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FOREWORD

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Under the terms of the Treaty establishing the European Atomic Energy Community, the Community, amongst other things, establishes uniform safety standards to protect the health of workers and of the general public against the dangers arising from ionizing radiation. The standards are approved by the Council, on a proposal from the Commission, established taking into account the opinion of the Group of Experts referred to in Article 31 of the Treaty. The most recent version of such standards is contained in Council Directive 96/29/Euratom of 13 May 1996 laying down basic safety standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiation.

The European Commission organises every year, in cooperation with the Group of Experts referred to in Article 31 of the Euratom Treaty, a scientific seminar to discuss in depth a particular topic of radiation protection suggested by the Group.

In 2006, the International Commission on Radiological Protection (ICRP) offered for public consultation a third draft of its future Recommendations. The European Commission organized, with the help of the Working Party on Research Implications on Health and Safety Standards (WP RIHSS), a seminar that took place in Luxembourg on 17 October 2006. The purpose of the seminar was to review this draft in the light of current insights in radiation risks.

Leading scientists in this area presented the latest information.

The Group of Experts discussed this information and drew conclusions that are relevant for consideration by ICRP and by the European Commission. The Commission will take into account both the Recommendations of ICRP and the conclusions of Experts in the process of revising Directive 96/29 into new consolidated Basic Safety Standards.

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1 NEW MODELS FOR EVALUATION OF THE RADIATION-INDUCED LIFETIME CANCER RISK

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1.1 Abstract

Generalised relative risk models are fitted to the latest Japanese atomic bomb survivor solid cancer and leukaemia mortality data, with the latest (DS02) dosimetry, by Bayesian techniques, taking account of errors in dose estimates and other uncertainties. Linear-quadratic and linear-quadratic-exponential models are fitted and used to assess risks for a current (2003) UK population.

Using a test dose of 0.1 Sv the solid cancer mortality risk using the linear-quadratic-exponential model is 3.3% Sv^{-1} (90% CI -1.3 - 7.0), and for the linear-quadratic model risk is 5.4% Sv^{-1} (90% CI 3.1 - 8.0). At 0.1 Sv, leukaemia mortality risk using the linear-quadratic-exponential model is 0.19% Sv^{-1} (90% CI -0.27 - 0.81), and using the linear-quadratic model risk is 0.58% Sv^{-1} (90% CI 0.13 - 1.15).

The slightly lower risks (at least at low test doses) produced by the linear-quadratic-exponential models are perhaps remarkable, and are a result of the incorporation of an exponential cell sterilization term, $\exp[\gamma \cdot D]$, in the dose response. Both for solid cancers and leukaemia the cell sterilization coefficient γ is not statistically significant, but its effect on the linear and quadratic coefficients is profound, resulting in the linear term becoming smaller (and generally not statistically significantly different from 0) and the quadratic term becoming much larger (and generally statistically significant). These effects are also observed in fitting of similar models by maximum likelihood techniques, and very similar central estimates of risk are produced.

1.2 Introduction

Epidemiological studies of cancer risks associated with internal and external exposure to ionizing radiation have been subject to extensive reviews by various scientific bodies (UNSCEAR 1994, 2000, US NRC 2006). In particular, the UNSCEAR 2000 Report assessed cancer incidence and mortality data relating to the Japanese atomic bomb survivor Life Span Study (LSS) cohort (Preston *et al.* 1994, Thompson *et al.* 1994, Pierce *et al.* 1996), as well as many other data relating to persons exposed occupationally, therapeutically or diagnostically. The cancer risk estimates calculated in the UNSCEAR 2000 Report were based on LSS data using the set of survivor dose estimates produced in the mid 1980s, the so-called DS86 dosimetry (Roesch 1987). For some time it was thought that the DS86 neutron dose estimates for the Hiroshima atomic bomb survivors were systematic underestimates, particularly for survivors beyond 1000 m from the hypocentre (Roesch 1987, Straume *et al.* 1992). The discrepancy was largely based on measurements of thermal neutron activation products derived from samples retrieved from Hiroshima (Straume *et al.* 1992). The DS86 gamma dose estimates were thought to be more reliable (Roesch 1987), as also were the Nagasaki neutron dose estimates (Straume *et al.* 1994). Recent analysis of the totality of data, including fast-neutron activation products, suggests that there are no appreciable

systematic errors in the DS86 Hiroshima neutron dose estimates (Cullings and Fujita 2003, Straume *et al.* 2003, Young and Kerr 2005). The most up-to-date set of dose estimates for atomic bomb survivors, the so-called DS02 dosimetry, differs slightly from the DS86 system, both for neutron and gamma doses, by generally no more than 20% for distances up to 1500 m from the two hypocentres, where survivors received the most appreciable doses (Cullings and Fujita 2003, Young and Kerr 2005). In both cities estimates of DS02 colon neutron dose were reduced, by no more than about 20% compared with DS86, with the ratio DS02:DS86 decreasing with increasing distance from the hypocentre; the decrease was particularly marked for Nagasaki (Preston *et al.* 2004). In Hiroshima DS02 colon gamma-ray estimates were reduced by about 10% compared with DS86 at all distances; Nagasaki colon doses were about 10% higher at ground range < 1800 m from the hypocentre, but a bit less than that at greater distances (Preston *et al.* 2004). Analyses of the RERF epidemiological data using the new dosimetry indicate that cancer risk factors might decrease by about 8% as a result, with no appreciable change in the shape of the dose response or in the age-time patterns of excess risk (Preston *et al.* 2004).

Although the resolution of dosimetric inconsistencies in the Japanese atomic bomb survivor data has reduced one source of uncertainty in the estimation of population cancer risks at low doses, a considerable number of other sources of uncertainty remain. A major source of uncertainty relates to extrapolation from the moderate dose but high dose-rate exposures received by the Japanese atomic bomb survivors (and in many therapeutically exposed groups) to the low doses and dose rates of interest to radiological protection. This has long been controversial. There is also uncertainty relating to the extrapolation of cancer risk to the end of life. In particular, about half of the LSS cohort is presently still alive (Preston *et al.* 2004); in estimating risks from this cohort it is vital to ascertain the pattern of variation of radiation-associated cancer risk for those exposed in childhood, who are presently reaching the age at which larger numbers of cancers would be expected spontaneously. Another source of uncertainty relates to transfer of radiation-induced cancer risk estimates between populations with different underlying cancer rates. For example, lung and breast cancer rates in the Japanese population tend to be rather lower than in many North American and Western European populations, whereas stomach cancer rates tend to be much higher (Parkin *et al.* 2002). The available evidence, most recently reviewed by UNSCEAR (1994), did not suggest that there is an easy resolution of this problem.

The present report re-assesses risks of cancer mortality from the LSS data, making use of the latest DS02 dosimetry and follow-up. A novel Bayesian methodology is used to assess uncertainty in cancer risk, taking account of uncertainties in dose estimates.

1.3 Data Used

The latest versions of the leukaemia and solid cancer mortality dataset for the LSS cohort of A-bomb survivors were employed (Preston *et al.* 2004). Following the example of Preston *et al.* (2004) in their analysis of this dataset, all survivors with shielded kerma dose > 4 Gy were omitted from the modelling of cancer (the second stage in the Bayesian MCMC model fitting: see below), because of possible systematic errors in the highest dose estimates and possible cell sterilization effects. However, the full dataset, including the survivors with shielded kerma dose > 4 Gy, was used to assess the dosimetric error structure (the first stage in the Bayesian MCMC model fitting: see below).

The organ dose used for the solid cancer data was that to the colon, whereas bone-marrow dose was used for leukaemia; in both cases a neutron relative biological effectiveness (RBE) of 10 was assumed, as used in previous analyses (Little et al. 2000, Bennett et al. 2004, Preston et al. 2004). All “nominal” organ doses were calculated using the latest dosimetry, the so-called DS02 dosimetry (Young and Kerr 2005). Individual data were not available, so that all analyses

used the stratified data. The stratification employed is very similar to that previously used (Preston et al. 2004), and is defined by time since exposure, age at exposure, attained age, city, sex, Nagasaki factory worker status, ground distance category and ("nominal") dose.

1.4 Models Used

Poisson disease models were used for all fitting to the LSS data. The models that are used in this paper are fundamentally functions of the (unobserved) "true" mean organ dose, $\overline{D}$, averaged over the survivors in the stratum. For convenience a relative risk models was used both for solid cancers and leukaemia, where the cancer risk in the stratum with city c, sex, s, attained age, a, age at exposure, e, and "true" average organ dose, $\overline{D}$, can be partitioned as:

$$h_0(a,e,c,s)\cdot[1+ERR(s,a,e,\overline{D})]\,. \quad (1)$$

The function $ERR(s,a,e,\overline{D})$ describes the radiation-induced excess relative risk (ERR), and is assumed to be zero for zero average organ dose; $h_0(a,e,c,s)$ describes the "baseline" (zero dose) cancer risk. Therefore, the number of cancer deaths in the stratum is a Poisson random variable with expectation:

$$PY\cdot h_0(a,e,c,s)\cdot\left[1+ERR(s,a,e,\overline{D})\right] \quad (2)$$

where PY is the number of person years of follow-up in the stratum. Previous dose-response relationships considered for these data include linear and quadratic models, and certain more general forms (Pierce et al. 1990, Little and Muirhead 1996, 1998, 2000, 2004, Little et al. 2000, Bennett et al. 2004). In modelling the latest solid cancer mortality data the following generalized ERR model was used, in which the cancer mortality rate for age a, age at exposure e, city c, sex s and "true" colon dose D is given by:

$$h_0(a,e,c,s)\cdot\left[\begin{array}{l}1+(\alpha\cdot\overline{D}+\beta\cdot\overline{D}^2)\cdot\exp[\gamma\cdot\overline{D}]\cdot\\ \exp[\kappa_1\cdot 1_{s=female}+\kappa_2\cdot\ln[a-e]+\kappa_3\cdot\ln[a]]\end{array}\right] \quad (3)$$

This is a generalized ERR model that is linear-quadratic-exponential in dose, and with adjustment to the excess relative risk for sex, s, attained age, a, and time since exposure, $a-e$. In addition, a variant of this model was fitted in which the cell sterilization term γ was set to 0, i.e., the model is linear-quadratic in dose.

Likewise, for leukaemia mortality the following generalized ERR model was used, in which the leukaemia mortality rate at age a, age at exposure e, city c, sex s and "true" average bone marrow dose $\overline{D}$ is given by:

$$h_0(a,e,c,s)\cdot\left[1+(\alpha\cdot\overline{D}+\beta\cdot\overline{D}^2)\cdot\exp[\gamma\cdot\overline{D}]\cdot\exp[\kappa_3\cdot\ln[a]+\kappa_4\cdot\ln[e]]\right] \quad (4)$$

This is a generalized ERR model that is linear-quadratic-exponential in dose, and with adjustment to the excess relative risk for attained age, a, and age at exposure, e. As for solid cancers, a variant of this model was fitted in which the cell sterilization term γ was set to 0, i.e., the model is linear-quadratic in dose.

It should be emphasised that the "true" stratum-average organ dose, $\overline{D}$, is not known; the only recorded dosimetric quantity in any stratum is the "nominal" stratum-average (DS02) organ dose, $\overline{d}$.

1.5 Model Fitting

Measurement error at individual level

The natural modelling of measurement error in Bayesian MCMC methods is at the individual level. The stratification creates groups of subjects, and so requires transfer of the modelling of measurement error on the *individual dose* to the measurement error on the *mean dose* over the stratum. At an individual level, the "true" dose distribution in each of the two cities (Hiroshima, Nagasaki) is modelled by a generalised Weibull distribution, in which the probability density of the "true" dose D in city c and sex s is given by:

$$w_{sc}(D) = \omega_{1sc} \cdot \left[\omega_{2sc} \cdot \omega_{3sc} \cdot D^{\omega_{3sc}-1} + \omega_{4sc}\right] \cdot \exp\left[-\omega_{2sc} \cdot D^{\omega_{3sc}} - \omega_{4sc} \cdot D\right] + [1-\omega_{1sc}] \cdot 100 \cdot 1_{D<0.01} \quad (5)$$

This is a superposition of an extended Weibull density function (similar to that used previously by Little (2002, 2004)), with an additional uniform density on the true dose interval [0.0, 0.01], and provides statistically significantly better fit than the standard Weibull distribution employed by Pierce *et al.* (1990). Jablon (1971) investigated the errors in the Japanese atomic bomb dosimetry and found that these errors were most likely to be log-normal, with a geometric standard deviation (GSD) of about 30%. Furthermore, a "classical" measurement error model is employed since the main component of the measurement error comes from the declaration by the survivor of their location and orientation with respect to the bomb at the time of explosion (Roesch 1987). Therefore, in this paper the distribution of the "nominal" dose, d, given the "true" individual dose, D, is assumed to be log-normal with median D. Following the example of Pierce *et al.* (1990), in this paper the "nominal" dose is assumed to be log-normally distributed with 35% GSD errors.

Bayesian modelling at the strata level

A two-stage method is used for modelling the stratum-specific dosimetric uncertainties, very similar to that used by Little *et al.* (2000) and Bennett *et al.* (2004), and described in more detail there. In the first stage, for each stratum i (defined by city, sex and age at exposure group) and dose group j, the distribution of the "true" mean dose $\overline{D_{ij}}$ is computed by Monte Carlo integration according to an iterative procedure (Little *et al.* 2000, Bennett *et al.* 2004). The FORTRAN program used to perform these calculations was written by the author, and is available on request. This procedure was necessitated by the grouped nature of the data, in particular by the fact that individual "nominal" doses were not available. In the second stage, the derived distribution of all the $\overline{D_{ij}}$ is then used together with the disease models (1)-(3) to derive the posterior distribution of the parameters of these models. The Bayesian sampling was performed using WinBUGS (Spiegelhalter *et al.* 2004), and the WinBUGS scripts for this are available from the author on request.

Sampling from the full posterior distribution of the various model parameters in the Bayesian method is achieved using MCMC simulation (Richardson and Gilks 1993a, b) since no analytic expression for the posterior distribution is available. Posterior samples of the "true" stratum-mean organ dose are assumed to apply for all members of the stratum defined by city, sex, age at exposure group and "nominal" dose group. In particular, they are assumed to be the same over all time periods of follow-up. This approximation neglects the fact that individuals are dying as follow-up proceeds. Given the aggregated nature of the dataset which is available, it would be difficult to adjust for this effect. However, this approximation would not be expected to result in appreciable bias in the estimates of mean organ doses, although it may result in a slight underestimation of their variance in later periods of follow-up and in older age groups.

Generally uninformative prior distributions were assumed for most model parameters. The parameters of the Weibull distribution were assumed to have gamma prior distributions with mean 0.001 and coefficient of variation 100; most other parameters have normal prior distributions with mean 0 and variance 100. Care was taken to check stability and convergence of the posterior MCMC samples, using Gelman-Rubin statistics (Gelman and Rubin 1992) and other graphical techniques. A total of 50,000 samples were taken for leukaemia and solid cancer, after 50,000 samples were discarded in each case to allow the Markov chains to reach their stationary equilibrium distributions. Each set of model parameter values from this sample was used to calculate a measure of population cancer risk, radiation exposure-induced death (REID) (see for example Thomas *et al.* (1992), Little *et al.* (2000) for a definition) for a current UK population. This sample of parameter values is therefore associated with a sample of population cancer risks for a current (2003) UK population (ONS 2004). The distribution of the risk is illustrated graphically in figures 1-2 for the two sorts of model (linear-quadratic, linear-quadratic-exponential).

1.6 Results, Discussion and Conclusions

Figure 1 illustrates the distribution of risk predicted by the optimal linear-quadratic and linear-quadratic-exponential models fitted to the solid cancer mortality data by Bayesian techniques. Using a test dose of 0.1 Sv the mean REID when employing the linear-quadratic-exponential model is 3.3% Sv^{-1} (90% CI -1.3 – 7.0). However, when a higher test dose is used risks increase appreciably for the linear-quadratic-exponential model: REID at 1 Sv is 7.1% Sv^{-1} (90% CI 5.7 – 8.7). The reason for this is that the quadratic coefficient, β, is about four times larger than the linear coefficient, α; the cross-over dose at which the linear and quadratic terms are of equal magnitude is 0.24 Sv. Using a test dose of 0.1 Sv the mean REID employing the linear-quadratic model is 5.4% Sv^{-1} (90% CI 3.1 – 8.0). When a higher test dose, of 1 Sv is used, REID increases to 6.7 Sv^{-1} (90% CI 5.3 – 8.1), a figure very much in line with that predicted by the linear-quadratic-exponential model. The generally lower risks produced by the linear-quadratic-exponential model (at least at low test doses) is perhaps remarkable, and is a result of the incorporation of an exponential cell sterilization term, $\exp[\gamma \cdot D]$, in the dose response. Although the cell sterilization coefficient γ is not statistically significant, its effect on the linear and quadratic coefficients is profound, resulting in the linear term becoming smaller (and generally not statistically significant) and the quadratic term becoming much larger (and generally statistically significant). These effects are also observed in fitting of similar models by maximum likelihood techniques, and very similar central estimates of risk are produced.

Figure 2 illustrates the distribution of risk predicted by the optimal linear-quadratic and linear-quadratic-exponential models fitted to the leukaemia mortality data by Bayesian techniques. Using a test dose of 0.1 Sv the mean REID employing the linear-quadratic-exponential model is 0.19% Sv^{-1} (90% CI -0.27 – 0.81). However, when a higher test dose is used risks increase appreciably: mean REID at 1 Sv is 1.28% Sv^{-1} (90% CI 0.85 – 1.84). The reason for this is that the quadratic coefficient, β, is positive and the linear coefficient, α, negative, and much smaller in absolute value; the cross-over dose at which the linear and quadratic terms are of equal magnitude is about 0.02 Sv. Using a test dose of 0.1 Sv the mean REID employing the linear-quadratic model is 0.58% Sv^{-1} (90% CI 0.13 – 1.15). When a higher test dose, of 1 Sv is used, REID increases to 1.14 Sv^{-1} (90% CI 0.74 – 1.73), a figure very much in line with that predicted by the linear-quadratic-exponential model. The slightly lower risks (at least at low test doses) produced by the linear-quadratic-exponential model is perhaps remarkable, and is a result of the incorporation of an exponential cell sterilization term, $\exp[\gamma \cdot D]$, in the dose response. As for solid cancers, although the cell sterilization coefficient γ is not statistically significant, its effect on the linear and quadratic coefficients is profound, resulting in the linear

term becoming smaller, indeed negative (and generally not statistically significantly different from 0) and the quadratic term becoming much larger (and generally statistically significant). These effects are also observed in fitting of similar models by maximum likelihood techniques, and very similar central estimates of risk are produced.

1.7 Acknowledgements

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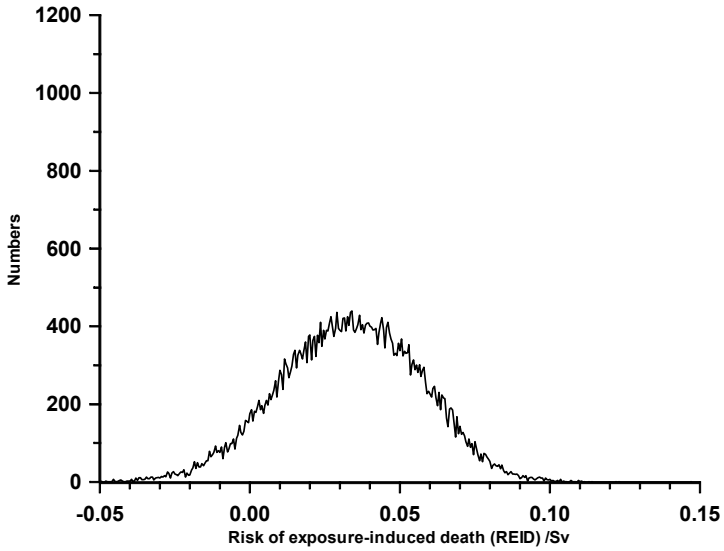
Figure 1. Solid cancer risk of exposure-induced death (REID) distribution for UK population, assuming a test dose of 0.1 Sv, using generalized linear-quadratic and linear-quadratic-exponential ERR models fitted by Bayesian MCMC. Risks are calculated for a population in equilibrium (mortality rates and population structure of current (2003) UK population) from various models fitted to LSS mortality data (Preston *et al.* 2004), assuming 35% GSD errors.

Linear-quadratic-exponential



Linear-quadratic

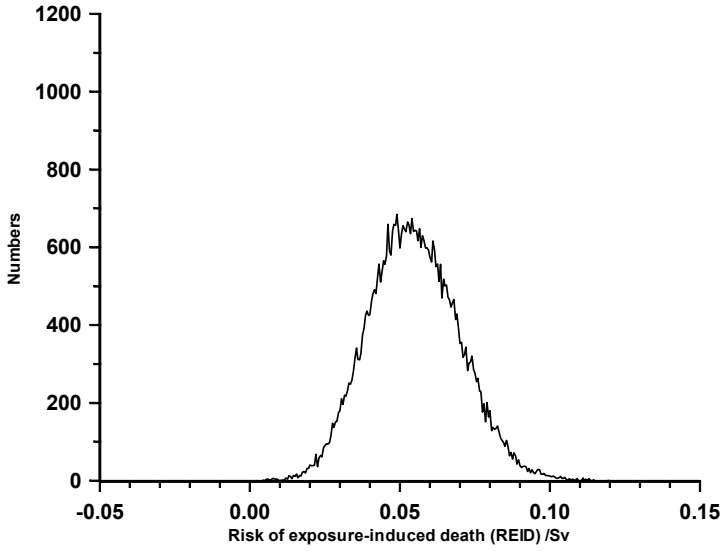
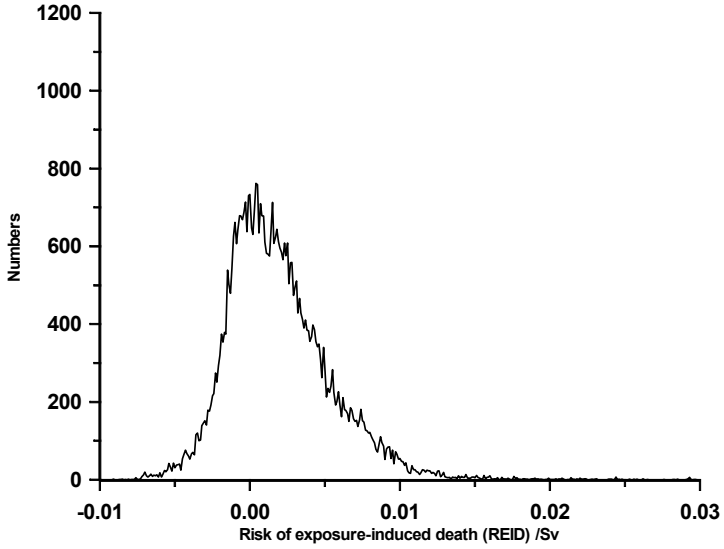
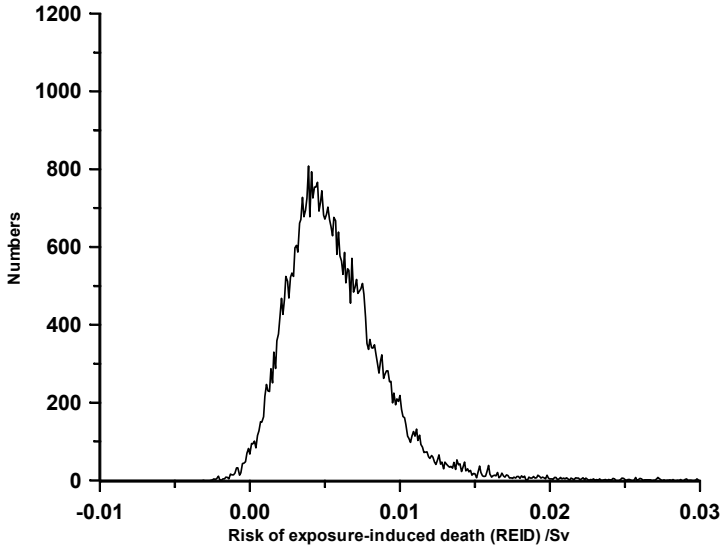


Figure 2. Leukaemia risk of exposure-induced death (REID) distribution for various current populations, assuming a test dose of 0.1 Sv, using generalized linear-quadratic and linear-quadratic-exponential ERR models fitted by Bayesian MCMC. Risks are calculated for a population in equilibrium (mortality rates and population structure of current (2003) UK population) from various models fitted to LSS mortality data (Preston *et al.* 2004), assuming 35% GSD errors.

