration induction provided a reliable tool for predicting the effects of different radiation types even at very low doses, of the order of 0.01 Gy or less. This can be of great interest for applications in radiation protection, especially if one takes into account that there exists a strong correlation between certain aberrations (typically reciprocal translocations) and some cancer types (typically leukaemia). Furthermore, since ratios between specific aberration types (e.g. dicentric to centric rings and complex exchanges to simple exchanges) can be regarded as biomarkers of the radiation quality, this can allow for applications in retrospective dosimetry, which can be of help in case of accidental exposures.

The mechanistic cancer models developed by this consortium can supplement the risk heuristic models from epidemiological studies for lung, bone, and liver cancer. The big differences obtained in some of the applications, e.g. when extrapolating the models for liver cancer from Thorotrast patients to low dose rates, stress the need for a better understanding of the carcinogenic process.

The research concentrated on radiation-induced cancer, but the mechanisms are likely to be to some extent universal for cancer induced by other environmental or nutritional agents. So the

proposal may also help in estimating small additional cancer risks due to other reasons than radiation. As the population is ageing in Europe, cancer as a cause of illness and death will become even more important in the future.