Linear-quadratic-exponential



Linear-quadratic



2 NEW EPIDEMIOLOGICAL DATA ON EFFECTS OF LOW DOSES OF RADIATION

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2.1 Abstract

The health effects of low doses of radiation have been the subjects of controversy for decades. Current radiation protection recommendations are mainly based on risk estimates from studies of populations with relatively high doses received at high dose rates, together with extrapolation models to predict risks associated with lower dose protracted or fractionated exposures. Recently, the US National Academy of Sciences BEIR VII committee has reviewed the epidemiologic and experimental evidence concerning low doses of low-LET radiation. It concluded that the risk would continue in a linear fashion at lower doses without a threshold and that the smallest dose has the potential to cause a small increase in risk to humans. Risk models were developed by BEIR VII for estimation of the effects of low doses and low dose rates such as those related to natural background radiation. For this, the committee derived a LSS-DDREF (Life-Span Study Dose and Dose Rate Effectiveness Factor) of 1.5 (with a credibility interval of 1.1 –2.3) to extrapolate solid cancer risk estimates derived from analyses of atomic bomb survivors data in the dose-range 0 to 1.5 Gy. This DDREF was not intended to be a universal DDREF value: different values would have been derived if the Committee had considered a different dose-range or a different population.

Two recent large-scale epidemiological studies provide direct information the effects of low dose, protracted exposures: the “15-Country study of cancer risk among radiation workers in the nuclear industry” and the Techa river cohort study. Both studies suggest the existence of a small risk of cancer following low doses of radiation. The risk estimates for solid cancers from both studies are higher than, but statistically compatible with, the linear extrapolations from a-bomb survivors. Both studies, however, have limitations: in the 15-country study, the exact magnitude of smoking confounding is not clear and it is difficult to evaluate the risk estimates one would obtain in the absence of confounding. In the Techa River study, errors in the dose estimates may have led to overestimation of the risk. It is therefore premature to draw any conclusion from the results about the adequacy of the DDREF values used by current radiation protection authorities to extrapolate from high dose rate studies.

Evidence that some individuals are more sensitive to radiation than others is increasing. While the effects of age at exposure have been well documented, a number of recent studies also suggest that variants in DNA repair and DNA damage recognition genes may increase the susceptibility to radiation-induced cancer. These findings, if confirmed, may have important implications for radiation protection, particularly in medicine.

2.2 Introduction

Current radiation protection recommendations are mainly based on risk estimates from studies of populations with relatively high doses received at high dose rates, together with extrapolation models to predict risks associated with lower dose protracted or fractionated exposures. These extrapolation models have been the subject of controversy for several decades.

2.3 BEIR VII and the Effects of Low Doses and Low Dose-Rates

Recently, the US National Academy of Sciences BEIR VII Committee conducted a critical review of the epidemiologic and experimental evidence concerning low doses of low-LET radiation. It concluded that the risk would continue in a linear fashion at lower doses without a threshold and that (although there is uncertainty in the exact magnitude of the effect) the smallest dose has the potential to cause a small increase in risk to humans[1].

Risk models were developed by BEIR VII for estimation of the effects of low doses and low dose-rates, such as those related to natural background radiation. The models use a DDREF (Dose and Dose Rate Effectiveness Factor) of 1.5, with a credibility interval ranging from 1.1 to 2.3, to extrapolate solid cancer risk estimates derived from studies atomic bomb survivors. This DDREF value, called the LSS-DDREF, was derived from a Bayesian analysis of the data on atomic bomb survivors who received doses below 1.5 Gy using a prior (i.e. an external estimate of the DDREF) derived from analyses of relevant data in mice. It should be noted that:

- the BEIR VII LSS-DDREF is not intended to be a universal DDREF factor, but rather the Committee's best estimate of the DDREF that should be applied to solid cancer risk estimates from current A-bomb data in the dose-range 0 to 1.5 Gy to estimate the risk following low-dose, low-dose rate exposures;
- different DDREF values would have been derived if the Committee had considered a different dose-range or a different population.

2.4 Information from Recent Epidemiological Studies

Direct information on the effects of low dose protracted exposures to ionising radiation can in principle be derived from epidemiological studies of populations with such exposures. For such studies to be informative, however, they must fulfil a number of important criteria. They should include observation of very large populations followed up over many years. The follow-up must be non-differential (i.e. not only restricted to persons who are ill or who have received high doses), virtually complete and the information on outcome (diagnosis) should be accurate. Precise and accurate individual dose/exposure level estimates must also be available for all persons in the study[2]. To emphasize the importance of these criteria, it is useful to note that the study of A-bomb survivors in Japan, which is the main basis for radiation protection recommendations today, includes about 86,000 subjects; to date, about 10,000 survivors have died from cancer and about 500 of these deaths are estimated to be attributable to their radiation exposure[3]. Thus, if a careful follow-up of this population, with individual estimation of radiation dose had not been conducted, and if the mortality of the residents of Hiroshima and Nagasaki prefecture had simply been compared to that of surrounding prefectures, it is unclear whether any increase in solid cancer mortality would have been observed … yet this is the population which, today, gives us the most information about radiation risks (including effects of age at exposure, gender, time since exposure and modifying effects of smoking).

As radiation protection today is generally concerned with lower doses, received at much lower dose-rates, even larger populations, with careful follow-up and individual dose estimations are needed to evaluate such effects.

A population of particular interest for the direct estimation of the effects of low-dose, low-dose rate protracted exposures to low LET ionising radiation is that of nuclear industry workers. This is because large numbers of workers have been employed by this industry

since its beginning in the early to mid 1940's (over 1 million workers in the world), because these populations are relatively stable and because, by law, individual real time monitoring of potentially exposed personnel has been carried out in most countries with the use of personal dosimeters (at least for external higher energy exposures) and the measurements have been kept.

2.5 Results of Recent Low-Dose Studies

Results of an international collaborative study, the "15-Country study of radiation workers in the nuclear industry" have recently been published[4;5]. Because the effects being studied were expected to be small, a priori, much effort went into assessing and ensuring comparability, including a common core protocol, agreed procedures, a detailed study of errors in dose estimates. A total of 400,000 workers were included in the analyses, 24,000 (5.9%) were known to have died during the study periods, 6,800 from cancer. The total duration of follow-up was about 5.2 million person-years and the total collective recorded dose (unlagged) 7,900 Sv. Most of the study population were men (90%) and they received 98% of the collective recorded dose. The overall average cumulative recorded dose was 19.4 mSv; the cohort specific average cumulative dose ranged from 3.8 mSv in CEA-COGEMA (France) to 62.3 mSv in Switzerland.
Table 1 shows the results of this study for mortality from all cancers excluding leukaemia, solid cancers and leukaemia excluding chronic lymphocytic leukaemia (CLL). The excess relative risk (ERR) for all cancers excluding leukaemia was elevated, at 0.97 per Sv, (95% confidence interval (CI) 0.14-1.97). This is higher than, but statistically compatible with, the corresponding estimate for male adult atomic bomb survivors (Table 1). For leukaemia other than chronic lymphocytic, the ERR was 1.93 per Sv with a very wide 95% confidence interval (<0 to 8.47); it was not statistically significantly different from 0. The estimate was intermediate between the linear and the linear quadratic extrapolation from the atomic bomb survivors (Table 1).

Analyses of smoking and non-smoking related causes of death indicate that, although smoking may play a role in the increased risk of all cancers excluding leukaemia, it is unlikely to explain all of the increased risk observed in the 15-country study. The study therefore suggests that there is a small increase in cancer risk even at the low doses and dose-rates typically received by nuclear workers in this study. Without further information on tobacco smoking, however, it is difficult to estimate the likely magnitude of the effect. The risk estimates from this study are consistent with those on which current radiation protection standards are based.

Another population of interest to estimate directly the effects of low-dose protracted exposures is that of the persons living along the Techa River in the Urals. In 1948, the Mayak Industrial Association started operations, producing and separating plutonium. Waste from the plant was released into the river and the population was also exposed to accidental and gaseous releases in the period 1950-1960. Exposure was both external from photons and internal due to incorporation of radionuclides (^{89}Sr & ^{90}Sr, ^{137}Cs, ^{103}Ru & ^{106}Ru, ^{95}Zr & ^{95}Nb). An epidemiological follow of a cohort of approximately 30,000 individuals born before 1.1.1950 and resident in any of the 41 exposed villages along the Techa River in 1950–1960 – the expanded Techa River Cohort – is underway.

This cohort is particularly interesting because it is an unselected population (it is composed of the inhabitants of the villages along the river) with a long period of follow-up (over 50 years). Exposure of the subjects is protracted and resulted both from external and internal radiation. The population also includes subjects with different ethnic backgrounds, and thus is potentially important to improve our knowledge of radiation risks.

Radiation doses to the subjects varied, with some subjects receiving doses of the order of 1.5 to 2 Gy to the large intestine and to the bone marrow; the mean doses are low however, although higher than those in the nuclear workers study, of the order of about 30 mGy to the stomach and 300 mGy to the red bone marrow in the most recent dosimetric system[6].

A recent paper[7] has considered solid cancer mortality in the Techa River cohort. It included 1,842 cancer deaths (1,796 expected – estimated excess: 46 deaths or 2.5%). The estimated ERR/Gy, based on the TRDS-2000 dosimetry system, was 0.92 (95% CI 0.2; 1.7) (Table 1), higher than, but compatible with, the comparable estimate from the atomic bomb survivors study. There was some indication of modification of risk by age at cancer mortality, ethnicity, and age at entry into the cohort. There was little evidence that departure from linearity in the dose-response. Analysis of leukaemia mortality in a case-control study nested within the Techa River cohort, yielded an ERR/Gy of 4.6 (95% CI 1.7, 12.3), based on 60 cases and 300 controls [8]. Again, this estimate is higher than, but statistically compatible with, the estimates from the atomic bomb survivors' study (Table 1) and there was no evidence for departure from linearity of the dose response.

There are a number of concerns about the Techa River dosimetry, however[6;9] and several authors have suggested that the estimated doses may be too low and hence the risk estimates too high. Current consensus is that dose estimates for Sr-90 and Cs-137 are reasonable. The role of short-lived isotopes in internal and external doses is currently under review: as there was no systematic monitoring before July 1951, there may be errors in the estimated amount of activity released and in the radionuclide composition.

In summary, both the nuclear workers and the Techa River studies suggest the existence of a small risk of cancer following low doses of radiation. The risk estimates from both studies are higher than linear extrapolations from a-bomb survivors. Both studies, however, have limitations: in the 15-country study, the exact magnitude of smoking confounding is not clear and it is difficult to evaluate the risk estimates one would obtain in the absence of confounding. In the Techa River study, errors in the dose estimates may have led to overestimation of the risk. The risk estimates from both of these bodies are compatible with extrapolation is from atomic bomb survivors that these and it is premature to draw any conclusion from the results about the adequacy of the DDREF values used by current radiation protection authorities to extrapolate from high dose rate studies.

2.6 Are Some of Us More Sensitive than Others?

Effects of age and gender

It is difficult to evaluate the effects of age at exposure and gender in the studies mentioned above. In the nuclear workers study at, the exposed workers were mainly men exposed as adults. In the Techa River cohort, there was some suggestion of an effect, although the solid cancer ERR appeared to increase with increasing age at first exposure (p=0.08).

Most of the evidence about the effects of age at exposure comes from studies of atomic bomb survivors, of medically exposed cohorts (including studies of children exposed *in utero*), and from studies of thyroid cancer following exposure to I-131 from Chernobyl[1]. These studies provide strong evidence that the risk of radiation induced cancer is greatest following exposures in childhood and adolescence.

Gene-radiation interactions

Associations have been reported between inherited breast cancer and pathogenic alleles in ten different genes involved in pathways critical for genomic integrity. BRCA1 and BRCA2

mutations confer very high risks of breast and ovarian cancer[10]. p53 and PTEN mutations lead to very high BC risks associated with rare cancer syndromes. Mutations in CHEK2, ATM, NBS1, RAD50, BRIP1, and PALB2 are associated with at least a doubling of BC risk. Because many of these genes are specifically implicated in the response to ionising radiation, women who carry pathogenic alleles in these genes might be more sensitive to radiation-induced BC than non-carriers. This hypothesis is supported by a number of recent studies [11-14]; methodological issues currently limit the conclusions that can be drawn from these studies, however[15]. The GENE-RAD-RISK project, a large European study, is currently underway to evaluate whether carriers of pathogenic alleles in DNA repair and damage recognition genes may confer an increased risk of breast cancer following medical irradiation[16]. If this project confirms the existence of an increased risk, this will have important implications for the protection of breast cancer patients and their close relatives. In particular, mutations carriers may wish to consider alternatives to X-ray for diagnostic purposes and the need for tailored cancer treatment strategies in carriers should also be evaluated carefully.

A study of meningioma risk in the tinea capitis study in Israel also suggests familial clustering of radiation induced meningioma[17]. Genetic analyses will be important to confirm these findings and identify the gene(s) involved.

As more mechanistic information on genetic susceptibility becomes available, the practice of risk assessment and the basis for radiation protection may need to be reconsidered.

2.7 Effects of Very Low Doses

Although there is no epidemiological evidence at present, there are arguments suggesting that a small risk exists even at very low doses. These include the observation of an increased risk of childhood cancer following *in utero* exposures to 6-10 mGy[18], a range of doses where cells in the exposed tissues see about one photon on average. Using a biophysical argument, if the dose is decreased, this will result in fewer electron tracks and proportionately fewer cells hit by a photon. Those cells that are hit, however, will be subject to the same types of electron damage, and will be subject to the same radiobiological processes[19]. Therefore, although the magnitude of risk uncertain, even the smallest dose has potential to increase the risk.

2.8 Conclusions

Careful recent studies of populations with low-dose protracted exposures suggest the existence of a small risk of cancer following the doses of radiation. The risk estimates are higher than, but statistically compatible with, the linear extrapolations from a-bomb survivors. These studies have limitations, however, and the exact magnitude of the ERR/Gy cannot be determined at present. No conclusion can be drawn therefore concerning the adequacy of the DDREF values currently used by radiation protection organisations.

Evidence that some individuals are more sensitive to radiation than others is increasing. While the effects of age at exposure have been well documented, a number of recent studies also suggest that variants in DNA repair and DNA damage recognition genes may increase the susceptibility to radiation-induced cancer. These findings, if confirmed, may have important implications for radiation protection, particularly in medicine.

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Table 1 – ERR Estimates per Sv for all cancers excluding leukaemia, solid cancers and for leukaemia excluding CLL; comparison of risk estimates: 15-country nuclear workers study, Techa river study and A-bomb survivors study (adapted from[4;7;8])[1]

	15-Country Study		Techa River cohort		Atomic bomb survivors *(men exposed between the ages of 20 and 60)*	
	N	ERR/Sv (95% CI)	N	ERR/Sv (95% CI)	N	ERR/Sv (95% CI)
All cancers excluding leukemia	5 024	0.97 (0.14-1.97)				
Solid cancers	4 770	0.87 (0.03-1.88)	1 842	0.92[2] (0.2, 1.7)	3 246	0.32[3] (0.01, 0.50)
Leukemia excluding CLL						
Linear model	196	1.93 (<0[4], 8.47)	60	4.6[5] (1.7, 12.3)	83	3.15[6] (1.58, 5.67)
Linear quadratic model						1.54[7] (-1.14, 5.33)

[1] Note: colon dose used for all cancers and solid cancer analyses, bone marrow dose for leukemia in nuclear workers and a-bomb survivors studies; stomach and marrow doses, respectively for the Techa river cohort.
[2] Based on TRDS2000 dose estimates.
[3] Analyses carried-out at IARC using an excess relative risk model that allows for age at exposure modification, adjusting for attained age, calendar period, and city. Estimate for men exposed at age 35[3]
[4] Estimate on boundary of parameter space.
[5] Based on TRDS96 dose estimates.
[6] Analyses carried-out at IARC using a constant excess relative risk model, adjusting for attained age, calendar period, and city.
[7] Analyses carried out at IARC - linear term of the linear-quadratic model – preferred model for describing leukemia mortality in analyses of A-bomb survivor data [20].

3 LARGE SCALE INDOOR RADON STUDIES: LUNG CANCER AND LEUKAEMIA RISK

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Radon-222 is a naturally occurring radioactive gas, and arises from decay chain of Uranium-238 present in the earth crust. If inhaled, radon is exhaled rapidly, but its short lived decay products may be deposited at bronchial epithelium level and cells be exposed to alpha radiation. This gas and its decay products may be inhaled by inhabitants in their homes.

Its concentration in the air is depending on the habits of life of the inhabitants, and its origin is mainly the underground soil, where the house is built on. This soil may be more or less rich of uranium, or radium, and the penetration of the radon gas is depending of many factors, like porosity of the soil, climate conditions, and isolation of the cellar of the house.

Its carcinogenic effect has been recognized since 1986, by IARC (WHO), based on the results of experimental data on animals, and the results of epidemiological studies having demonstrated an excess of lung cancer in cohorts of uranium miners. These results were observed mainly on miners having experienced in the past high individual exposure to radon decay products.

Assessment of the cancer risk linked to low exposures, protracted over life, needs either extrapolation of the risk coefficient from the high to the low exposed populations, with different hypotheses on the effects of low protracted alpha exposure on the lung or direct in field studies on the population concerned.

During the last twenty years, a large number of epidemiological studies have been launched both in the field of occupational exposure and on general population. Two types of studies have been used: analytical or ecological studies.

For lung cancer risk, only analytical studies have been evaluated, as they are the only ones that may demonstrate a dose-response relationship based on individual information of exposure and disease. Major results come from a large number of national case-control studies. All studies have registered individual information on smoking habits, and have measured radon in the different houses occupied by each person (case or control) during the 25-30 years preceding the date of diagnosis of the disease of the case. A joint analysis has been realized on all the European data during several European programs (under FP4 and FP5). During FP6, a large project has been launched, focusing on the major populations exposed to alpha emitters. In this project, called Alpha-risk, one work package is dedicated to the joint analysis of all available data from European studies of uranium miners, and a second one is realising pooling of all data related to lung cancer and domestic radon exposure, from European, North American and Chinese studies with the aim to estimate as precisely as possible the effect of radon decay products on lung cancer risk, in absence or presence of tobacco smoking and other possible co-factors. Calculation of the organ dose, taking in account all pollutants of the mining environment is another part of this program. Finally, a synthesis of the available results and corresponding methodology for risk assessment of radon risk in general population is another objective of this European collaboration.

Leukaemia risk has been studied both in adults and in childhood, through ecological and analytical studies. Results of the follow-up of uranium miners will be necessary for assessing any dose-response relationship, taking in account not only radon exposure, but also external gamma exposure and probably other chemicals that may be present under precise mining conditions. Childhood leukaemia has been studied either on geographical level, or through case-control studies.

3.1 Lung Cancer Risk

Results of a case-control study of indoor radon and lung cancer in France:
(in Epidemiology 2004, 15, 6)

It is as multi-centre study launched in parallel with other studies in Germany, England,…all of them were based on the same protocol.

In France the study has been conducted in four radon prone areas: Bretagne, Limousin, Auvergne, Languedoc-Roussillon and interviewing was conducted in 10 hospitals

Final results are based on:
- 486 Cases (diagnosed with lung cancer)
- 984 Controls (free of respiratory disease at time of hospitalisation)
- Paired by sex, age, and hospital

Risk factors studied**:**
- two measurements of radon concentration (6 months duration of measurements) were realized in each house occupied during the last 30 years: total exposure was estimated for the last twenty five years, considering a minimum latency period of 5 years
- Questionnaire focused also on other risk factors (occupational exposures, detailed smoking history, previous medical history, socio-educational status…).

Statistical analysis (based on logistic regression analysis and a log linear model) demonstrated that lung cancer risk increases with exposure to radon:

RR (relative risk) = 1.04 per 100 Bq.m-3 CI 95% = [0.99 – 1.11],
after adjustment on age, sex, region, smoking and occupational exposure.

This risk coefficient is increasing if only those cases and controls are retained, for whom all the houses over the whole period of 25 years have been measured**:**

RR = 1.07 per 100 Bq.m-3 CI 95% = [1.00 – 1.15]

Conclusion: This risk is low when compared to the risk associated to smoking, but it is persisting after precise adjustment on smoking. The risk is increasing with cumulated exposure over the last twenty years. This result is concordant with those from previously published studies and with the risk extrapolated from miners studies. See joint figure, based also on French uranium miner studies with following assumptions: 25 WLM in mines is equivalent to 230 Bq/m³ per year cumulated over 25 years. As the RR from French miner studies is close to 1.008 per WLM, the corresponding equivalent risk is 1.09 per 100 Bq/m³ . This value, calculated through rough approximation, is nevertheless very close to the observed results from our case control study.

The **joint European analysis** made it possible to analyse the radon risk on a total of 7148 cases and 14208 controls. Analysis of these data has been published by Darby et al in *BMJ.2005;330:223-7*

- Results show a clear linear dose-response relationship : Lung cancer risk is increasing with cumulated radon exposure : **RR = 1.08 for 100 Bq/m³** CI 95% = [1.03 – 1.16]
- There is a significant relationship even if limited to those exposed =< 200 Bq/m³
- There is also a significant increase in the non-smokers population
- For smokers, precise stratification on smoking habits increases the value of the risk coefficient linked to radon
- There is no evidence that the effect of radon differs between studies
- Influence analysis : analysis repeated by omitting each study in turn (ref Darby et al, *Scan J Work Environ Health 2006, vol32 suppl 1*) showed that the estimated linear relationship (after stratification by study, age, sex, region of residence and smoking history) changed by less than 10% for 11 of the 13 studies
- There is no significant difference, when considering lung cancer diagnosis over clinical versus death certificates, study with hospital or population based studies, whether or not surrogates interviews were used and radon measurements detectors were open or closed.

Conclusion: results of this large pooled studies are convincing, they have used best information of major cofactor, tobacco smoking, and demonstrated a clear linear dose-response relationship between radon in houses and lung cancer risk. In parallel, Krewski and al have published major studies from North America (*Krewski et al. Epidemiology 2005 and J Toxicology Environ Health, 2006):* Their results confirm those observed on European data.

3.2 Leukaemia Risk

A review on this subject had been realized by Laurier and al in 2001: it concluded that there was no clear evidence for an association between radon exposure and leukaemia.

During the last 5 years, we have to mention three studies concerning leukaemia risk after exposure as an adult; these results come from uranium miners studies of the Czech Republic and of Germany:

- A retrospective case-cohort study was published by Rericha and al in *Env Health Perspectives 2006 114 (6),* it is an incidence study, with 84 leukaemia cases (including 53 chronic lymphatic leukaemia CLL cases)
  The author considers that a leukaemia risk may be associated with cumulative radon exposure. But considering that CLL risk may associated with cumulative radon exposure raises some question, as up to now most publications consider that CLL is not related to radiation.
- In the Czech uranium miners cohort study Tomasek and al published in *IRPA-Europe proceedings 2006* a mortality study, notifying 30 leukaemia deaths: risk increased with duration of work, but the risk was not significantly associated with cumulative radon exposure. Calculation of equivalent RBM dose of the U miners indicates that long lived radionuclides contributes for more than 60% of the total dose, radon contributing for less than 10%; the risk of leukaemia was associated with the cumulated equivalent RBM dose.
- It is not evident if the data of the two studies are two independent sets of data or if some of the leukaemia cases may be present in both studies.

- Another case-control study has been published by Möhner and al in *Am J Ind.Med. 2006, 49 (4)* based on 377 leukaemia cases and 980 controls coming from the former uranium miners cohort of East Germany. This incidence study showed an elevated risk for employees with a very long duration of work; but in contrast to the previous paper, calculation of equivalent RBM dose indicates that radon contributes for more than 75% to the total dose. No association was found with exposure to short-lived radon decay products.

Conclusion**:** the Uranium miners studies give some indication of a leukaemia risk in those having experienced a long duration of work, but more information is needed before any conclusion can be drawn on a relationship between a red bone marrow dose attributable to domestic radon and leukaemia risk.

Radon measurements have been realized in France for 13 000 dwellings; in parallel a register of childhood leukaemia has been installed: it regroups all the cases registered from 1990-2001: a PhD has considered the possible correlation between different components of natural radioactivity and childhood leukaemia risk: major results were published by AS Evrard in *Health Physics*; *2006,* 90(6) *and in Eur J Cancer Prev. 2005 14(2)*

- A significant positive association between indoor radon and AML (acute myeloid leukaemia) incidence was observed, it remained significant in a multivariate analysis, including either terrestrial gamma dose or total gamma dose.
- Association with AML seems limited to those less then 14 years old.
- After adjustment on rural areas, proportion of managers, proportion of university graduates, average net income : the association between radon and childhood leukaemia persists , the standardized incidence ratio SIR is multiplied by 1.20 for 100 Bq/m³ increase

In 2002, the large UK Childhood Cancer Study published in *Br J Cancer. 2002 5;86 (11)* showed no evidence of increased risk between domestic radon exposure and childhood leukaemia.

These two studies, with different results continue to pose the problem of a possible small risk of leukaemia linked to natural background radiation

- In the UK study, radon concentrations considered were measured close to time of diagnosis and the houses of controls had intrinsic features resulting in higher than average indoor radon concentrations.

  If radiation risk estimates considered by COMARE, the Committee of Medical Aspects of Radiation in Environment, suggests that approximately 14% of leukaemia incidence in childhood in UK may be linked to natural background radiation, what is the power of a case-control study to demonstrate clearly this excess? The number of cases in high exposed regions may be small? What are the cofactors we have to adjust for? For childhood leukaemia most of them are still unknown.

Conclusion: we have not yet enough evidence of a link between childhood leukaemia and indoor radon, but this subject should be studied in future in order to learn more on all possible risk factors in relation to childhood leukaemia.

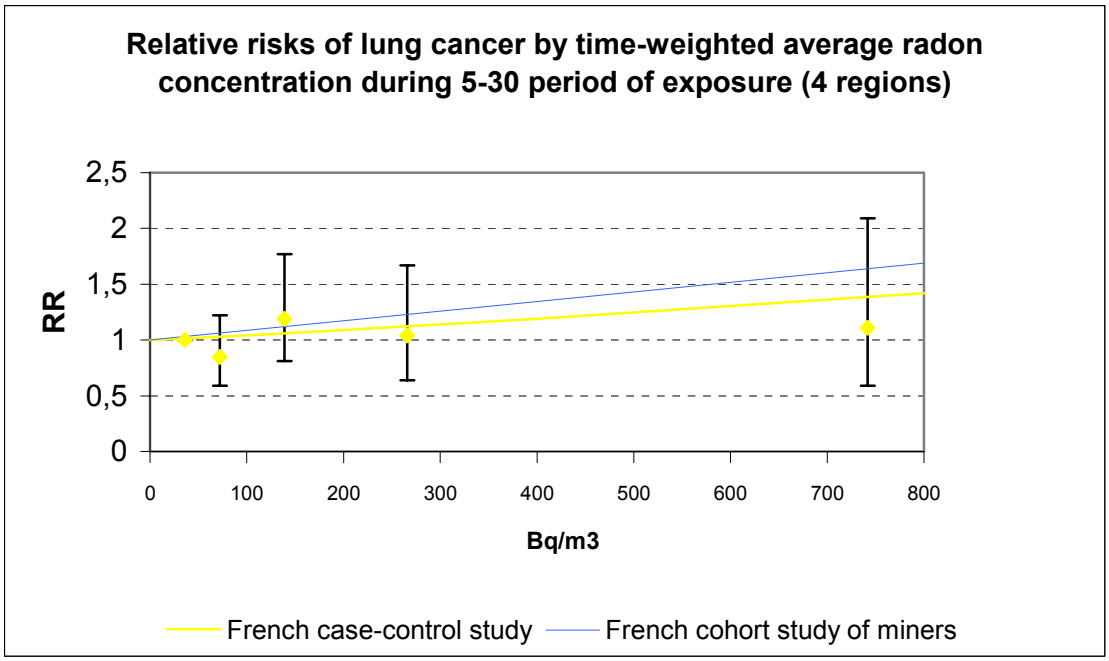

Fig 1 : comparing lung cancer risk based on French case-control studies in homes and cohort study of French uranium miners.

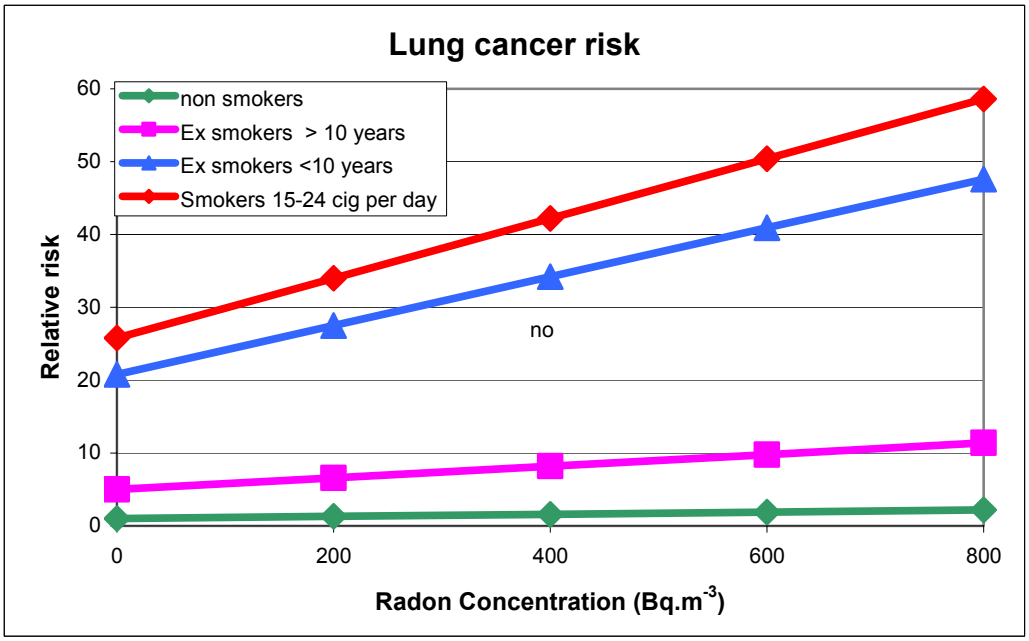

Fig 2 : lung cancer risk in relation to radon in homes, for non-smokers and different groups of smokers : from Darby and al *Scan J Work Environ Health 2006, vol32 suppl 1.*

4 BIOLOGICAL ASPECTS IN RELATION TO AGE/GENDER SENSITIVITIES AND DISCUSSION OF POTENTIAL IMPLICATIONS

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4.1 Introduction

Epidemiology tells us that there are differences in radiosensitivity among different age groups and among women and men. These differences must have a biological basis. In the following some of the factors and mechanisms responsible for age and gender differences as to radiosensitivity will be introduced. This list is, presumably, not exhaustive, but may be looked at as a starting point of experimental studies addressing the age and gender problem in radiation protection.

4.2 Examples where Specific Age Groups Show a Higher Radiosensitivity Compared to Middle-Aged Adults

There are many epidemiological studies that point to an age dependence of radiation risk. Some examples of specific age groups showing a higher risk to acquire a specific tumor after radiation exposure are mentioned in the following list:

- Foetus: leukaemia [1;2]
- Young children: thyroid carcinoma [3]
- Children and teens: basal cell carcinoma [4]
- Girls during puberty: breast cancer [5]
- Women under 30 years: breast cancer [6;7]
- Individuals older than 50 years: lung cancer [8;9]A specific example of age

dependence is shown in Fig. 1. Clearly, radiation risk for basal cell carcinoma diminishes with age. Similar dependences have been observed for other cancers as well.

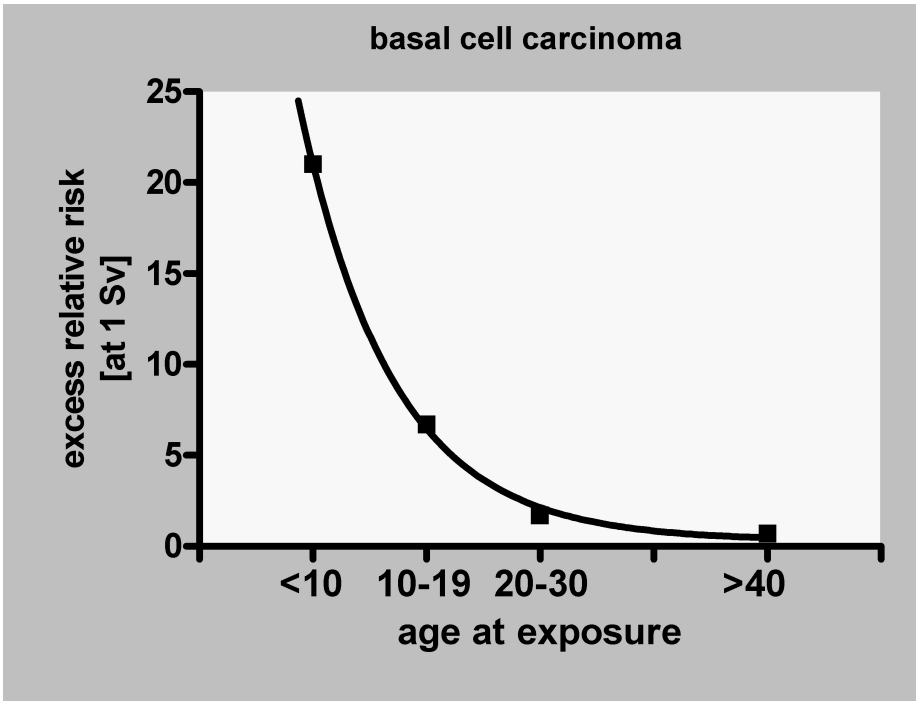

Fig. 1: Specific example of age dependence: basal cell carcinoma

(Data source: [4], p. 422)

An interesting and as yet not completely understood example of age dependence, however, is the observation that radiation induced lung cancer risk seems to increase at an age of around 50 years [9]. This indicates that age dependence might not be as simple as: young individuals = radiosensitive; old individuals = radioresistent. Taking into consideration the many biological mechanisms that are involved and which will be discussed in this paper, such an observation is not as surprising as it might look at a first glance.

4.3 Examples Where Higher Radiation Risks Have Been Shown for Women Compared to Men

Meanwhile, it is well known that women have a higher relative radiation risk to acquire a tumor than man. This is not only true for the age at exposure (Fig. 2), but also for the attained age (Fig. 3). In addition the higher sensitivity does not only depend on the high sensitivity of the female breast, but is observed for almost all cancer entities (Fig. 4).

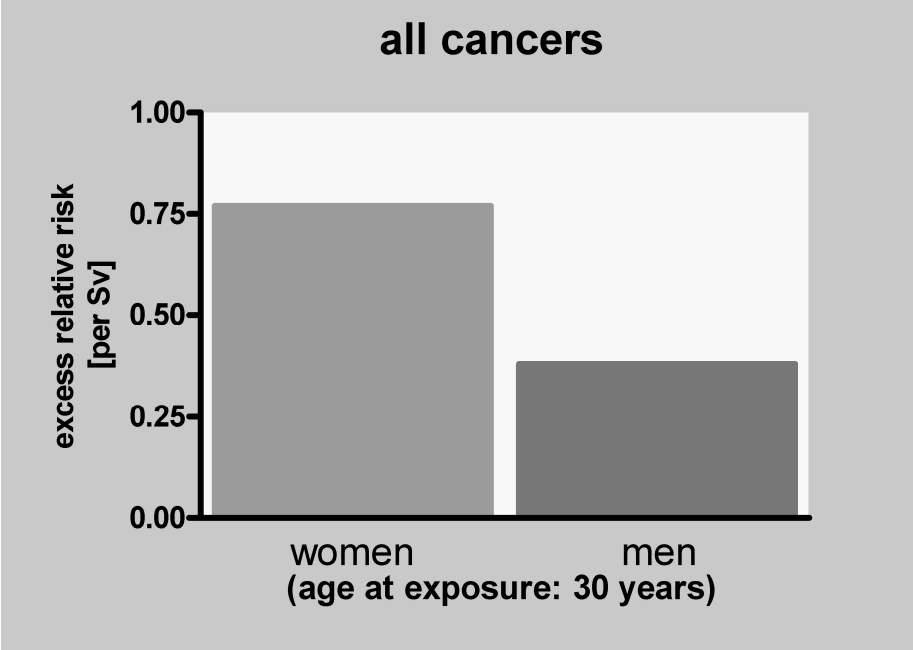

Fig. 2: Difference in relative risk for all cancers between women and men (age at exposure = 30 years)

(Data source: [4], p. 425)

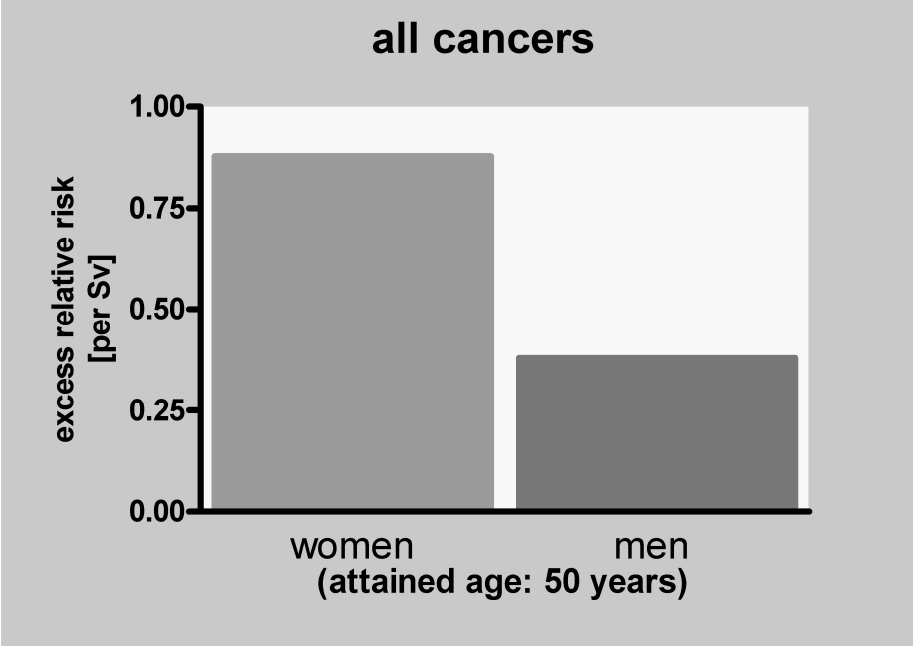

Fig. 3: Difference in relative risk for all cancers between women and men (attained age = 50 years)

(Data source: [4], p. 425)

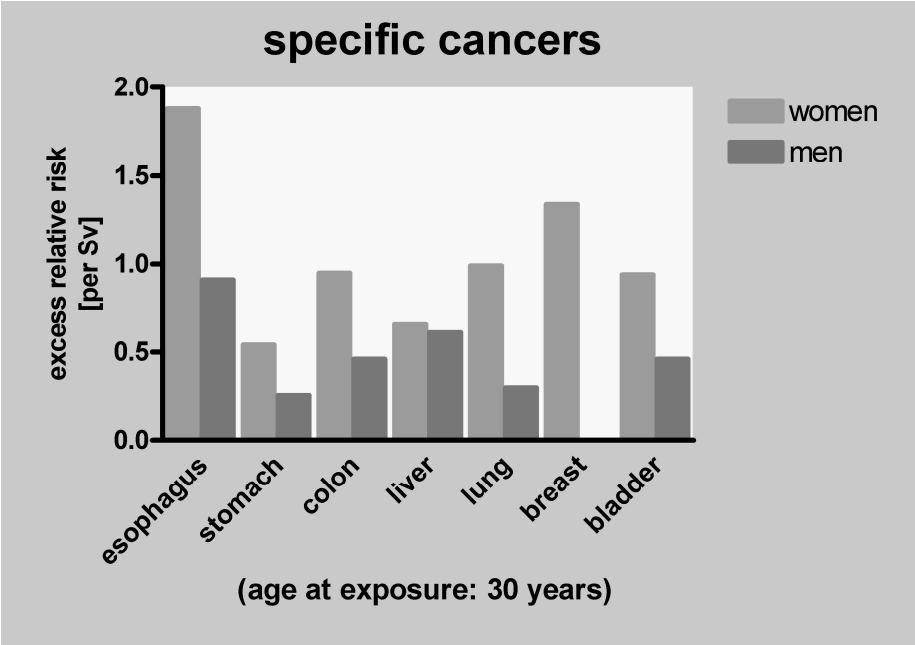

Fig. 4: Difference in radiation-induced tumor risk for various tumor entities between women and men

(Data source: [4], Annex I, p. 425)

One must keep in mind, however, that the spontaneous frequency of cancers is lower in women compared to men, so that the absolute risk is similar between women and men.

4.4 Still a Helpful Rule: Bergonié and Tribondeau (1906)

According to Bergonié and Tribondeau [10] those tissues are most radiosensitive with regard to cell death whose cells divide rapidly and whose cells are more or less undifferentiated. An important exception has to be kept in mind: lymphocytes. Although they usually do not divide in the peripheral blood and although they are highly differentiated, they belong to the most radiosensitive cells of the body. No explanation for this observation has been found till now. The rule of Bergonié and Tribondeau helps to assess deterministic effects, but not stochastic ones!

4.5 Some Known Biological Factors that Determine Radiosensitivity

The following list gives an impression on the many biological mechanisms that play a role in individual radiosensitivity. This list is in alphabetical order, thus avoiding any subjective preference as to the importance of a specific mechanism.

- **Adaptive response**

  Describes the observation that in some cases a low “adapting” radiation dose reduces the effects of a high “challenging” dose; many experiments show that this is not a universal phenomenon.

- **Apoptosis**

  In each single cell a program is implemented that forces, when triggered, the cell to die without provoking an inflammatory response. This mechanism allows the tissue to get

rid of potentially dangerous cells, like transformed cells that might be the origin of a tumor.

- **Bystander effect**

  Many experiments demonstrate that a cell may show radiation effects without being exposed to ionizing radiation directly. This can be demonstrated either by hitting a specific cell and proving radiation effects in neighbouring cells or by radiation exposure of a cell culture and transfer of the medium of this culture to unexposed cells.

- **Cell-cycle checkpoints**

  At various positions of the cell-cycle specific proteins check, whether all the prerequisites for a successful execution of the next cell-cycle phase is fulfilled. Only after establishing that this is the case, the cell is allowed to enter the next stage. Serious problems can emerge, when such a controlling protein is modified due to a mutation in the corresponding gene.

- **Cell proliferation**

  Cell proliferation is important in the development of tumors. In addition, pronounced cell proliferation increases radiosensitivity with respect to cell death (see chapter 4.4).

- **Cell migration**

  Cell migration is a frequent process during embryonic and foetal development and known for its high sensitivity in the context of radiation exposure.

- **Cell differentiation**

  (See chapter 4.4)

- **Enzyme status**

  There are pronounced differences in the enzyme status at various ages. A well-known example is lactase, an enzyme that is necessary for the degradation of lactose; practically all children have this enzyme and can thus consume milk without problems, whereas about one third of all adults lack this enzyme and run into problems when drinking milk. Similar situations might exist with regard to enzymes that are important after radiation exposure.

- **Genetic predisposition**

  Tumor formation is a multistep process. Thus, several modifications within the genome are necessary. A person who already shows four of, let’s say five necessary steps on the way to a tumor, has a higher probability to develop a tumor after radiation exposure than an individual with cells that show only two of the five steps required. This is described by the term “genetic predisposition”.

- **Genomic instability**

  The stability of the genome after exposure to potentially hazardous agents is different from individual to individual. An increase in genomic instability can be induced by, among others, radiation exposure.

- **Hormone status**

  Many hormones stimulate cell proliferation. This is one of the mechanisms how they can increase radiation risk. Thus, a high fraction of proliferation-stimulating hormones has a marked impact on radiosensitivity.

- **Immune competence**

  The immune system takes care that transformed cells are eliminated from the tissues of the human body. This protection is not perfect and the failure rate is increased, when the immune system is compromised to some extent, due to, for example, diseases or age.

- **Oxygen supply**

  Cells with a very low oxygen concentration, so-called hypoxic cells, are by a factor of 3 more radioresistent than fully oxygenated (euoxic) cells.

- **Repair characteristics**

  DNA repair mechanisms are crucial for unimpaired survival of cells after radiation exposure. There is, however, always the danger that errors occur in the course of the repair process. The efficiency of repair seems to be age-related.

Right now, this list is more or less a reminder of what to take into consideration, when it comes to the question of how to assess individual radiosensitivity. Unfortunately, we lack quantitative measurements with regard to all of these mechanisms, a problem that prevents any conclusions on the final outcome of the interplay of these processes.

The factors just mentioned do not only affect individual radiosensitivity, but they are also responsible for differences in radiosensitivities among groups like:

- embryo/foetus – children – teens – young adults – elderly
- women – men,

because there are systematic differences among these groups with regard to the factors and mechanisms just mentioned.

There are, however, additional factors that are important. Some of these factors are not directly related to radiosensitivity, but cause different doses to younger compared to older ages, although the conditions of exposure are the same (see, for example, “organ mass” or “sensitive organs” in the following list).

- **Persistence of embryonic cells in children**

  In some cases, embryonic cells with a high potential of proliferation persist in children giving rise to tumors; radiation risk of these children may be increased by the presence of such cells.

- **Differences in anatomy:**

  - **Distribution of bone marrow**

    The distribution of bone marrow is different between children and adults; this may play a role in leukaemia induction.

  - **Organ mass**

    Organ mass is critical in case of incorporation of radionuclides that accumulate in specific organs. With very few exceptions, organ masses are less in children than in adults resulting in higher doses after incorporation of the same activity.

  - **Sensitive organs that are closer to frequently exposed body regions**

    This applies, for example, to the thyroid and the ovaries which are closer to frequently examined body regions in medical radiation diagnostics.

- **Differences in physiology:**
  - **Growth processes**

    This is the reason why Sr-90 accumulation is higher in young children than in adults, because Sr-90 is accumulated in bone and bones are growing extensively in young children whereas growth of bones is low in adults.
  - **Metabolic turnover**

    Metabolic turnover is, in general, higher in children than in adults. This has various implications which are not always harmful to children. Excretion rate of certain radionuclides, for example, might be higher in some cases in children.
  - **Breathing rate**

    Air pollution with radionuclides has different effects on children and adults due to differences in breathing rate.
- **Time available for expression of damage**

  Solid tumors, in particular, show latency periods in the range of several decades. Thus, the probability to live long enough for manifestation of a tumor is much greater for young people than for old ones.
- **Accumulation of genetic damage**

  Although repair processes are working all the time, an increase of genetic damage in the course of time is unavoidable, because not all defects in DNA are recognized by the repair enzymes and some defects are “repaired” incorrectly. A pre-damaged genome is more prone to develop a tumor than an intact one (see, e.g., “genetic predisposition” and “genomic instability” in chapter 4.5). An example of accumulation of genetic damage with age is given in Fig. 5. The number of micronuclei in peripheral lymphocytes is more than double as high in people older than 50 years compared to children below the age of 10.

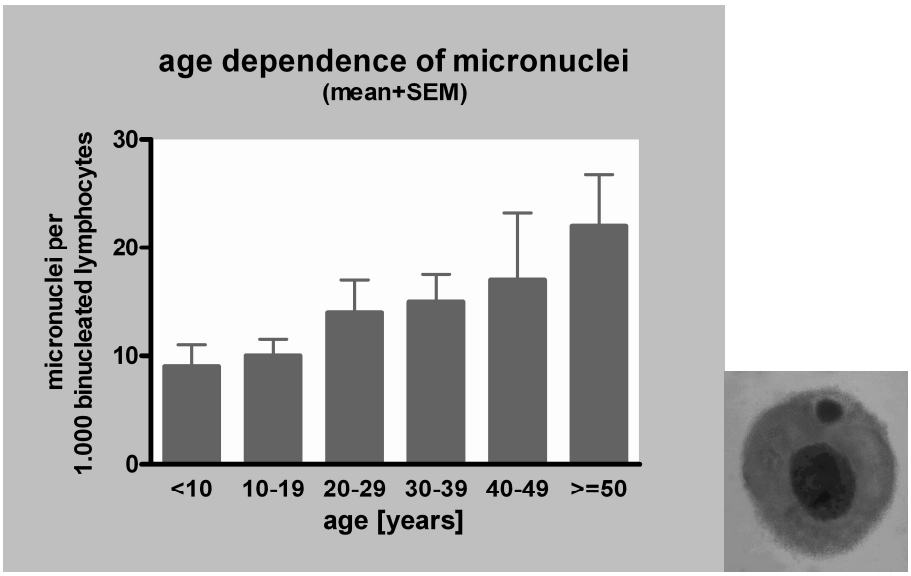

Fig. 5: Increase of genetic damage with increasing age exemplified by the increase in micronucleus frequency with age (a lymphocyte with cell nucleus and micronucleus is shown on the right)

(Source: Own unpublished data)

4.6 It Depends on the Conditions whether a Factor is Harmful or Beneficial!

The high number of mechanisms involved results already in a very complicated situation. An aspect that contributes to an even more complicated picture is quite frequently overlooked: it very much depends on the specific conditions, whether a mechanism is harmful or beneficial. Two examples may illustrate this statement:

- **First example: Cell proliferation in the foetus**
  The harmful aspect in this context is the pronounced radiosensitivity of proliferating cells mainly due to the possibility of hitting a sensitive cell cycle stage. The beneficial aspect, however, relates to the possibility to replace killed cells rapidly due to the high flexibility of foetal cells in certain stages of development. Both aspects differ in the various stages of embryonic/foetal development, with flexibility extraordinarily high in the early stages. Thus, rapid cell proliferation is not always an indicator of an increased radiation risk.**Second example: no repair combined with cell death can be beneficial or disastrous**

  In general, it is believed that repair after radiation exposure is urgently required in order to prevent radiation effects. In the case of mis-repair, however, repair generates problems. There are indications that mis-repair is responsible for a fraction of radiation-induced cancers. Therefore, a more reasonable approach for the organism is, to avoid any repair processes and to send the cells into apoptosis. Those eliminated cells can easily be replaced by cell divisions of neighbouring cells. Quite obviously, this strategy has its limitations, when it comes to higher radiation doses, because in that case too many cells will be lost with a pronounced risk of acute effects for the organism. Thus, no repair can be beneficial or disastrous. It depends on the conditions.

4.7 Biological Mechanisms that Might Be Responsible for Differences in Radiosensitivity at Various Ages

In this chapter an attempt will be made to associate those mechanisms mentioned above with specific age groups. This allocation (Tab. 1) is somewhat subjective and may need adjustments in the future. It should be looked at as a first step in the identification of mechanisms that are responsible for age dependences of radiosensitivity.

When looking at Tab. 1, one has to be very careful with the interpretation. “Cell proliferation”, for example, plays a role at all ages, but its role is different. Whereas in younger ages cell proliferation is high, thus pointing to an increased radiosensitivity, proliferation will be lower at older ages and presumably be accompanied by a decreased radiosensitivity. “Immune competence” is another, very complicated issue. Early in life, full immune competence is not acquired, middle aged healthy individuals show a relatively efficient immune response, whereas older people may have problems with the efficiency of their immune system.

Tab. 1: Biological mechanisms and factors that affect radiosensitivity at various ages

Mechanism/factor	embryo/ foetus	children	teens	adults	elderly
cell proliferation	X	X	X	X	X
cell migration	X	X			
cell differentiation	X	X	X	X	X
persistence of embryonic cells		X			
genetic predisposition	X	X	X	X	
genomic instability			X	X	X
hormone status	X	X	X	X	
enzyme status	X	X	X	X	X
immune competence	X	X	X	X	X
oxygen supply	X	X	X	X	X
repair characteristics	X	X	X	X	X
distribution of bone marrow	X	X			
organ mass	X	X			
growth processes	X	X	X		
metabolic turnover	X	X			
accumulation of genetic damage			X	X	X
time available for expression of damage	X	X	X		

One should always keep in mind: Children are not just young adults! They differ not only with respect to quantities, but some characteristics are basically different. For example, some enzymes only exist in children and are not observed in adults.

4.8 Biological Mechanisms that Might Be Responsible for Differences in Radiosensitivity of Women and Men

There are no systematic quantitative studies addressing the question why women are more radiosensitive with regard to relative tumor risk. At least three mechanisms or factors may play a role:

- **Hormones**

  Young women, in particular, show a hormone pattern that is characterized by a high fraction of proliferation-stimulating hormones. This is understandable in the context of a potential pregnancy. The situation changes between about 30 to 40 years, when proliferation-stimulating hormones become less prominent.

- **Higher cell proliferation in some tissues**

  Uterine tissue, for example, shows pronounced cell proliferation. At a first glance, this aspect seems to be covered already by mentioning hormones in the previous chapter, but hormones are not the only molecules responsible for stimulation of cell proliferation. Wound-healing processes are stimulated by non-hormonal growth factors.

- **Sex-linked proto-oncogenes and tumor-suppressor genes**

  No systematic study of potential differences between women and men as to the pattern of proto-oncogenes and tumor-suppressor genes has been published up to now. But it is very conceivable that such differences exist with the implication that after mutation different oncogenes and mutated tumor-suppressor genes might show up in women and men.

4.9 Future Research Requirements

In the future, it will be necessary to collect as many as possible quantitative information on the factors and mechanisms mentioned above. Subsequently, the use or development of modern information technologies will be required in order to put all the quantitative information together and come up with a final estimate of risk.

4.10 Potential Implications for Radiation Protection, in particular, in relation to the Recommendations Envisaged by ICRP

The current radiation protection system does not take into consideration sufficiently age and gender dependent differences in some contexts. This is understandable against the background of the attempt to keep the regulations simple and pragmatic. Nevertheless, it is worth to re-think the regulations from time to time whether it might be reasonable to improve radiation protection, because it turned out that the rules were too simple to guarantee efficient protection. (The following comments refer to the ICRP draft 02/276/06 - 5 June 2006.)

4.10.1 Problem no. 1:

The concept of the „effective dose“ is not applicable to the embryo/foetus due to the impossibility to generate tissue-weighting factors for the foetus;
and
adult tissue-factors are used for children.
Possible solution for children:
Use of organ doses and dose limits based upon organ doses (and not on effective dose).

4.10.2 Problem no. 2:

The dose constraint of 100 mSv (acute or per year) in interventions. This constraint also applies to sensitive subgroups like embryo/foetus and children.
Possible solution:
Markedly lower dose constraint for sensitive subgroups.

4.10.3 Problem no. 3:

Medical exposure; in paragraph 81 ICRP states: „...in utero exposure should not be a specific protection case in prolonged exposure situations where the dose is well below about 100 mSv“. Such a statement may make some physicians very careless.
Possible solution:
Avoidance of such a statement.

4.10.4 Problem no. 4:

Averaging of female and male risk.
Possible solution:
Stick to the pure scientific mandate and leave social decisions (like the gender question) to the policy makers.

4.11 References

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5 NEW BIOLOGICAL DATA IN RELATION WITH LOW DOSE RISK

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5.1 Introduction

Human beings are exposed during life time only to low dose ionizing radiation (IR), with the exception of radiation therapy or radiation accidents. Thus, the evaluation of risks for human health at low dose and low dose rates constitutes an important issue in radiation protection. The deterministic and stochastic effects of high dose and high dose rate IR exposures and the corresponding risks for human health are fairly well established, whereas the effects of low doses and chronic exposures are still a matter of debate.

In most cases, epidemiological studies do not have sufficient statistical power to determine risks from very low dose and low dose rate exposures. Therefore, fundamental mechanistic studies are essential to understand biological short and long term effects and to help evaluating risks at those low exposure levels.

Actual regulations concerning such low exposure levels are based on the linear no- threshold hypothesis (LNT) assuming that there is a linear relationship between IR exposure and the risk for human health (Brenner DJ et al. 2003, Tubiana M 2003, Tubiana et al. 2006a,b).

However, recent research developments and in particular, molecular approaches have lead to new findings that put into question some radiobiological paradigms and concepts and thus the basis of LNT. This has become especially evident from recent reports concerning radiation protection such as the Report of the French National Academies of Science and Medicine 2005 (Tubiana et al. 2005, 2006a,b), the BEIR VII report 2006 (BEIR VII 2006) and the ICRP recommendations 2006 (ICRP 2006a,b).

Recently evolving new facts concern the interaction of IR with living matter, radiation-induced lesions, the signalling and repair of radiation damage in relation to the genotoxic consequences, i.e. the induction of mutation and cancer as well as general phenomena such as low dose radiation hypersensitivity, adaptive responses, the bystander effect and genetic instability. Most of these cellular findings specifically modify previous views on low dose and low dose-rate effects.
The present review outlines what we got to know recently, what we still like to know of low IR dose and low IR dose rate effects, and the possible consequences for radiation protection.

5.2 Interaction of Ionizing Radiation (IR) with Living Matter

In recent years some new findings have alerted radiation biologists:

High LET- and low LET ionizing radiation may give rise to quite deleterious lesions including locally multiply damaged sites (LMDS) in DNA (Ward 1988, Goodhead 1994; Nikjoo H. et al. 2001) and may involve processes such as K-shell activation by low and high LET IR (Fayard

et al. 2002, Boissière et al. 2004, Gobert et al.2004) and even the action of very low energy electrons (Boudaiffa et al. 2000, Huels et al. 2003). Indeed, using synchroton irradiation, an increase by a factor of > 2 in lethal events, chromosome aberrations, and DSB could be observed in Chinese hamster V79 cells with photons of ultrasoft X irradiation of 350 eV in comparison to 250 eV, which could be explained by an activation of inner shell activation of carbon atoms giving rise to secondary and AUGER electrons (Boissière et al. 2004). The contribution of K shell activation appeared to be relatively low but significant (10%) for low LET, but high (nearly 100%) for high LET radiation (heavy ions) (Fayard et al. 2002). The phenomenon of K shell activation also could explain the "paradox of high LET radiation", i.e. the abrupt change in biological efficiency of high energy particles at high energies (Chetioui et al. 1994).

Furthermore, low energy electrons (4 to 6 eV) have been shown to be able to induce single and double-strand breaks in plasmid DNA, apparently due to dissociative attachment of these electrons by generation and loss of molecular resonances of anions in DNA. At higher electronic energies (15 to 100eV) due to local excitation, dissociations or dissociative ionizations of DNA components in groups of multiple DSBs (« multiple strand break » clusters) were observed (Huels et al. 2003). Since IR exposures are expected to induce about ten-fold more electrons of energies below 15eV than above it could be possible that such effects of low energy electrons are also relevant at the cellular level (Huels et al. 2003).

5.3 Endogenously versus IR-Induced DNA Lesions

Knowing that mutations, cellular transformation and cancer can originate from IR induced DNA damage, much effort has been put in recent years on the characterization and identification of lesions in DNA and specifically those induced by IR (see Cadet et al. 2004).

Endogenously, due to cellular (oxidative) metabolism, one finds already fairly high amounts of modified DNA bases and single strand breaks in DNA. For instance, in the steady state the order of one base modification per 106 nucleotides has been found in cell and organ extracts considering most abundant lesions such as 8-oxoguanine and abasic sites (AP)(De Bont R et al. 2004) which correspond to approximately 10000 per human genome per day and about 6000 AP sites in the steady state (De Bont R et al. 2004, Barnes DE and Lindahl T 2004). Although during DNA replication, mitosis and meiosis, many double strand breaks may arise, these are generally enzymatically induced and of transient nature, and just very few (8) are estimated to be induced per genome per day (Burkart et al. 1998). Furthermore, metabolically induced lesions are expected to be single lesions and thus relatively more easily to be taken care of by cellular DNA repair systems.

In contrast to what is known for endogenously induced lesions, IR induces a large variety of lesions in DNA including base modifications, single-strand breaks (SSB) , intra-strand and inter-strand DNA crosslinks, DNA-protein crosslinks and complex and clustered lesions such as double-strand breaks (DSBs) and locally multiply damaged sites (LMDS). The latter have been predicted from biophysical model (Monte Carlo) calculations (Goodhead 1994, Nikjoo et al. 2001) and are defined as lesions formed within one or two helical turns of the DNA molecule at the end of a single radiation track. (Ward 1988, 1995). They are thought to consist of closely spaced SSBs together with base damages or of complex DSBs. As said in the BEIR VII report 'LMDS (clustered damage) may be viewed as complex lesions associated with IR and not with endogenous oxidative processes. If they are refractory to repair, the risk to humans posed by IR may be viewed as greater than that posed by endogenous oxidative stress.' (BEIR VII 2006). Because LMDS may thus represent specific IR-induced DNA damages which, as DSBs, are expected as being highly deleterious for cells, many recent studies have focussed on the detection and characterization of this type of clustered damage. Basically three approaches have been used. One consists of treating the

DNA from IR-irradiated cells with specific DNA glycosylases such as Fpg and endonucleases such as Nth or Nfo in order to introduce selective cuts at sites of oxydatively damaged DNA bases and thus transforming the sites of single SSBs present in the same LMDS in opposite DNA strands into DSBs which are detectable by conventional pulsed-field gel electrophoresis (Sutherland et al. 2000). Another consists of constructing artificial oligonucleotides containing closely spaced lesions such as defined oxidatively damaged bases, abasic sites etc. together with SSBs in the opposite DNA strand in order to test their reparability in vitro using by DNA repair enzymes or cell extracts (David-Cordonnier et al. 2000, Harrison et Malyarchuk 2002, Eot-Houllier et al. 2005, Budworth et al. 2005). The third approach used bacterial and mammalian cells in order to determine the effects of under – or overexpression of glycosylases and/or endonucleases on IR-induced survival and mutagenesis (Blaisdell and Wallace 2001, Yang et al. 2004, 2006).

Using the first approach, considerable amounts of clustered lesions (up to 3-4 fold higher yields than DSBs were found after high and even after low LET-radiation (Sutherland et al. 2000, 2001 2002) and even in unirradiated viral and human DNAs (Sutherland et al 2003). However, a recent set of experiments demonstrated in irradiated mammalian cells that the apparent level of LMDS after low and high LET irradiation was reduced close to background levels after low and high LET-radiation when avoiding artificial oxidation of DNA during lysis and DNA extraction from the irradiated cells and there was no dose dependence (Boucher et al. 2006). The second approach showed that indeed in vitro DNA repair can be compromised and may show lethal and mutagenic potentials depending on the position and type of oxidative base damage close to DNA single strand breaks in opposite DNA strands (Harrison et al. 1999, David-Cordonnier et al. 2002, Eot-Houllier 2005, Dianov et al. 2006). The third approach showed that in irradiated cells abortive repair events associated with increased lethality may occur due to the activity of glycosylases or endonucleases producing additional DSBs (Blaisdell and Wallace 2001, Yang et al. 2004, 2006; Pearson et al. 2004). Overexpression of glycosylases in a human lymphoblastoid TK6 cell line led to increased DSBs induction which apparently correlated to increased sensitivity to IR-induced cytotoxicity and mutagenicity (Yang et al.2004) although mutagenicity was not compared at the same survival levels. Nevertheless, using this last approach in a subsequent study, overexpression of enzymes involved in the excision of oxidative base damage resulted in higher yields of DSBs and lethality thus providing some evidence for the existence of LMDS in living cells exposed to IR (Yang et al. 2006). At the present state, it is difficult to distinguish in such experiments between repair attempts on lesions located in one or two helical turns of DNA (IR-specific LMDS) or the interference of the repair of more widely spaced lesions interfering one or another with the repair of others. Interference of the presence of different types of lesions with the repair of one another has been observed previously for bulky photochemically induced lesions (Papadopoulo et al. 1988).

From this, it appears that IR-induced LMDS may well exist. However, if so, the methods actually available for their detection are at present inadequate and need to be refined. The available data from irradiated cells (Yang et al. 2006) suggest that LMDS exert mostly lethal and if at all, relatively low mutagenic effects. LMDS are somewhat very similar to complex DSBs which are refractory to DNA repair and mostly lethal (Löbrich et al. 2000).

At the present state of our knowledge, LMDS can thus not be taken as IR-specific and the most relevant lesions for radiation-induced mutagenesis and carcinogenesis as previously suggested in the BEIR VII report (BEIR VII 2006) and the recent ICRP Committee I Task Group report (ICRP Recommend. 2005, ICRP Publ. 99, 2006).

LMDS are thus unlikely to contribute significantly to mutagenic and carcinogenic risks of IR for humans.

5.4 Importance of Cellular Signalling in Cellular IR-Responses Involving Cell Cycle Arrest, DNA Repair and Apoptosis

In recent years, molecular analysis of cellular responses has shown that IR activates distinct cellular signalling pathways involving genes and gene products (proteins) which, if mutated, may predispose to cancer in humans. Cellular IR impacts are recognized by sensor proteins capable of transmitting signals from damaged membranes, cytoplasm and nuclear DNA. IR induces damage to all cellular compartments. Apart from direct effects it induces free radicals such as reactive oxygen species which generate radical mediated reactions in all cellular compartments including membranes, cytoplasm, mitochondria and nucleus. Membrane damage activates a cascade of protein network signalling involving MAP kinase directed pathways leading to the activation of transcription factors such as NF-κB and the induction of genes (Yang J et al. 2003, Dent et al. 2003).

Damage to DNA activates signalling cascade involving phosphoinositidyl 3kinases such as ATM (which is mutated in the human syndrome ataxia telangiectasia) and ATR (ATM and RAD3-related) leading to cell cycle arrest, DNA repair or apoptosis (Abraham 2001, 2003, Sancar 2004). In fact, IR induced DSBs are first recognized by the MRE11/RAD50/NBS1 complex, the 53BP1 (p53 binding) protein, some replication proteins (Rfc, Rpa,TopBP1), the helicase BLM (deficient in the human Bloom's syndrome) and the BRCA1 protein (if mutated, it causes predisposition to breast cancer). The DSB signals are rapidly transmitted by transducers to effector proteins (Shiloh Y 2003, Bakkenist and Kastan 2003,2004). A dose of 500 mGy of gamma irradiation (producing 18 DSB per cell) induces within 5 minutes an intermolecular autophosphorylation of serine 1981 of the pre-existing ATM protein in the form of dimers or multimers. Thus, active ATM molecules are liberated which migrate to DSB sites and bind to them. Also, other proteins such as p53 (the guardian of the genome) controlling cell cycle and apoptosis are activated. Already during the first minutes after IR, ATM phosphorylates (at serine 139) the histone H2AX, an important component of chromatin and visible foci of γ-H2AX (detectable by immunofluorescence using specific antibodies) are formed corresponding to DSB sites (Rogakou EP et al. 1998, Sedelnikova OA et al. 2002, Celeste A et al. 2003, Rothkamm and Löbrich 2003).

5.5 Dose Dependence of DSB Signalling and DNA Repair

The recent discovery that γ-H2AX (phosphorylated H2AX protein) can be taken as an indicator for the formation of DSBs was used by Rothkamm and Löbrich (2003) to examine the induction and repair of DSBs in human fibroblasts at very low IR doses (Rothkamm and Löbrich 2003). Combining the detection of DSBs by PFGE with that by γ-H2AX formation, they were able to show that with both methods, DSBs are induced linearly with dose in the range from 1.2 mGy up to 2 Gy. This provided clear evidence that DSBs can be precisely measured at very low doses approaching doses of diagnostic X-ray machines and annual natural background radiation levels. The method is so sensitive that it allowed the detection of DSBs and their repair in lymphocytes from patients which underwent a CT scan (at doses < 20 mGy) (Löbrich et al. 2005).

A major finding of this work is the observation that the number of γ-H2AX foci, i.e. number of DSB induced in human fibroblasts by the very low dose of 1.2 mGy, remains stable for several days (Rothkamm and Löbrich 2003). In fact, the DSBs induced at that dose level were not subject to repair and apparently the few damaged cells in the population just died off. From this, it has been concluded that there is complete absence of DSB repair at this very low dose. Interestingly, at slightly higher doses (5 mGy) DNA repair took place although

with somewhat slow kinetics, whereas at again higher doses (20 mGy) repair of DSBs was very efficient. It is thus evident that the activation of DNA damage signalling and the repair of DSBs is highly dependent on the dose level of IR: at very low dose (1 mGy) there is no initiation of repair and cells are going to die (probably because of the absence of proper DNA repair signalling). At slightly higher doses (5-20 mGy) DNA repair is clearly initiated (Rothkamm and Löbrich 2003). According to BEIR VII a dose of 5 mGy corresponds to approximately 1 electron track per cell resulting in 5- 10 damaged bases, 2.5-5 SSBs and 0.25 DSBs (BEIR report VII, 2006). It is likely that at higher doses DNA repair (which may be error-prone and mutagenic) and/or apoptosis are fully activated.

Although γ-H2AX foci can be induced in many other experimental conditions (stalled replication forks, after UV-irradiation, inhibitors of topoisomerases, etc.) the validity of this method for the detection of low levels of DSBs at low doses or dose rates of IR has been confirmed in other, independent studies (Collis et al. 2004a, Löbrich et al. 2005, Bouquet et al. 2005).

These experimental data obtained at the cellular level clearly show that the physical impacts of IR leading to the induction of DSBs are linear with dose. However, direct extrapolations from high dose to very low dose biological responses do not correspond to the actual reactions observed in living cells after IR exposure.

These findings are likely to have important consequences at tissue levels. Mammalian cells are usually embedded in tissues. Thus, at very low IR doses, when just a few cells damaged by IR are unable to survive and are eliminated, important tissue functions are not yet compromised. At higher doses, however, a substantial fraction of cells is damaged and normal tissue functions cannot be assured. Thus, there is a certain need to repair damaged cells even as mutated cells so that tissue functions can be fulfilled even if the presence of mutations may initiate genomic instability, malignant cell transformation and cancer.

5.6 Dose-Rate Effects on Cell Survival and the Induction of DSBS in Mammalian Cells

Dose-rate effects of ionizing radiation and the effects of pro-tracted exposures in mammalian cells have been documented since 1963 (Bedford and Hall 1963, Bedford and Mitchell 1973). Although the underlying mechanisms are not yet fully understood, low dose-rate exposure usually results in higher cell survival than high dose-rate exposure and this phenomenon appears to be highly dependent on DNA repair (Price 1993, Dhermain et al. 1995, Dikomey and Brammer 2000, Boucher et al. 2004). In fact, it has been shown that the increased survival after low dose rate exposures involves efficient DSB repair by non homologous end-joining (NHEJ) (Dhermain et al. 1995, Dikomey and Brammer 2000, Boucher et al. 2004). Curiously, inverse dose-rate effects have been observed at certain chronic exposure levels (Brenner DJ et al. 1996) and the probability for mutation induction may vary strikingly with dose rate (Vilenchik and Knudson 2000). Recent work provided evidence for the involvement of DSB signalling in very low dose rate effects (Collis et al. 2004a). Taking the formation of γ-H2AX foci as indicator for IR-induced DSBs, these authors convincingly showed that at very low dose-rate of 1.5 mGy/min DSBs are recognized by detector proteins such as MRE11-RAD50-NBS1 (the MRN complex) but they are not repaired because of lack of activation by ATM . The absence of repair leads to decreased survival, in other words, very low dose chronic IR may cause more cell killing than that estimated from extrapolation from higher dose-rate exposures. Activation of DSB repair was found absent at the very low dose rate of 1.5 mGy/min but fully present at higher dose rate (4.15 mGy/min) and at high dose-rate (750 mGy/min). The results clearly demonstrate that signalling of DNA induced

damage (DSBs) and DNA repair is highly dependent on dose-rate. Furthermore, these data suggest the existence of a threshold for ATM dependent signalling towards DNA repair.

This clearly questions the general application of the DDREF value of 2 for high dose rate exposures proposed by ICRP (ICRP Publ.99, 2006).
Also, a recent paper by Elmore et al. 2006 indicates that the DDREF is very dependent on dose rate and approaches at very low doses and dose rates infinity taking neoplastic transformation *in vitro* as relevant biological endpoint.

5.7 Differences in Gene Induction and Protein Activation depending on IR Dose and Dose-Rate

Recent analysis of transcriptional responses and protein activation following IR have provided new and very informative insights into cellular radiation responses. In yeast (*Saccharomyces cerevisiae*) some gene families were induced by IR (rays) at very low dose rates (0.1 to 20 mGy/h), whereas the induction of genotoxic effects required much higher dose rates (100 mGy/h) (Mercier et al. 2004). In mammalian cells, different gene families were found induced at different levels of radiation dose and dose-rate (Bishay et al. 2001, Amundson et al. 2003, Franco N et al. 2005). An IR dose-rate-dependent induction was observed for the genes p21 and GADD45 A involved in cell cycle arrest as well as for the UV radiation responsive XPC gene, whereas induction of the ERCC1/XPF gene involved in nucleotide excision repair was independent of dose-rate (Amundson et al. 2003). What this induction really means in terms of IR-responses is however not yet clear. It seems possible that this is part of an adaptive response.

Furthermore, it has been demonstrated in cultured primary keratinocytes that a low dose (20 mGy) elicits the induction of different gene sets than a high dose (2 Gy) of gamma-irradiation (Franco N et al. 2005). Among 10500 gene probes tested on microarrays, 853 were modulated by IR and, respectively, the expression of 214 and 370 genes, was specifically modulated by low dose (10 mGy) and high dose (2Gy) exposure. In addition, the kinetics of modulation differed for low and high dose responsive genes with maximum expression at 48 and 72 hours or already at 3h, respectively. The low dose response (140 known genes) concerned mostly genes of homeostasis, cell communication, signalling, membrane, cytoskeleton, RNA and protein synthesis, chromatin, energy metabolism, stress, cell death and transport but rarely DNA repair genes.

As stated above, IR exposures cause activation of important effector proteins via the phosphorylation by specific kinases. A recent analysis of the phosphoproteomic profiles of human fibroblasts after low and high dose X-irradiation revealed that at a high dose (4 Gy) important effector proteins are phosphorylated that are involved in cell cycle checkpoint control, DNA damage signalling, DNA repair and apoptosis, whereas a low dose (2 mGy) activates proteins involved in global metabolism (such as a cyclin dependent kinase) but not specific genotoxicity-related proteins. (Yang F et al. 2005). Thus, low and high dose IR exposures give rise to different phosphoproteomic profiles.

Thus, the radiation response at low dose appears to be clearly different from that obtained at high dose.

As in the case of radioadaptation, it is likely that gene induction corresponds to the mobilisation of cellular maintenance and defence systems in order to better cope with forthcoming exposures and genotoxic stress (Mitchell SA et al. 2004).

5.8 Role of DNA Repair Systems in the Maintenance of Genomic Integrity

During evolution efficient DNA repair pathways have been developed in order to cope with endogenously or exogenously (IR) induced DNA damage and to assure maintenance of genomic integrity and survival of living organisms (Hoeijmakers 2001, Christmann et al. 2003, Sancar 2004). Most of the repair systems are error- free and can reconstitute the original DNA sequences. However, some are error–prone and produce cellular mutations that may initiate the development of cancer. Apart from simple enzymatic repair activities such as the direct reversion of damage, for example, the direct ligation of 3' OH-ended DSBs by DNA ligase I, demethylation of methylated bases by methyltransferase, reversion of ultraviolet light-induced dimers by photolyase, there are five main multienzymatic repair systems which are commonly in charge of most endogenously or exogenously induced lesions: (1) mismatch repair (MMR) deals with DNA replication errors (1×10^{-8}), (2) base excision repair (BER) with modified (oxidized) bases or single strand breaks (SSB), (3) nucleotide excision repair (NER) with the repair of damaged nucleotides (bulky lesions and adducts), (4) homologous recombination (HR) with the repair of DSBs during S/G2 phase and (5) non homologous end-joining (NHEJ) with the repair of DSBs (independent of cell cycle stage). Furthermore, cells may call upon polymerases able to tolerate certain types of DNA lesions and to perform translesional DNA synthesis across lesions definitely fixing them as mutations.

Most repair systems are error free (reversion of SSB, MMR, BER, NER and HR), however, NHEJ includes DSB end trimming which may yield deletions (Collis et al. 2004b). Owing to the complexity of radiation induced damage, DNA repair systems are somewhat overlapping and act in concert. Although even after high doses of gamma rays (80 Gy) DNA repair is generally not yet saturated (Löbrich et al. 2000, Dikomey and Lorenzen 1993), in some instances, local density of IR-induced lesions is likely to prevent correct repair processing of one or the other lesion by different repair systems. At present, little is known on the possible mutual interaction or interference of DNA repair systems working on IR-induced lesions.

The importance of DNA repair systems is shown by the fact that absence of their activation or presence of genetic deficiencies causes individual radiation sensitivity and increased susceptibility to spontaneous cancers and to cancers induced by radiation or other genotoxic agents (Hoeijmakers 2001, Christmann et al. 2003). Several human syndromes of DNA repair defects are known to be associated with cancer predisposition. For example,(1) defects in NER in Xeroderma pigmentosum (XP) patients give rise to predisposition for sun-light induced skin cancers (2) deficiencies in MMR (genes hMLH1, hMSH1) predispose to colon hereditary non polyposis colon cancers (3) deficiencies in damage signalling and repair of DSB in ataxia telangiectasia (ATM), Fanconi's anaemia (FA) or Bloom's syndrome (BLM) give rise to leukaemia or other cancers (4) defects in the repair of DSBs by homologous recombination (BRCA1 and BRCA2 genes) predispose to breast and ovarian cancers (5) deficiency in ligase IV (needed in the repair of DSBs by NHEJ) gives rise to acute leukaemia. Several genes of the NHEJ pathway (genes KU70/80, DNA-PKcs, DNA ligase IV, XRCC4 , Artemis and Cernunnos-XLF) if mutated give rise to immunodeficiency because of defects in V(D)J recombination and immunoglobulin maturation. Defaults in the DNA-PK gene are associated with strong radiation sensitivity and immune deficiency in mice (severe combined immunodeficiency or SCID syndrome). Similarly, mutations in the Artemis or Cernunnos (XLF) gene are associated with severe immunodeficiency in humans (Ma Y 2002, Jeggo and Löbrich 2005, Buck et al. 2006, Revy et al. 2006,Callebaut et al 2006, Ahnesorg et al. 2006). Also, defaults in translesional synthesis (mutation of the polymerase □ gene in XP variant patients) give rise to skin cancer predisposition.

Moreover, deficiencies and mutations in the general regulatory gene p53 (guardian of the genome) are associated with Li-Fraumeni syndrome and in 50% of the cases with various types of cancers.

In addition, polymorphisms of certain DNA repair genes have been found to be associated with tissue overreactivity in radiation therapy, for example, XRCC1 involved in the repair of abasic sites by BER, ATM involved in signalling and repair of DSBs, hHR21 involved in DSB repair, DNA ligase IV involved in NHEJ, FANCC involved in the repair of DSB by homologous recombination (Fernet and Hall 2004). Lymphocytes from workers in nuclear power station carrying a Ser/Cyt polymorphism of the OGG1 gene (involved in the repair of SSB and oxidative damage by BER) showed reduced DNA strand repair after ionizing radiation (Aka P et al. 2004). The list of DNA repair polymorphisms which may affect individual radiation sensitivity and cancer predisposition in the general population is still increasing. However, individual sensitivity is rare and usually not detectable in population studies (epidemiology) (Fernet and Hall 2004). Molecular analysis of specific mutations or polymorphisms may help to clarify the situation in specific cases, for example, when patients after radiodiagnostic (tomographic) analysis or radiotherapeutic treatments are found with decreased DSB repair capacity (Löbrich et al. 2005). XRCC1 and glutathion S transferase gene polymorphisms were found associated with radiotherapy-related malignancies in survivors of Hodgkin disease (Mertens et al. 2004).

5.9 Dose-Effect Relationships in Radiation Biology are Affected by non Targeted and Delayed Effects Involving Low IR Doses

Several radiobiological phenomena have been brought to light which specifically concern low dose IR responses. Among these are the radioadaptive response, the bystander effect and low dose hypersensitivity. Although not yet fully understood, very intriguingly, these phenomena modulate dose-effect relationships preferentially at low doses (or dose-rates).

5.9.1 Adapative responses

Adaptive responses have been shown to reduce DNA damage, mutation induction, chromosomal aberrations, micronuclei and cell transformation (Rigaud and Moustacchi, 1996). They are based on the observation that IR pre-exposure of cells to a low priming dose (between 5 mGy and 200 mGy, usually delivered at a low dose-rate) reduces significantly the damaging effect of high doses delivered several hours later (Wolff 1998). For example, in human fibroblasts an adaptive response on micronuclei production was found after a priming dose of 1 mGy and a 2 Gy challenging dose (Broome et al 2002). Adaptive responses observed in human lymphocytes appear to be quite variable. Occupational exposures of 2.5 mGy/year for up to 21 years resulted in variable adaptive responses in lymphocytes challenged with 2 Gy (Barquinero et al. 1995).

The molecular mechanisms of radioadaptation are not yet well understood. The phenomenon is absent in cells treated with an inhibitor of poly(ADP-ribosyl) transferase (PARP) indicating that repair of single strand breaks by BER may be involved. Also, cell cycle changes (Zhou and Rigaud 2001, Cramers P et al. 2005), low dose IR induced G1-arrest (Seo et al. 2006) as well as induction of antioxydant cellular defences such as MnSOD, catalase, glutathion peroxidase, glutathione-S-transferase induced by the priming dose at low dose rate *in vitro* and *in vivo* may be involved (Bravard et al. 1999, Otsuka K et al. 2006). The adaptive response seems to depend on IR damage signaling (Szumiel 2005) possibly involving some DNA repair activities induced by the first low dose exposure.

In this context, it is of interest that recent data show suppression of neoplastic transformation at low doses (up to 100 mGy) for a variety of low LET radiations and support the notion of a threshold dose for neoplastic transformation *in vitro* by low LET radiation (Ko et al. 2006).

Obviously, the underlying mechanisms are not yet known, however, the existence of these adaptive type of IR responses is not compatible with the LNT model.

5.9.2 Bystander effects

It is an accepted paradigm in radiation biology that the DNA in the nucleus is the critical target in irradiated cells, that DSBs are most critical IR-induced lesions and that this radiation damage will give rise to cytotoxicity, mutations and malignant transformation in the irradiated cells themselves. Recent data show that this is not always the case. One example is the so-called bystander effect where IR effects on single cells influence the responses of adjacent non-irradiated cells in the surrounding population (Little 2000). Two types of bystander effects can be distinguished : signals from IR damaged cells are transmitted to the non-irradiated cells (1) in confluent cell cultures and tissues by signals via cell-to-cell communication and intercellular gap junctions and/or (2) by soluble factors released into the surrounding medium from the irradiated cells. In the first case, cell-to-cell contacts are required, in the second case, the bystander effect is caused by the surrounding medium. The effects are termed « non-targeted » since the « bystander » cells have not received any radiation (Mothersill and Seymour 2004). Radiation effects involve also non-irradiated neighbouring cells. Thus, the notion of target size in radiation effects needs to be reconsidered.

At very low fluences of alpha particles (corresponding to 10 to 20% traversal of cell nuclei), the efficiency of HPRT locus mutation induction in CHO cells increased by a factor of 3 to 4 in comparison to that of cells directly traversed. The nuclei of non directly traversed cells showed 90% of point mutations, whereas directly traversed cells showed mostly deletions due to the induction of complex DNA lesions (Nagasawa et al. 2003). At low doses, the bystander effect resulted thus in a supra-linear response for the induction of mutations. However, this does not seem to be always the case. Following IR exposure, the phenomenon of apoptosis appears to be more frequent than the induction of mutations, and also increased proliferation and protective adaptive effects by bystander-induced differentiation have been reported (Little 2006 and Lyng et al. 2006, Belyakov et al. 2006). A threshold at around 2 mGy of □-irradiation for a medium transfer bystander effect was shown for a human skin cell line (Liu et al. 2006). However, a significant bystander effect was absent after exposures to neutrons in the dose range of 1-33 mGy. At low dose gamma-irradiation (10 mGy) as yet undefined protein factors appeared to be released into the culture medium which reduced cloning efficiency and increased apoptosis in unirradiated cells when exposed to this medium (Mothersill and Seymour 2004a). This should diminish the risk for the induction of genetic instability and cancer.

Also, the repair capacity of the bystander cells may play a role. A larger number of bystander cells were found at risk for the induction of mutations and chromosomal aberrations in NHEJ-deficient than in NHEJ-competent cells (Little 2006). In tissues, the bystander effect involves preferentially intercellular gap junctions which allow the transmission of cell damaging or inflammatory signals. In fact, low LET irradiation induces enhancement of intercellular gap junctions correlated with increased expression of Connexin 43 (Little 2006).

Since bystander effects are mostly seen after low dose exposures in cells outside the actual radiation field and are dependent on the oxygenation and proliferative state of the tissue, they could be well of importance in certain radiotherapeutic protocols.

Thus, IR-induced bystander effects can modify dose-effect relationships in cells and tissues. They may involve membrane modifications and the release of long lived reactive oxygen species and cytokines. This may be beneficial (proliferation) or detrimental (apoptosis) depending on the cell system and the range of doses used.

Non targeted effects such as the bystander effect may also play a possible role in the induction of genomic instability (Seymour and Mothersill 2004a, Morgan 2003a,b) and may be involved in IR-induced transgenerational changes in mutation rates and DNA damage (Barber RC et al. 2006).

As shown here, the actual role of the bystander effect for the induction of cancer and the consequences for risk evaluations at low IR doses and dose-rates are not yet clearly established.

5.9.3 Low dose hypersensitivity

IR-induced low dose hypersensitivity (Joiner et al. 2001, Chalmers et al. 2004, Marples et al. 2004) has been observed in many cell types. It consists of high lethality at a few hundred mGy followed by radioresistance at doses above 500 mGy. It involves poly(ADP-phosphoribosyl)transferase activity (PARP 1), ineffective cell cycle arrest in G2-phase cells and DNA repair.

Apparently, the hypersensitivity response depends on the activity of poly(ADP-ribosyl)polymerase (PARP) and the presence of antioxidants in the irradiated cells. It is suspected that PARP is involved in DNA damage recognition leading to the activation of signaling pathways and the repair via NHEJ in cells arrested in G2 phase after irradiation (Marples et al. 2004). In some cell lines, low dose hypersensitivity appeared to be associated with a reduction in DNA-PK activity which is very important for DSB repair by NHEJ (Vaganay-Juéry et al. 2000). Some data revealed hyper-radiosensitivity for signal transduction (200-500 mGy), mutagenesis and genomic instability (50 mGy) (Schiestl et al. 1994). The low dose hypersensitivity for mutation induction was shown in melanocytes by the disproportional increase (4 fold) in mutation frequency at 10 mGy as compared to that observed at 1 Gy (100 fold).

The adaptive radiation response, bystander effect and low dose hypersensitivity response have some common features. They appear to involve cellular signaling, the production of oxidative reactive species and effects on cell cycle control and DNA repair at low and very low doses.

However, in all three cases the importance of these phenomena for radiation-induced carcinogenesis is not yet understood.

5.10 Conclusions

IR can be distinguished from many other genotoxic insults by the induction of a large variety of lesions in cellular DNA. Among these are rather complex lesions such as DSBs and locally multiply damaged sites (LMDS). In mammalian cells, DSBs are linearly induced with IR dose and are undoubtedly very deleterious lesions. LMDS are predicted to consist of local clusters of different types of lesions but still remain difficult to be properly defined and quantified. There is some evidence that LMDS are high obstacles for repair and thus, are much more lethal than mutagenic. Thus, in contrast to previous expectations (see BEIR VII, ICRP report) conceivably, their impact on radiation-induced mutagenesis and carcinogenesis should be relatively low or even absent.

Recent studies led to the emergence of a new concept concerning high and low dose and dose-rate IR effects. It has been demonstrated that mammalian cells very sensitively react to IR exposure. In response to IR, genes are induced and proteins activated that are, apart from general metabolism, involved in DNA damage signaling, cell cycle control, DNA repair and apoptosis. However, very strikingly, living cells react differently at high and low doses or dose-rates of IR and there are striking differences in low dose and dose rate versus high dose and dose-rate responses. At low doses and dose-rates a multitude of parameters influence the cellular fate and IR responses involve wide ranging metabolic networks, whereas at high doses and dose rates, cellular responses are more directly channelled towards survival, genomic instability and malignant transformation or cell death.

As outlined in the present review, different gene families are induced and different types of phosphoproteins activated at high and low doses or doses-rates. Most strikingly, at very low doses (1 mGy) or dose rates (1.5 mGy/min) of low-LET radiation, DNA damage signaling towards the activation of DNA repair is compromised, and the few IR damaged cells in the population are eliminated by cell death. At higher doses (5 mGy) and dose-rates (4 mGy/min) DNA damage signaling pathways towards cell cycle arrest and DNA repair and/or apoptosis are activated. Owing to these results, a unique DDREF as proposed by ICRP is not applicable.

Obviously, the observed dose and dose-rate dependent cellular radiation responses do not correspond to what is predicted from the LNT hypothesis which solely concerns the physical IR energy deposition and the induction of molecular damage. Furthermore, the results suggest that cells are generally better protected at very low than at high dose levels of IR, and thus, human risks from low dose exposures are likely to be much lower than expected from LNT calculations.

Non-targeted IR effects such as adaptive responses, radiation hypersensitivity, bystander effects and genomic instability, preferentially expressed at very low doses, are likely to influence dose-effect relationships for mutation induction and carcinogenesis of IR, particularly, at low doses and dose-rates. However, the mechanisms involved and their actual quantitative impact need to be clarified. In some cases, they are reported to be protective.

There is evidence for individual radiation sensitivity due to individual defaults in DNA damage signaling and repair. This may indeed have some bearing on individual radiation risk. Because cases of individual radiation sensitivity are rare they are not predictive for IR risks of the general population.

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6 IONIZING RADIATION, GENETIC RISKS AND RADIATION PROTECTION

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6.1 Abstract

This paper provides a brief overview of the concepts, assumptions and method used in the estimation of genetic risks associated with the exposure of human populations to ionizing radiation and presents the estimates published by the United Nations Scientific Committee on the Effects of Atomic radiation (UNSCEAR) in 2001. These risk estimates are of interest since they have recently been used by the International Commission on Radiological Protection (ICRP), in the document on new recommendations (expected to be published in 2007) for deriving genetic risk coefficients to assess the relative contribution of genetic effects to total detriment. A major difference between genetic risk coefficients in this new ICRP document and those used in the ICRP Publication 60 (1991) is the following: in the new document, the estimated genetic risks for the first two post-radiation generations have been used for the calculations whereas in Publication 60, the 5-fold larger predicted risks at equilibrium (discussed in the 1988 UNSCEAR report) constituted the basis. The implication of this conceptual change in the new document is that the relative contribution of genetic effects to the total detriment is now much lower than was assumed previously. The reasons for this conceptual change are discussed.

6.2 Introduction

The discovery that exposure to x-rays could cause transmissible genetic effects was made in 1927 by the American geneticist, H. J. Muller (Muller, 1927). Muller used Drosophila (commonly known as fruit flies) for his experiments. In the years that followed, a prodigious amount of confirmatory evidence in Drosophila and other biological systems accumulated and Muller started alerting the medical profession and the public at large about the possible genetic hazards of exposure of human populations to ionizing radiation.

However, concern over the potential genetic consequences of exposures of large numbers of people to low levels of radiation first arose in the aftermath of the detonation of atomic bombs over Hiroshima and Nagasaki in World War II, some 20 years after Muller's discovery. This concern was launched into the orbit of regulatory policy only in the mid-1950s, when a Committee, called the BEAR committee set up by the U.S. National Academy of Sciences (NAS, 1956) recommended that the principal limitation of radiation exposure of people should be set by the genetic hazards involved.

In a historical sense, the BEAR committee's recommendation was a major milestone in radiation protection for at least two reasons: first, until that time, the emphasis was on what we now call 'deterministic effects' to radiation workers and the principles that were formulated were aimed at keeping individual doses below the relevant thresholds. Now,

'stochastic effects' (of which genetic effects constitute one type) in the general population began to receive attention (Clarke and Valentin, 2005).

Second, the several general radiation principles that emerged mainly from Drosophila radiation studies guided the BEAR and other Committees in their deliberations at that time. One of these is the concept of linear relationship between radiation dose and mutation frequency. In current terminology, it is the linear, non-threshold model (LNT model). Of note is the fact that this model has been, and still is, used by ICRP and other scientific committees for predicting genetic as well as carcinogenic effects at low doses from data at moderate and high doses. This model is thus a legacy from Muller's Drosophila studies.

We have come a long way in genetic risk estimation in the 50 years since the BEAR Committee's work. The reports of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) published in 2001 (UNSCEAR, 2001) and of the Committee on the Biological Effects of Ionizing Radiation (BEIR VII) of the U. S. National Research Council- National Academy of Sciences (NRC, 2005) provide recent estimates of genetic risks. In the new set of recommendations for radiological protection expected to be issued in 2007 by the International Commission on Radiological Protection (ICRP), the estimates of genetic risk presented in the 2001 UNSCEAR report have been used to derive genetic risk coefficients and estimate the relative contribution of genetic effects to total detriment (due to both cancers and genetic effects).

The aim of this paper is to (a) provide a background to genetic risk estimation; (b) discuss the 2001 UNSCEAR risk estimates and their use in the new ICRP recommendations; (c) indicate how the situation has changed, relative to the one that prevailed at the time of the ICRP Publication 60 (ICRP, 1991) and (d) provide answers to some of the questions raised at the 2004 meeting of the working party of the Article 31 group of experts involved in RIHSS (Research Implications of Health and Safety Standards) in the European community. A draft of the discussions at the above meeting was made available to me by Dr. Patrick Smeesters.

6.3 Background

From about the mid-1950s until now – the major concept that has driven the efforts at genetic risk estimation by the various national and international scientific committees has been that radiation-induced mutations in human germ cells would give rise to genetic diseases of the types known to occur naturally as a result of spontaneous mutations. However, thus far, no radiation-induced genetic diseases have been found. Genetic epidemiological studies initiated in Japan in the aftermath of the detonation of the Atomic bombs over Hiroshima and Nagasaki (and which have been carried over for over 4 decades) have not provided any evidence for radiation-induced adverse effects in the children of A-bomb survivors (Neel and Schull, 1991).One should hasten to add, however, that the endpoints used in the Japanese studies were not, what would be formally called "genetic diseases".

The fact that radiation is a proven mutagen and the view that human germ cells are unlikely to be an exception with respect to induced mutations prompted the scientific committees to devise methods early on to estimate genetic risks indirectly using the baseline frequencies of genetic diseases in human populations, mouse data on induced mutations and population genetic theory. One such method is referred to as the 'doubling dose (DD) method'. The method, which enables us to predict and express risks in terms of inducible genetic diseases, has evolved over the years (Sankaranarayanan and Chakraborty, 2000a).

The doubling dose method. The equation currently used for risk estimation with the doubling dose method is:

$$\text{Risk per unit dose} = P \times 1/DD \times MC \times PRCF$$

where P is baseline frequency of the disease class under study; DD is doubling dose, 1/DD is the relative mutation risk per unit dose; MC is the mutation component and PRCF is the *p*otential *r*ecoverability *c*orrection *f*actor.

It suffices to note here that the estimates of P for different classes of genetic diseases come from epidemiological studies. The diseases of interest in this context are Mendelian diseases (which include autosomal dominant, X-linked and autosomal recessives), multifactorial diseases (which include congenital abnormalities and chronic diseases), and chromosomal diseases (which are due to gross chromosomal abnormalities). DD is the amount of radiation that is required to produce as many mutations as those occurring spontaneously in one generation. It is now estimated from human data on spontaneous mutation rates and mouse data on induced mutation rates. The present DD estimate is 1 Gy for chronic low LET irradiation conditions, and has not changed over the years.

MC provides a measure of the extent to which different classes of genetic disease will increase in frequency as a result of new radiation-induced mutations. MC is highest for autosomal dominants, followed by X-linked, is very much less for autosomal recessives and far less for chronic diseases. The mathematical models used for estimating MCs for the different classes of genetic diseases have been discussed (Chakraborty et al, 1998; Denniston et al, 1998; ICRP, 1999).

PRCF enables us to bridge the gap between induced mutation rate estimates for mouse genes and the risk of inducible genetic diseases in humans; it is a fraction. For details of the rationale and the procedure used to estimate PRCF, see Sankaranarayanan and Chakraborty (2000b).

The equilibrium theory. The genetic theory that underlies the use of the DD method for risk estimation is what is referred to as the equilibrium theory which population geneticists use to explain the dynamics of mutant genes in populations. The theory postulates that the stability of mutant gene frequencies (and thus of disease frequencies) in a population is the result of the existence of a balance between the rate at which spontaneous mutations enter the gene pool of the population in every generation and the rate at which they are eliminated by natural selection i.e., through failure of survival or of reproduction. Under normal conditions (i.e., in the absence of radiation exposures), the population is assumed to be in equilibrium between mutation and selection. So, the quantity P in the risk equation is the equilibrium frequency of genetic diseases before radiation.

When the mutation rate is increased as a result of radiation, say, in every generation, the balance between mutation and selection is disturbed by the influx of induced mutations, but the population will eventually attain a new equilibrium (over a number of generations) between mutation and selection, depending on a number of factors.

The equilibrium theory predicts that for a population under conditions of radiation in every generation, when there is an x% increase in mutation rate, there will be an x% increase in disease frequency at equilibrium. It should be remembered that we talking about several tens or hundreds of generations into the future.

6.4 Major Advances Incorporated in the 2001 UNSCEAR Report

The major advances incorporated in the 2001 UNSCEAR are the following: (1) the development of the concept of potential recoverability correction factor (PRCF) (to bridge the gap between rates of radiation-induced mutations empirically estimated from mouse studies and the risk of inducible genetic diseases) and (2) the development of the concept that the effects of germ cell irradiation in humans are more likely to manifest themselves in the progeny as multiple developmental abnormalities than as single gene diseases. These two advances stem from progress in molecular biology that have been incorporated for the first time in genetic risk estimation. In addition, the baseline frequencies of Mendelian diseases have been revised upwards from 1.25% (in UNSCEAR, 1993) to 2.40%. Further, the doubling dose is now estimated using human data on spontaneous mutation rates and mouse data on induced mutation rates (in 1993, mouse data were used for both spontaneous and induced mutation rates). Finally, mathematical methods have now been developed and used to estimate MC for both Mendelian and chronic diseases. As a result, for the first time in the history of genetic risk estimation, it became possible to present risk estimates for all classes of genetic diseases and they support the view that the genetic risks of radiation at low doses are certainly not as high as Muller feared they might be.

6.5 2001 Risk Estimates

Table 1 summarizes the 2001 UNSCEAR risk estimates. The risks are expressed as the predicted number of additional cases (i.e., over the baseline) of different classes of genetic disease per million progeny per Gy for a population exposed to low LET, low-dose or chronic irradiation generation after generation. Estimates are given for the first and up to the second post-radiation generation. As will be evident, the total risk to the first generation is of the order of about 3,000 to 4,700 cases per million progeny per Gy which represent about 0.4 to 0.6% of the baseline risk. The risk up to the second generation is slightly higher, namely, 3,930 to 6,700 cases per million per Gy, or about 0.5 to 0.9% of the baseline risk.

Table 1. The UNSCEAR (2001) estimates of genetic risks from continuing exposure to low LET, low- dose or chronic irradiation. Assumed doubling dose = 1 Gy

Disease class	Baseline frequency (per million live births)	Risk per Gy per million progeny	
		1st generation	up to 2nd generation
Mendelian			
Autsomal dominant & X-linked	16,500	~ 750 to 1,500[a]	~1,300 to 2,500
Autosomal recessive	7,500	0	0
Chromosomal	4,000	B	b
Multifactoria			
Chronic	650,000[c]	~ 250 to 1,200	~ 250 to 1,200
Congenital abnormalities	60,000	~ 2,000[d]	~ 2,400 to 3,000[e]
Total	738,000	~ 3,000 to 4,700	~ 3,930 to 6,700
Total per Gy expressed as per cent of baseline		~ 0.41 to 0.64	~ 0.53 to 0.91

[a] The ranges reflect biological and not statistical uncertainties
[b] Assumed to be subsumed in part under autosomal dominant and X-linked diseases and in part under congenital abnormalities
[c] Frequency in the population
[d] Estimated from mouse data without using the DD method
[e] On the assumption that between 20% and 50% of the abnormal progeny in the first generation may transmit the damage to the second generation.

6.6 How Do the UNSCEAR 2001 Risk Estimates Differ from those Presented in UNSCEAR 1988?

The reason for making comparisons of the UNSCEAR 2001 risk estimates (Table 1) with those presented in the UNSCEAR 1988 report (Table 2) is that the latter were used in ICRP Publication 60 (ICRP 1991). A comparison of the entries in Tables 1 and 2 will reveal that, despite the use of the same doubling dose of 1 Gy (a) the main difference between the estimates is that in 1988, risk estimates could not be made for multifactorial diseases (i.e., congenital abnormalities and chronic diseases) whereas in 2001, risks could be estimated for all classes of disease including multifactorials [Of note here is that the ICRP, in its Publication 60, presented estimates also for multifactorials, but emphasizing the very tenuous nature of the assumptions used] and (b) in 1988, the risk estimates were for the first and second post-radiation generations and at equilibrium; in 2001, however, the estimates (for all classes of genetic disease) pertained to the first and second post-radiation generations only (The fact that the baseline frequencies of Mendelian diseases in 1988 were estimated to be lower than those in 2001 and the risk estimates consequently slightly lower is a relatively minor point).

Table 2. The UNSCEAR (1988) estimates of genetic risks from continuing exposure to low LET, low-dose or chronic irradiation. Assumed doubling dose = 1 Gy

Disease class	Baseline frequency (per million live births)	Risk per Gy per million progeny		
		1st generation	up to 2nd generation	Equilibrium
Mendelian				
Autsomal dominant & X-linked	10,000	1,500	2,800	10,000
Autosomal recessive	2,500			
-- Homozygous effects		No increase	No increase	1,100
-- Partnership effects		Negligible	Negligible	400
Chromosomal (due to structural anomalies)	400	240	340	400
Sub-total (rounded)	13,000	1,800	3,200	11,500
Early acting dominants	Unknown		Not estimated	
Congenital anomalies	60,000		Not estimated	
Other multifactorial diseases	600,000		Not estimated	
Heritable tumours	Unknown		Not estimated	
Chromosomal (due to numerical anomalities)	3,400		Not estimated	

6.7 How Does the ICRP Use the 2001 UNSCEAR Genetic Risk Estimates in its Recommendations Expected to Be Issued in 2007?

In the ICRP's scheme of things, the estimates are expressed as percentages per Gy. As mentioned earlier, in the 2007 document, estimates up to generation 2 were used. The upper and lower limits of each of the estimated ranges are first used to obtain average estimates and the latter are then combined (Table 3). For example, for

Table 3. Risk coefficients for the reproductive and the total population (all values expressed in percent per Gy) and are up to 2 generations when the population sustains radiation exposure generation after generation.

	Reproductive population		Total population
Disease class	Range	Average[a]	Average[b]
(a) Mendelian diseases	0.13 to 0.25	0.19	0.08
(b) Chronic diseases	0.03 to 0.12	0.08	0.03
(c) Congenital abnormalities	0.24 to 0.30	0.27	0.11
Total for all classes		0.54	0.22

[a] Average of the limits of the indicated ranges
[b] 40% of that for the reproductive population

Mendelian diseases, the 1300 to 2500 cases per million per Gy become 0.13 10^{-2} to 0.25 10^{-2} per Gy with an average of 0.19 10^{-2} per Gy. A similar procedure is applied to the other classes. The total risk (i.e., for all classes combined) thus becomes $0.54.10^{-2}.Gy^{-1}$.

The above estimates are applicable when the radiation doses received by all individuals in the population are genetically significant i.e., for a reproductive population However, when the total population of all ages is considered, the genetically significant dose will be markedly lower than the total dose received over a lifetime. Genetic damage sustained by germ cells of individuals who are beyond the reproductive period, or who are not procreating for any reason, poses no genetic risks. On the assumption that the average life expectancy at birth is of the order of 75 years, the dose received by 30 years of age (i.e., the mean reproductive age) is 40% (i.e., 30/75 = 0.4) of the total dose. The risk coefficients for the total population, therefore, are estimated to be 40% of the above values. Note that the total risk coefficient of 0.22% per Gy for the whole population is 40% of that for the reproductive population.

6.8 How Do these Estimates of Risk Coefficients Differ from those Estimated in ICRP Publication 60 (1991)?

Table 4 provides these comparisons. As can be noted, the risk coefficients up to the first two generations for the whole population presented in 1991 and 2007 are nearly the same. One must hasten to add that this is nothing more than pure coincidence. The main difference is that ICRP 2007 uses the first two generation risk coefficient to assess the relative contribution (estimated at 3 to 4%) of the total detriment. In 1991 however, the ~ 5-fold higher new equilibrium risk coefficient was used for this purpose giving a higher relative contribution of some 18% to the total detriment The equilibrium estimates were discussed in 2001, but not used in detriment calculations.

Table 4. Comparison of genetic risk coefficients in ICRP Publication 60 (ICRP 1991) with those in ICRP (2007)

	Risk coefficients in % per Gy	
	Reproductive population	Total population
Up to 2 post-radiation generations		
ICRP (1991)	0.53	0.19
ICRP (2007)	0.54	0.22[1]
New equilibrium		
ICRP (1991)	2.40	1.00[2]
ICRP (2007)	Not used	

[1] Used to assess the relative contribution of genetic effects (~3 to 4%) to total detriment
[2] Used to assess the relative contribution of genetic effects (~ 18%) to total detriment

6.9 Answers to the Questions of the EU Working Party of RIHSS

Question 1: " …When evaluated on comparable bases (risk for the first generation, for the first two generations…) the genetic risk is not reduced in the 2001 UNSCEAR report by comparison with the UNSCEAR1993 report. Nevertheless, based on the existence of large uncertainties, ICRP takes now the effect on the generations farther than the second as being zero. Can this position be considered as a balanced acceptable position according to present knowledge?" (note: the authors actually meant the 1988 UNSCEAR report and not the 1993 UNSCEAR report).

Answer: yes, it is as balanced as it can be. The reason why the risk estimates in 2001 for Mendelian diseases did not differ much from those presented earlier has already been explained. But, it was never stated that the risks to generations beyond the second is zero. The reason for limiting attention to the estimates for the first two generations and not on those at the new equilibrium is simply this: although it is mathematically possible, it is not biologically feasible.

First, risk predictions at the new equilibrium imply the totally unrealistic and untestable assumptions that the (a) circumstances (e.g., demographics, advances in medicine and health care) of human populations will remain constant over very long periods of time and (b) the estimates of the various parameters used to estimate mutation component and other quantities in the risk equation will remain unchanged over tens or hundreds of human generations. Second, advances in molecular biology have raised strong doubts about the concept that radiation will induce genetic diseases that are similar to those that arise as a result of spontaneous mutations.

There is a much more general philosophy here. When in the mid-1950s, Muller argued that the principal limitation of radiation exposure of people should be set by the genetic hazards involved, this was the correct argument then in the light of Drosophila data that were then available. When we now argue that genetic risks are smaller than what Muller feared they might be, we are guided by the data currently available. We did not gloss over the uncertainties, and in fact have explained strengths and limitations of these estimates in these reports and more importantly why they may be on the high side.

Question 2. "…According to the challenging speaker, the main problem is the radiation induction of *small* deletions leading to recessive mutations and diseases of the *phenotypes* might frequently not be *recognized* by the physicians. Such cumulative small genetic

disorders may propagate in the future generations with the risk of leading to more important pathological consequences…"

"…This is hardly taken into account by ICRP (and UNSCEAR) in the risk coefficient, as they are of the opinion that the major contribution to the genetic risk comes from large deletions expressing themselves essentially in the first generations …The basic question is whether we know enough about radiation-induced hereditary effects to close the matter…"

Answer: An important concern certainly. There are two parts to the answer. First, in Sweden, the late Prof. Lüning conducted studies in the late 1960s and 1970s to answer the question whether radiation-induced recessive lethal mutations would accumulate in mouse populations subject to radiation in every generation. He went up to 15 generations of radiation but could not demonstrate accumulation of recessive lethal mutations over time (summarized in UNSCEAR 1972). Several other population genetic studies by others focused on fitness in irradiated mouse populations also came up with negative results and were therefore abandoned. This is in contrast to studies with irradiated Drosophila populations which were carried out in the 1960s where good evidence for accumulation of recessive lethal mutations could be obtained. Why is this so? In part, this could be explained by the sample sizes and techniques that could be used in the mouse for screening for recessive lethals which were not as efficient as in Drosophila. It now seems probable that this difference in part may also be related to differences in genomic architecture between Drosophila and the mouse and their response to radiations.

Second, in the mathematical models that were developed in the late 1990s to examine the extent to which the incidence of recessive diseases will increase in human populations exposed to radiation in every generation, we considered situations where the mutation rate will be permanently double that of spontaneous rate (Chakraborty et al., 2000). The results, obtained through computer simulations, show that the increase in disease frequency will be incredibly slow. By generation 15 or so the number of generations which Lüning used, one would not be able to demonstrate an increase.

While small deletions are certainly among the types of damage induced by radiation, multi-gene deletions appear to be the principal ones. They are expected to manifest themselves phenotypically as multi-system developmental abnormalities. Individuals with these are expected to be severely handicapped and the chances of propagation beyond the first few generations is therefore predicted to be small. Obviously, we do not know enough about radiation-induced hereditary effects and we are not closing the matter.

6.10 The Future

We are in an exciting transition period between the accepted dogma that radiation will induced genetic diseases of the types that occur naturally and what we can now predict with the knowledge and capabilities that human genome research provides. As I look at the situation now in the early years of the 21st century, I see unprecedented and challenging opportunities to examine the question of genetic risks from the molecular perspective through the use of genome-based technologies. And I wish to end my talk on that positive note.

6.11 Acknowledgement

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7 NEW DATA ON GENETIC RISK - MUTAGENESIS OF REPEATED DNA REGIONS TO ASSES THE RISK OF CHEMICAL AND PHYSIC GENOTOXICS AGENTS

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7.1 Introduction

Detection of increased germline mutagenesis in human populations exposed to ionising radiation of various types. This type of exposition modifies the genetic material of the somatic and germ cells. In the latter case, the modification may be detected in the offspring.

1. A first investigation was performed on families from rural areas of the Ukraine which were heavily contaminated by after the Chernobyl accident. Mutagenesis of the germline was compared in families with children conceived before and after the accident. A 1.6-fold increase in mutation rate was found in the germline of exposed fathers, whereas the maternal germline mutation rate remained nearly constant (Dubrova et al., 1996).

2. The population living at the Semipalatinsk nuclear test site in Kazakhstan present increased mutation rates in the exposed population. This effect tend to disappear after the decay of radioisotopes in the late 1950's, and after surface and atmospheric nuclear testing stopped (Dubrova et al., 2002).

3. Families living in the Techa River area, a region contaminated by radioactive waste produced in the weapon-grade plutonium separation combine, show a 1.7-fold rise in germline mutation rates compared with unexposed families from the rural area of Chelyabinsk and Kurgan Oblasts (Dubrova et al., 2006). Recently, strong evidence for elevated cancer incidence and increased somatic mutation rate has been reported among the residents of this contaminated area (Ostroumova et al., 2006).

These observations prompted the establishment of an experimental model to understand the molecular basis of this induced mutagenesis in the human germline.

7.2 Mutation Rate Determination

In the work mentioned above, the mutation rate was determined by genotyping minisatellite loci. This is a novel approach used to elucidate the variation that may occur in a genome. Cytogenetics methods have ben widely used to detect structural modifications of human chromosomes in the range of several million base pairs. In contrast, novel molecular biology approaches made possible to analyse sequence changes in the range of 5000 to 20 000 base pairs. Certain of these regions present extremely high mutation rates, some of them mutating at a frequency 1000 times higher than other well-known marker genes (Bouffler et

al., 2006). These loci were initially named "minisatellites" and were first reported by Dr Alec Jeffreys in 1984 in Leicester University (Jeffreys, 2005; Jeffreys et al., 1985a; Jeffreys et al., 1985b). Molecularly, "minisatellites" are composed of tandemly repeated DNA sequences (TRDL). This type of repeated DNA is detectable in prokaryotic and eukaryotic cells. This offers the possibility to use the repeated DNA like probes to genotype DNA samples for forensic identification, and also in fundamental genetic research. Today we will focus firstly on the use of tandemly repeated DNA regions to determine germline mutagenesis in mice. Secondly, we will present new data indicating that germline mutagenesis depend on the integrity of DNA repair systems. This was possible by using genetically modified mice mimicking well-known cancer-prone human diseases like *Xeroderma pigmentosum*, in which cancer incidence is 1000-fold higher than in the European population (Cleaver, 2005; Friedberg et al., 2006).

Although DNA tandem repeats have a similar structure overall, their total number differs between organisms. They also differ in the number of base pairs forming the repeat unit and the size of the array. During cell division, the number of repeats preset in the array may decrease or increase, creating contraction or expansion. Interestingly, ionising radiation particularly affects the mutagenesis of certain DNA repeats. This property was used by Dr. Dubrova to facilitate the determination of the mutation rate of human germline affected by ionising radiation. It is important to note that initially, repetitive DNA sequences were considered to lack any functional significance, and for this reason were called "junk DNA". However, today several diseases are associated with changes in these DNA arrays. In the particular case of microsatellites, there are more than 40 neuropathies associated with contraction or expansion (Ding et al., 1999; Jeffreys, 2005; Larson et al., 1999; Pearson et al., 2005; Stead et al., 2000; Stead and Jeffreys, 2000).

The schematic representation of the contraction/expansion of a minisatellite is shown in the left side of the slide. This representation fits well with the real modification detected by gel electrophoresis. The right panel shows the genotype of human DNA from a Caucasian family as determined by DNA gel electrophoresis. This pattern of bands corresponding to different fragments of DNA allows determination of the mutagenesis rate in the germline of one of the progenitors. Under our experimental conditions, DNA fragments are separated from high (top) to low (bottom) molecular weight. Standard DNA fragments of well-known molecular weight are used to determine the precise molecular weight of these bands. After separating the human DNA from a mother, father and child, the fragments were hybridised with four different minisatellite radioactive DNA probes. Different types of mutants were detected by directly comparing the size of these bands. It is clearly seen that the DNA pattern of only one of the genitors is modified, indicating that the mutagenic event took place in the paternal (or maternal) germ line. If the mutated band is shorter or longer than the parental band, this will indicate whether this is a contraction or expansion. Based on these results, Dr. Dubrova optimised an extremely simply and reliable method of determining mutation patterns and to provide the first experimental evidence that ionising radiation increases the mutation rate in a human population living in contaminated areas (Dubrova et al., 1996).

The fact that repeated DNA regions are widely present in eukaryotes, and that several humans and mouse loci conserve a high mutagenic rate in the germline, open the question as whether mouse models may be used to monitor the effects of genotoxic agents. A first step was to compare standard methods used to determine germline mutagenesis with the genotype of mouse repeated sequences. Previously, Russel monitored the phenotypic changes produced by 7 dominant genetic markers. Using this test he characterized the mouse germline mutagenesis induced by the chemical carcinogen Ethyl-N-nitrosourea (ENU) (Russell et al., 1979). ENU is an alkylating agent that forms O-alkylated and N-alkylated products. O6-alkylguanine and O4-alkylthymine are potentially mutagenic lesions because they can mispair during DNA synthesis. These lesions are specifically removed by the

methyltransferase. However, chronic treatment with ENU specifically results in neural tumours in experimental animals.

The Paternal mutation rate determined by the phenotype of 7 genes was plotted against the used ENU doses. The Russel test detected increased germline mutagenesis in the range of 50 to 250 mg of ENU/kg. Using similar doses the genotype of mouse repeated DNA sequences detect clear differences in the range of 0 to 75 mg of ENU/kg. Doses as small as 12.5 mg/kg are sufficient to induce a 2.8-fold significant increase in the mutagenesis rate of the mouse germline. The number of animals used for the Russell test clearly indicated the experimental efficiency of the new method to determine germline mutagenesis. These data also show that it is possible to monitor the genetic effects of chemical mutagens at low-dose exposure (Vilarino-Guell et al., 2003).

To further test the accuracy and the sensitivity of this method, acute X-rays doses were used to determine the germline mutation rate at intermediate doses of ionising radiation. The results showed that the induction of ESTR mutations by acute X-rays occurs mainly at premeiotic stages of mouse spermatogenesis, and that the mutation rate increases linearly with radiation dose. As expected, the number of mutations found in the offspring of irradiated males would require an extremely high number of DNA lesions per genome (single- and double-DNA strand breaks or other type of damage). The number of DNA lesions exceeds by far the lesions that are induced by direct targeted events (Dubrova et al., 1996). This indicate that ESTR loci themselves are not the direct target of irradiation. Interestingly, the doubling dose estimate for paternal ESTR mutation rate is 0.33 Gy (95% confidence interval 0.2-0.95 Gy), which is very close to that of previous studies using the 7-locus test. The low number of descendents used to determine the doubling dose by ESTR-genotyping highlights the unique advantage of ESTR-genotyping in analysing mouse germline mutagenesis. It appears that mutation induction reflects radiation-induced structural damage in DNA at the premeiotic stages of spermatogenesis. Sequential mitotic and meiotic divisions amplify the primary effects, making possible to use a very small number of samples to determine germline mutation rate (Dubrova et al., 1998).

The comparison of acute and chronic exposure indicated a similar number of germline mutations, as judged by comparison of a 0.5 Gy dose delivered at a dose rate of 0.5 Gy/min and at a dose rate of 0.000166 Gy/min. This indicate that the elevated mutation rate is independent of the ability of the cell to repair the direct damage immediately (within 2 minutes) or over a period of up to 100 hours. Current estimates of the genetic hazards of low-dose radiation have been extrapolated from data obtained using high doses of radiation, and assuming that chronic radiation exposure induces 25-33% as many germline mutations as an acute dose. The data presented here on the similar mutagenicity of chronic and acute low-LET exposure indicate that this extrapolation does not hold true. The risk factors for chronic and acute irradiation for this dose range should be regarded as similar. This observation may be particularly important for the estimation of the genetic hazards to the human germline, which are supposed to be essentially low doses (Dubrova et al., 2000). However, clear differences are apparent when comparing the mutation rate at low- or high-LET. As expected, chronic exposure to high-LET fission neutrons further increases the mutation rate. (Dubrova et al., 2000).

Conclusions

We conclude that genotyping of DNA using repeated loci is a robust and convenient method to monitor mouse germline mutagenesis. This may help to determine the potential genetic effects of chemical and physical genotoxic agents, since they induce a similar increase in the mutation rate. Moreover, the detected increase is similar when comparing the coding and noncoding regions of the genome, indicating that this is not a process exclusively linked to

repetitive sequences. These results raise the possibility of testing low doses of genotoxic agents that were impossible to analyse before. The relatively small number of samples, and the simplicity of the method, prompted us to ask whether the inactivation of a particular metabolic pathway might affect the ESTR mutagenesis of the germline. This kind of experiment is now possible using mice in which a given gene is inactivated by using recombinant techniques. We assumed that the induction of DNA damage is the primary and most important biological effect of ionising radiation. It is also well established that cells respond to DNA damage by activating genes involved in maintaining homeostasis, and in preventing metabolic imbalance that may lead to development of cancer. The so called "DNA-damage response" is a complex, highly branched signalling network, which involves the induction or activation of several hundred genes and proteins.

It is well established that lesions provoked by ionising radiation severely modifies DNA structure, creating single- and double-strand breaks, bulky adducts, base modifications, DNA-protein links, and locally multiply damaged sites (LMDS). All these lesions trigger the DNA-damage response. We therefore tested whether the inactivation of some of the genes coding for proteins involved in lesion recognition affects germline mutagenesis. We focused our experimental work on a mice deficient in the recognition of bulky adducts. We compared it with those in mice mutated in the gene coding for the DNA-dependent protein kinase (DNA-PK), which enables the repair of DNA double-strand breaks (called SCID mice)(Friedberg and Meira, 2006) and a mutant mouse in which recognition of single-strand breaks (and partially base excision repair) is affected (named PARP-1 KO mice) (Barber et al., 2004). Our observations were further confirmed by the analysis of a mutant mouse unable to resolve bulky lesions, because *Pol*□a DNA polymerase involved in translesion synthesis, is inactivated (Burr et al., 2006). Finally, a mutant mouse in which a general signalling pathway controlled by p53 protein had been inactivated was also analysed (Burr et al., 2005).

7.3 DNA-Repair Deficiencies Increase Spontaneous Germline Mutation Rate

Significantly increased mutation rates were detected in the germline of all the analysed mice deficient in lesion recognition. In contrast, the loss of p53 function does not affect the spontaneous ESTR mutation rate in the mouse germline. The precise molecular mechanism by which the rate of germline ESTR mutations increases remains to be established. However, taken together, all these data confirm the hypothesis that prolonged pausing of replication forks affects mutation rate. The demonstration of enhanced mutation rates in the germline of mice unable to deal with bulky lesions (XPC and *Pol*□mutant mice) is interesting, considering that XPC KO mice mimic the human cancer prone syndrome xeroderma pigmentosum (XP) (Burr et al., 2006). The group C is one of the most common forms of this DNA repair disease, and has a 1000-fold increase in cancer incidence over the normal population (Friedberg et al., 1995). In the mouse XPC KO model the deficiency in global genome repair contribute to the development of lung tumours, the majority of which are adenomas, at the age of six months. Later, 100% of mice will have lung tumours (Hollander et al., 2005). It should also be mentioned that some offspring of *Pol*□ mutant mice spontaneously manifest various disease states, including diabetes insipidus, vitiligo, and neurological abnormalities. Apparently these disease states are not directly linked to the *Pol*□ mutant state It is likely that the increased rate of mutations in the germline of these animals results in heritable disease states (Burr et al., 2006).

7.4 XPC Deficiency Increases Germline Mutation Rate

We further analysed the effects of ionising radiation and a chemical carcinogen on the germline mutation of mice unable to recognize bulky adducts. Spontaneous and radiation-induced mutation rates in the mutated males were significantly higher than those in the wild-type mice. In contrast, exposure to the monofunctional alkylating agent, ethylnitrosourea (ENU), resulted in similar increases in ESTR mutation rates in wild-type and mutated mice. This is not surprising, since XPC protein does not recognize alkylated DNA bases and is not concerned with their repair. Therefore, ENU and ionising radiation similarly increase ESTR germline mutagenesis, indicating that the ESTR instability may only be explained by the inability to repair bulky adducts

7.5 Radiation-Induced Mutation Rate in the Germline of DNA-Repair Deficient Mice

Comparison of the ESTR mutation rates in the germline of mice strains unable to recognize DNA breaks or bulky lesions shows that spontaneous mutation rates exceed those in wild-type isogenic animals in all three cases. In contrast, the irradiation of mutants has different effects. Mutants unable to recognise single- or double-strand breaks (PARP-1 and SCID, respectively) do not further increase the mutation rates after irradiation. However, the mutant unable to recognize bulky lesions (XPC KO) presents a clear ionising radiation-induced increase in the mutation rate. These data are consistent with the fact that inactivation of the recognition of single- and double-strand breaks on DNA is highly deleterious for the cells. These types of lesions are incompatible with DNA replication, and therefore a substantial proportion of cells in the germline of deficient males is eliminated by apoptosis. This situation is completely different with the recognition of bulky lesions of DNA, which are repaired by global genome repair and by transcription-coupled repair, two subpathways of the nucleotide excision repair (NER) system. Furthermore, bulky lesions may also be resolved by another mechanism called trans-lesion synthesis (TLS), in which an error-prone DNA polymerase, like *Pol*□, is able to bypass a DNA lesion, leading to a “quasi normal DNA replication” without compromising cell survival. Therefore, XPC gene inactivation only partially affects the repair of radiation-induced bulky damage, and it does not affect the survival of irradiated germ cells. However, it has been reported that XPC KO mice present elevated mutation rates in germinal and somatic tissues. This means that even if the XPC KO mutant is only partially deficient in repair of bulky lesions, this incapacity is sufficient enough not only to increase ESTR mutagenesis, but also to contribute to enhanced carcinogenesis across multiple tissues. Indeed, 100% of XPC KO mice develop spontaneous lung tumours, indicating that XPC deficiency in mice results in a cancer risk for a variety of tissues, as is the case in the human syndrome.

7.6 Conclusions

Our results indicate that ESTR mutagenesis may be a convenient biomarker of radiation exposure in the mouse. These data further support the idea that a delay in repair of DNA damage in mice may slow down replication fork progression, which, in turn, affects ESTR mutation rate. The increased instability in the germline of genetically modified mice allows the identification of two genes coding for proteins that recognise single- and double-strand breaks as being involved in the enhanced ESTR mutagenesis induced by ionising radiation. This raises the possibility of defining the molecular basis of ESTR mutagenesis.

Furthermore, ESTR mutagenesis allows the direct characterisation of recessive mutations and modification of noncoding DNA regions. This contrast with most of the approaches used until now, in which recessive mutation induction is estimated by extrapolation from dominant mutations. The possibility that small DNA modifications induced by IR destabilise replication and produce a “cascade effect” leading to a general enhanced mutagenesis can now be tested using specific mutants. This will define the mechanisms involved in ESTR mutagenesis, and will certainly help to improve germline risk estimates (long-term storage of radioactive waste).

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8 RADIATION CATARACT

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8.1 Introduction

One of the more crucial yet ill-defined effects associated with ionizing radiation exposure is its effect on the transparency of the eye lens, a pathology called radiation cataract. The uncertainties surrounding dose relations to cataract development are of considerable interest to the risk assessment community (UNSCEAR, 1988; BEIR V 1990; ICRP, 1991; NCRP, 1993). Present ocular guidelines are predicated on the view that cataractogenesis is a deterministic event and requires a threshold radiation dose (currently 2 Gy) before lens opacities will develop (NCRP, 1993, 2000). Yet, in populations exposed to far lower doses of radiation, including those undergoing CAT scans (Klein, 1993), radiotherapy (Wilde, 1997; Hall, 1999), the astronaut pool (Cucinotta, 2001; Rastegar, 2002), atomic bomb survivors (Minamoto, 2004; Nakashima, 2006), residents of contaminated buildings (Chen, 2001) and the Chernobyl accident "liquidators" (Worgul, 2003, 2007), dose-related lens opacification at exposures significantly lower than 2 Gy was reported.

The concept of a dose threshold is critical not only to risk assessment but also to theories regarding the patho-mechanism(s) of radiation cataract. Initial presentation of radiation cataract usually involves a posterior superficial opacification of the lens, termed a PSC or *posterior subcapsular cataract*. While psc may also arise from other causes, such as corticosteroid treatment, chronic uveitis, diabetes or galactosemia (Hiller, 1986; Pau, 1989), radiation exposure is most closely associated with this form of lens opacification (Cogan, 1952; Worgul, 1976; Otake, 1982; Merriam, 1983). The time in which a psc develops is inversely related to radiation dose and also depends on the rate at which damaged lens epithelial cells divide, aberrantly differentiate and migrate to the posterior pole (Merriam, 1957, 1973, 1984; Worgul, 1975, 1989). Many early studies of radiation cataract, which generally had short follow-up periods, failed to take into account the increasing latency period as dose decreases, did not have sufficient sensitivity to detect early lens changes and had relatively few subjects with doses below a few Gy (Leinfelder, 1936; Cogan, 1953a,b; Merriam, 1962). More recent experimental animal and human epidemiological studies suggest that cataract can occur following exposure to significantly lower doses of ionizing radiation.

8.2 The Eye Lens and Cataract

The lens is an optically clear, a vascular tissue which continues to grow in size and cell number throughout life and whose primary pathology is opacification or "cataract" (Kleiman, 1994). Lens transparency depends on the proper morphogenesis of lens fibre cells, the anuclear, amitochondrial, differentiated progeny of a proliferating subset of a single layer of epithelial cells on the lens anterior surface.

The lens is entirely dependent on the surrounding aqueous and vitreous fluids for nourishment (Harding, 1984). Its anatomy is unique, with a single epithelial cell layer on the

anterior, corneal facing surface that contains the progenitors of the underlying lens fibre cells (Horwitz, 1992). It is completely encased by a basement membrane, termed the lens capsule. Throughout life, epithelial cells located at the periphery of the lens, in the germinative zone, divide and differentiate into mature lens fibre cells. These terminally differentiated cells do not contain nuclei or mitochondria and are greatly dependent on the overlying epithelial cell layer for nutrient transport, energy production and protection from insulting agents. The process of lens epithelial cell division and differentiation slows considerably at about the time of puberty. Nevertheless, the lens continues to grow throughout life, eventually tripling in weight (Kleiman, 1994). In the central zone of the lens epithelium, along the lens visual axis, the mitotic index is negligible (von Sallmann, 1962; McAvoy, 1978). The cells in the central zone, however, play an important role in maintaining the metabolism of the lens, providing much of the required homeostatic mechanisms (Kuck, 1970). Because of the unique anatomy of the lens, disruption of the integrity of the epithelial cell layer is likely to lead to cataract (Cogan, 1952; von Sallmann, 1957a; Worgul, 1989b).

The principal pathology of the lens is its opacification, termed cataract, (van Heynigen, 1975). There are three predominant forms of cataract, depending on their anatomical location in the lens: cortical; involving the outer, more recently formed lens fibre cells, nuclear; developing first in the inner embryological and foetal lens fibre cells and posterior subcapsular; developing from the dysplasia of transitional zone epithelial cells and resulting in an opacity at the posterior pole (Kuszak, 1994).

From early in embryogenesis, all growth of the lens is restricted to proliferation of a band of epithelial cells termed the germinative zone (GZ). The cells more anterior to the GZ, the central zone (CZ) population, proliferate very slowly and play little or no role in lens growth. The GZ is approximately 60 cells wide occupying a position immediately anterior to the lens equator. Following terminal cell division, GZ cells migrate towards the equator. After traversing a ten cell wide region where mitoses is absent, they queue-up in precise registers called meridional rows (MR). There they begin to differentiate into mature lens fibre cells. Since mitosis is only 1 hour in duration and given that the human lens epithelial population remains constant after the age of 2 weeks (von Sallmann, 1957b) approximately one layer of new fibre cells is created every 8 hours. Qualitatively, the same phenomena are true for all mammalian lenses. As aging proceeds, the rate of fibre cell formation decreases but never stops. (reviewed by Harding, 1971.)

8.3 Radiation Cataract

For more than 100 years, investigators have known that cataracts develop following exposure of the lens to ionizing radiation (*e.g*, see reviews by Desjardins, 1931; Poppe, 1942; Bellows, 1944; Ham, 1953; Lerman, 1962; Radnot, 1969; Bateman, 1971; Merriam, 1972; Worgul, 1977; Koch, 1980). The clinico-histopathological changes accompanying radiation cataractogenesis, while not pathognomic, are characteristic and similar in all vertebrate lenses. At least four readily distinguishable stages are identifiable by slit-lamp biomicroscopy and these form the basis for a classification system to gauge cataract severity (Merriam, 1962). The rate at which these changes develop is strongly dose dependent with an age modulating component (Merriam, 1962, 1972, 1973, 1975).

While other environmental insults may also result in psc formation, radiation cataract is generally associated with this form of lens opacification (Cogan, 1952, Merriam, 1983). The time in which cataract develops is inversely related to radiation dose and also depends on the rate at which damaged lens epithelial cells divide, aberrantly differentiate and migrate to the posterior pole (Worgul, 1975). Although the mechanism of radiation induced cataracts is not precisely known, genomic damage resulting in altered cell division, transcription and/or

abnormal lens fibre cell differentiation is considered to be the salient injury, rather than cell killing.

Histopathologically, the earliest changes following irradiation involve the dividing cells of the germinative zone (GZ) of the lens epithelium. This is typified by mitotic inhibition, the duration of which is dose and species dependent (von Sallmann, 1952, 1955, 1957b; Harding, 1965). During the weeks following irradiation, but well before the appearance of a cataract, the meridional rows of the epithelium become disorganized and lose their typical cytoarchitecture (von Sallmann, 1951a; Worgul, 1975, 1976). Since this region encompasses cells which beginning to internalize and form mature lens fibres, it would be expected that the bow region should also demonstrate some effect. Indeed, when viewed in sagittal section, bow organization is disrupted (von Sallmann, 1951a; Cogan, 1952; Worgul, 1976). These events are followed by the appearance and accumulation of abnormally shaped lens fibre cells beneath the posterior capsule which often assume a rounded bladder-like appearance. The nuclei of such "Wedl" cells (Wedl, 1860) are often pycnotic. As the accretion of abnormal cells continues in the posterior cortex of the lens, deeper fibres, as judged by light microscopy, remain morphologically unaffected (Worgul, 1976). Eventually, Wedl cells begin to appear anteriorly and collect in a fashion similar to the posterior changes. The continued accumulation of such cells completely surrounds the deeper cortex of the lens (Cogan, 1952; Worgul, 1976). It is this altered cortical cytoarchitecture that is considered the basis for at least the early stages of radiation induced lens opacification (Cogan, 1951).

8.4 Cataract and World Blindness

Cataract, accounts, by far, for the majority of cases of blindness worldwide. The World Health Organization estimates more than 25 million blind and 119 million visually impaired individuals are affected (Thylefors, 1995, 1999; Arnold, 1998; WHO, 2004). It is estimated that evidence of lens opacities can be found in greater than 96% of the population over 60 years old (Luntz, 1992). Currently, the only treatment for cataract is surgical removal, a procedure that, in the United States alone, consumes 12% of the overall Medicare budget and 60% of all Medicare costs related to vision (Stark, 1989; Ellwein, 2002). In the third world, such surgical treatment is often unavailable, (WHO, 1989; Thylefors, 1999; Shichi, 2004). Given the increasing human lifespan, the societal burden of cataract surgery is expected to worsen in future years (Kupfer, 1985; WHO, 1997; The Eye Diseases Prevalence Research Group, 2004).

8.5 Oxidative Stress and Cataract

Oxidative stress is believed to be a major early or initiating event in the development of cataract induced by a variety of different agents (*e.g.*, Matsuda, 1981; Worgul, 1981; Babizhayev, 1988; Padgaonkar, 1989; Spector, 1993, 1995a). In human lenses, oxidation of lens constituents is a consistent finding (Augusteyn, 1981; Bhuyan, 1983; Spector, 1984). Experiments with lens organ and cell cultures have demonstrated that such stresses result in rapid metabolic and cellular changes similar to those observed in human cataract (Giblin, 1995; Kleiman, 1990a, 1993; Spector, 1995b, 1998; Zigler, 1989). Changes in cellular redox potential, membrane function, mitochondrial viability and DNA damage have been shown to be the earliest events following oxidative stress (Giblin, 1987, 2000; Kleiman, 1990a; Spector, 1995b).

8.6 DNA Damage and Cataract

Because DNA is so easily damaged by oxidative stresses or direct photochemical action of ultraviolet light, many investigators have suggested that unrepaired DNA damage to the lens epithelium ultimately results in cataract (Bellows, 1975; Jose, 1978; Courtois, 1981; Bloemendal, 1984; Rink, 1985; Spector, 1989; Worgul, 1989b). Two major mechanisms are proposed, namely (a) damage to the central zone cells could result in failure of the epithelium to provide sufficient metabolic regulation of the underlying cortical fibre cells and (b) damage or mutation in the germinative region, where defects in the dividing cell population would result in aberrant formation of new cortical lens fibre cells. The latter is believed to be most important to the development of radiation-induced posterior subcapsular opacification.

8.7 DNA Damage and Repair

Double strand breaks (DSB) are one of the most serious types of radiation-induced DNA damage (Bryant, 1985) and, if unrepaired or misrepaired, can lead to cell transformation or cell death. The cell has two main pathways for DSB repair, non-homologous end rejoining (NHEJ) and homologous recombination (HR) (Jackson, 2002). A large number of proteins are involved in regulating these processes.

In addition to DNA strand breaks, ionizing radiation induces various types of DNA base damage (Hutchinson, 1985, Wallace, 2002. Analysis of different kinds of base damage following low- or high-LET irradiation may provide important supporting data for the hypothesis that unrepaired or misrepaired DNA base damage is related to cataractogenesis and may also permit comparisons between rates of DSB repair and repair of specific damaged bases (Breimer, 1990).

In contrast to the relatively rapid repair of DNA SSB and/or oxidative base damage induced by low-LET radiation, repair of high-LET induced DNA damage is slower (Taucher-Scholz, 1996; Sternerlow, 2000) and may result in prolonged or aberrant cell cycle arrest (Goto, 2002). The slower rate of repair may reflect the complexity of the DNA damage, the difficulty of binding DNA repair proteins to multiple numbers and types of sites and the generation of alternative repair pathways (Pastwa, 2003).

The suggestion that low doses of high-LET irradiation results in some percentage of cells with heritable or long-lasting DNA damage is consistent with the finding that high-LET radiation is generally more effective in causing cataract (Worgul, 1986, Brenner, 1981, Hall, 2006). Radiation cataract formation is, *a priori,* dependent on survival and potential division and/or differentiation of lens epithelial cells with compromised genomes (Worgul, 1989b). The recent observations that astronauts are at increased risk for cataract, despite being exposed to relatively low levels of ionizing radiation, lend further support to this hypothesis (Cucinotta, 2001; Rastegar, 2002).

8.8 Space Radiation and Health

Radiation standards for space exploration have followed a somewhat different path from those on Earth. Exposures are potentially much higher than terrestrial irradiation due to galactic cosmic radiation, trapped radiation belts near the earth and solar particle events (Robbins, 1997). Radiation exposures in space are relatively difficult to reduce (compared

with radiation from human activities on the ground) and impossible to eliminate entirely. At the same time, other risks to humans in the hostile environment of space may be more acute or drastic than those of radiation. This puts a different perspective on radiation hazards and is one reason, together with the limited number of individuals involved, why larger annual dose limits have been tolerated for astronauts than are recommended for radiation workers on the ground (though career limits of risk have been roughly equalized). The purpose of radiation protection is to prevent deterministic effects of clinical significance and limit stochastic effects to levels that are acceptable, modulated by societal concerns.

Acute effects from solar flares or from increased doses of high-LET radiation also need to be guarded against and some limitation on organ exposures is needed to avoid direct deterministic effects. This assumes increased importance because of the recently reported association between increased incidence and earlier appearance of cataracts in astronauts exposed to higher heavy ion doses (Cucinotta, 2001) and the association between posterior subcapsular opacification and space flight (Rastegar, 2002). At present, cataract is the only unequivocal long-term degenerative effect reported for astronauts exposed to space radiation. Given that their exposure to heavy ions was very small and assuming radiation was the cause of their cataracts, this issue is of considerable health concern.

Although the mechanism of radiation induced cataracts is not known precisely, genomic damage resulting in altered cell division, transcription and/or abnormal lens fibre cell differentiation is considered to be the salient injury, rather than cell killing. For this reason, the classification of cataracts as a deterministic effect must be called into question, although they currently satisfy the definition of “an effect which has a threshold in dose, above which the severity of the injury increases with dose” (ICRP 1991; NCRP 2000).

8.9 Dose Threshold

Considerable uncertainty surrounds the relationship between radiation dose and cataract development, which is of concern to the risk assessment community, (UNSCEAR, 1988; ICRP, 1991; NCRP, 1993). Present ocular guidelines are predicated on the view that cataractogenesis is a deterministic event and requires a threshold radiation dose (currently 2 Gy) before lens opacities will develop. Yet, in populations exposed to far lower doses of radiation, including those undergoing CAT scans (Klein, 1993), radiotherapy (Hall, 1999), the astronaut pool (Cucinotta, 2001; Rastegar, 2002), atomic bomb survivors (Minamato, 2004, Nakashima, 2006), residents of contaminated buildings (Chen, 2001) and the Chernobyl accident “liquidators” (Worgul, 2003, 2007), dose-related lens opacification at significantly lower doses than 2 Gy was reported. The concept of a dose threshold is critical not only to risk assessment but also to theories regarding the patho-mechanism(s) of radiation cataract.

The ocular-radiation protection standards formulated by National Council on Radiation Protection (NCRP) and the International Council on Radiation Protection (ICRP) are all predicated on the assumption that radiation cataracts are deterministic and only appear when a high-dose threshold is exceeded. However, several lines of evidence from experimental and epidemiologic studies strongly suggest a stochastic basis for radiation cataracts. If this is the case, then the radiation safety standards set for workers, as well as the general population, may be inadequate. Furthermore, if radiation cataract is proven to be stochastic (implying that some fraction of a population will develop them even at low doses) its utility for dose reconstruction will be enhanced. Therefore, it is important for the risk-assessment community to know whether cataract formation is indeed a stochastic response to radiation, a question which may be resolved by studying a dose-defined subset of the Chernobyl population, the cleanup workers or “Liquidators”.

8.10 Animal Models for Radiation Cataract

Animal studies are well suited to examine the relationship between radiation and cataract development at both tissue and cellular levels. These model systems have great relevance to human radiation exposure and subsequent health outcomes. Extension of the presumed radiation cataract threshold in animal models to even lower doses is likely to be important to the development of appropriate guidelines for national radiation risk policy.

8.11 Genetic Models for Radiosensitivity

Individuals that are haplo-insufficient for multiple genes involved in DNA damage repair and/or cell cycle checkpoint control may be more susceptible to the cataractogenic effects of ionizing radiation than wild-types or those haplo-insufficient for only one such gene. Using animal models, Atm, Brca1 and Rad9 heterozygotes demonstrate enhanced sensitivity to radiation-induced cataract formation (Worgul, 2002, 2005a, 2006). Dual haplo-insufficiency for Atm and Rad9 increases the frequency of cataractogenesis as compared to each mutation alone (Worgul, 2006). Curiously, haplo-insufficiency for both Atm and Brca1 does not increase the frequency of cataract formation as compared to each heterozygous mutation alone. The roles of Atm, Rad9 and Brca1 in the cell cycle and during DNA repair are consistent with a genotoxic basis for radiation cataractogenesis.

These studies are among the first to study the effect(s) of multiple haplo-insufficiency on biological response in a highly organized tissue. The lens was the first higher level system to demonstrate that heterozygosity for the Atm or Rad9 gene alters the late response of a normal tissue to radiation. In addition, these findings may have important implications for radiosensitive subsets of the human population and for the astronaut core.

8.12 Low-Dose Radiation Exposures

Recent findings demonstrate dose-related significant lens opacification within a reasonable fraction of the lifespan of the mouse or rat after exposure to as little as 100 mGy X-rays or 32.5 cGy ^{56}Fe (Worgul, 2005a,b). Preliminary data suggests that there may be an even lower threshold dose for cataractogenesis, if one exists at all. These animal models have great relevance and similarity to human response to radiation exposure and determination of appropriate human exposure guidelines. Furthermore, any extension of the presumed radiation cataract threshold in this model to even lower doses is likely to be important to the development of appropriate guidelines for national radiation risk policy.

Because rodents are highly predictable surrogates for radiation cataractogenesis in humans, the observation that 100 mGy is cataractogenic in rats is of concern. This finding is supported by the ongoing UACOS study of ~8,600 Chernobyl clean-up workers which indicates that exposures far less than that suggested by current guidelines are cataractogenic.

8.13 The Ukrainian/American Chernobyl Ocular Study (UACOS)

The Chernobyl nuclear reactor accident in 1986 and resultant explosion and fire caused radioactive contamination of large areas of Belarus, the Russian Federation and Ukraine.

More than 250,000 individuals (Liquidators) were involved in clean-up and maintenance activities at the site. Many thousands were exposed to low doses of ionizing radiation.

The Ukrainian American Chernobyl Ocular Study (UACOS) was established in 1996 to monitor the effects of this radiation exposure on the eyes of clean-up workers. Among eye tissue, the lens is most radiosensitive. Time and dose dependent development of posterior subcapsular cataracts (psc) following radiation exposure is well established as a marker of radiosensitivity. The goals of the UACOS are to monitor development of psc in a subset of the Liquidator population that undergoes periodic health and ophthalmological examinations and for whom there is good bio-dosimetry data associated with the clean-up efforts.

In particular, this multi-decade, longitudinal study measures radiation cataract incidence, non-subjectively grades and records lens opacification using conventional slit-lamp biomicroscopy and Scheimpflug imaging and, in cases where cataracts are surgically removed, stores lens capsule-epithelial fragments for biochemical and molecular biological analysis.

The most recent findings from this study support a significant lowering of the supposed cataract “threshold” radiation dose and call into question the prevailing view of radiation cataract as a deterministic event (Worgul, 2007). At a minimum, the data indicates that the threshold for radiation cataract is far lower than current guidelines imply. The broader scientific implications of this study point to development of radiation cataract as a stochastic event and lend additional support to a re-evaluation of current risk-assessment standards.

More specifically, UACOS is a cohort epidemiological study (with a nested case control subset) of cataract onset and progression using standardized subjective parameters in 8,607 “*Liquidators*” responsible for the cleanup of radioactive materials after the Chernobyl nuclear power plant accident in 1986 (Worgul, 1999). Twelve and fourteen years after the accident, comprehensive ophthalmic examinations were conducted on individuals who averaged 33 years of age at exposure and thus were at low risk for any lens opacification. Analyses focused on data collected at the first examination (prevalence) and second examination two years later (incidence) utilizing a variation of the Merriam/Focht radiation cataract scoring method (Worgul, 2003). Using corrected gamma dose estimates, the individual beta dose values were calculated and individual uncertainty distributions simulated. At the first exam, 12 years after exposure and at an average age of 45, a 30% prevalence of pre-cataractous changes was noted with a 20% prevalence for stage I opacification. While not visually disabling, these early lens changes in a relatively youthful population at low risk for cataract suggest that the small doses to which most Liquidators were exposed has already begun to cause pre-cataractous lens changes. Even though most study subjects had not yet progressed to stage 2 cataract or greater, with accompanying visual loss, the evidence to date already points to a dose threshold no greater than 700 mGy with ongoing study likely to lower the threshold response in future years. For stage I posterior subcapsular radiation cataracts, the maximum likelihood estimate of a dose threshold was 350 mGy.

Most importantly for the risk assessment community, the latest findings strongly indicate that the current ICRP guidelines following fractionated or prolonged exposures of a 5 Gy threshold for detecting opacities and 8 Gy for visual impairment (ICRP, 1991) are much too high. Similarly, NCRP and UNSCEAR recommendations for eye exposures of 2 Gy-Eq per year also appear unwise (UNSCEAR, 1988; NCRP, 1993). Even current ICRP occupational guidelines for exposure limits to the lens, currently 150 mGy/year, may need to be re-evaluated in light of the present findings and any continued follow-up and presumed cataract progression in the Liquidator population.

If, indeed, there is a threshold for cataracts, it may be as much as an order of magnitude lower than current guidelines permit. Knowing that the latent period for radiation cataract is

inversely related to dose, continued follow-up of the UACOS cohort will likely result in a further lowering of the presumed radiation cataract threshold. As the average age of the Liquidators is only 53 years and 94% received exposures less than 400 mGy, it is certain that continued ophthalmological examination over the next 20 years or longer will provide additional statistical support for this hypothesis. This conclusion is further supported by re-analysis of the atomic bomb cataract data 56 years after exposure (Nakashima, 2006) which, at its lower limit for statistical significance, reported a zero dose threshold for cataract development. The findings in the Liquidator population are also in agreement with those from experimental low-dose radiation cataract animal studies (discussed above) and the types of opacities observed are compatible with the generally accepted pathomechanism of radiation cataract.

8.14 Conclusions

New data from animal models and from exposed human populations suggests that lens opacities occur at doses far lower than those generally assumed to be cataractogenic and these observations are consistent with the absence of a dose threshold. Given that all national and international risk standards for ocular exposure are predicated on a relatively high threshold, current risk guidelines for ocular radiation safety require reassessment.

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9 CONCLUSIONS AND POTENTIAL IMPLICATIONS

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9.1 Introduction

This document provides the background, summarizes the presentations and presents the main conclusions and potential implications of the Scientific Seminar on New Insights in Radiation Risk and Basic Safety Standards organized by the European Commission in Luxembourg on 17 October 2006. It takes into account the discussions that took place during the Seminar and during the subsequent meeting of the Article 31 Group of experts on 18 October 2006, although it is not intended to report in an exhaustive manner all the opinions that were expressed. The document has been submitted for comments to the lecturers, as far as their contributions were concerned.

9.2 The Article 31 Group of Experts and the Rationale of the RIHSS Seminars

The Article 31 Group of experts is a group of independent scientific experts referred to in Article 31 of the Euratom Treaty, which assists the European Commission in the preparation of the EU Basic Safety Standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiation. According to the Euratom Treaty and to their Code of Ethics, this group of experts has to give *priority to the protection of health*, to the safety and to the development of the best available operational radiation protection. They may express views on political, economical, financial and liability matters but the health and safety considerations must always be clearly identifiable in their opinions, proposals, guidance and statements.

Therefore when there is scientific plausibility of the existence of a risk of serious harm for health, even if there is still uncertainty, they should alert the EU Commission (precautionary principle).

Although the worldwide international genesis of the regulation regarding radiation protection (UNSCEAR, ICRP, IAEA …) is very useful and stimulates harmonisation and cross-validation, the national and EU regulators and their advisers still have to take their *own responsibility* for the evaluation of the data and the regulatory framework for the safety of the population of their countries. Regulations always include *value judgments*, which can vary between countries and cultures, and, even *within* the scientific evaluations, ethical issues are sometimes deeply interwoven. In this context, the RIHSS Working Party of the Article 31 Group of experts was set up, with the task of helping to identify the potential implications of recent research results or new data analysis on the European Basic Safety Standards (BSS) Directive and on the related Recommendations and guidance.

* This summary was prepared by the Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of experts. The following members of the Working Party contributed to the preparation of this document: L. Lebaron-Jacobs, W-U Müller, P. Olko, S. Risica, P. Smeesters (Chairman of the WP), R. Wakeford. They were assisted by external experts (J. Piechowski and A. Susanna) and by the following official of the European Commission: J. Naegele.

On the basis of input from the Directorate General Research of the European Commission and of information provided by individual members of the Article 31 Group of experts, each year the RIHSS Working Party proposes relevant themes to the Article 31 Group that could be discussed during a subsequent seminar. After selection of the theme and approval of a draft programme by the Article 31 Group, the RIHSS Working Party deals with the preparation and the follow up of the seminar. The seminars involve invited speakers who are asked to synthesize clearly the state-of-the-art in the field, with special attention paid to new information. Additional experts, identified by members of the Article 31 Group from their own country, take part in the seminars and act as peer reviewers. The Commission convenes the seminars on the day before a meeting of the Article 31 Group, in order that members of the Group can discuss the potential implications of the combined scientific results on the European legislation and related documents.

9.3 Background and Purpose of the 2006 Seminar

9.3.1 Background

In 2004, the International Commission on Radiological Protection (ICRP) offered for public consultation a second Draft of its future Recommendations (Draft 2005 ICRP Recommendations). To perform a critical review of this draft, the EU Commission organized, with the help of the RIHSS WP, a Conference that took place in Luxembourg on 4 November 2004. A list of highlights of this Conference has been identified and submitted to ICRP for consideration.

Recently, ICRP issued a third draft of Recommendations for public consultation. The purpose of the 17 October 2006 seminar was to review this new proposal in the light of current insights in radiation risks.

9.3.2 Questions and concerns

P. Smeesters, chairman of the RIHSS WP, introduced the Seminar by listing scientific and regulatory concerns related to – and challenging - the new draft ICRP Recommendations, many of which having already been mentioned during the 2004 Conference and being addressed by the speakers invited to the 2006 Seminar.

- Regarding cancer risk estimation, the main issue is the value of the **DDREF**: as a lot of recent epidemiological data seem to point consistently to a DDREF lower than 2, a DDREF of 2 is currently more difficult to justify. From a Radiation Protection point of view, we need indeed *strong evidence* for assuming a lower risk per unit dose at low or protracted exposures than for high acute doses.
- Regarding the issue of the **inter-individual differences**, as the radiation-induced cancer risk is significantly different for women (v/men) and for children (v/adults), particularly for certain tissues (breast cancer in women, thyroid cancer in children ...), it seems more appropriate, based on equity considerations, to promote **age** and **gender** specific protection measures, such as specific w_T or specific organ dose constraints/limits. Similarly, it seems to be appropriate to proactively take into account differences in **genetic susceptibilities** to radiation-induced effects, particularly in high dose situations (some medical exposures, rescue workers …).
- Regarding genetic risk, based on the current evidence, one can still doubt if the radiation-induced genetic risk is really practically limited to **2 generations**. Taking the numerous uncertainties into account, we should not neglect the possibility of significant long term risks. The genetic consequences of **continuous** exposure of many consecutive generations (as may be the case after a major accident or increased use of medical IR) seem to be insufficiently taken into account.

- Regarding **pregnancy**, the **uncertainties** in the evaluation of the effects of in utero exposures are still insufficiently emphasized and taken into account. It is unwise from a radiation protection perspective to present or suggest the figure of **100 mGy** to the embryo/foetus as being a kind of "limit of concern" in medical exposures and prolonged exposure situations.
- Regarding radiation-induction of **cataract**, new data challenging the current dose threshold are available. ICRP should explicitly take these data into account in its judgements and recommendations, particularly in the relatively high dose situations that can occur in medical applications and in prolonged exposures. If the scientific evidence is sufficient, ICRP could already now propose revised dose limits for the lens of the eye.
- There are also new data available pointing to the possible induction by radiation of **non-cancer diseases** at much lower doses than currently assumed. While it is clear that no definite answer can be given regarding the shape of the dose-response at low dose and regarding the numerical values of possible threshold doses, that situation is to a certain extent comparable with the situation ICRP faced in the past regarding cancer induction. ICRP should take these data explicitly into account in its judgements and recommendations, particularly in the relatively high dose situations that can occur in medical applications and in prolonged exposures.
- Regarding exposures to **radon indoors,** the European pooled analysis showed that lung cancer risk for residential exposures to Rn is statistically significant even at concentrations lower than 200 Bq/m³. Are the two unchanged values for the constraints for Rn (600 Bq/m³ for dwelling and 1500 Bq/m³ for work places) still enough protective?
- Globally, in the perspective of public health protection, major concerns are related to the question whether ICRP underlines enough the uncertainties and adopts sufficiently the **precautionary approach**.

9.4 Main Points Arising from the Presentations

9.4.1 Radiation-induced cancers and age/gender sensitivities

M. P. Little: *New models for evaluation of the radiation-induced lifetime cancer risk*

M. P. Little was consultant for the soon to be available UNSCEAR Report. His new radiation-induced lifetime cancer risk evaluations are based on current A-bomb dosimetry (DS02) and data (10 years more follow up of cancer mortality and incidence) and on new risk models. *UNSCEAR 2006 mortality models give risks a bit lower than, but consistent with, many previous sets of cancer risk estimates,* mainly as a result of the form of (solid cancer) models used, with only a small contribution (about 10-15%) from new dosimetry (DS02).
For a test dose of 0.1 Sv, the UNSCEAR 2006 cancer mortality risk (applied to current UK population) is 6.0 % per Sv (using a linear-quadratic model implying a DDREF of 1.2-1.3). This is a bit lower than the last BEIR estimation (8.33-10.6) but similar to the current ICRP evaluation at low dose and dose rate (5 % per Sv with a DDREF of 2). Substantially lower risks, 3.5% per Sv, are predicted by models using a linear-quadratic-exponential model, a result of the linear term in the dose response becoming substantially smaller (and statistically non-significant) when the exponential term is added. Whether the linear-quadratic-exponential model is correct depends on whether one would expect repopulation of the appropriate stem cell population after exposure at the dose levels received by the atomic bomb survivors.

E. Cardis: *New epidemiological data*

E. Cardis presented the conclusions of BEIR VII regarding the DDREF (Dose and Dose Rate Effectiveness Factor). Based on A-bomb survivor solid cancer incidence data below 1.5 Gy, in conjunction with animal data, the committee adopted a DDREF of 1.5, with an uncertainty interval of 1.1 - 2.3. This estimation was referred to as "LSS DDREF" (not a universal figure). The BEIR VII committee concluded also, based on a comprehensive review of the literature, that the risk would continue in a linear fashion at lower doses without a threshold and that the smallest dose has the potential to cause a small increase in risk to humans.
E. Cardis presented also recently published results of large epidemiological studies. The "15-Country study of cancer risk among radiation workers in the nuclear industry" (a retrospective cohort study) gives direct estimations of the effect of low dose, protracted exposures to external-photon radiation. Estimates are statistically consistent with extrapolations from A-bomb data. Confounding by smoking cannot be ruled out but is unlikely to explain all the increase.
The Techa River cohort study addresses a large population exposed to external and internal radiation due to the releases from the Mayak Industrial Association (protracted exposure and long follow up). Risk estimates are higher than linear extrapolations from A-bomb survivors, but there are still dosimetric uncertainties (role of short lived isotopes currently under review). Both studies have limitations but they suggest the existence of a small risk at low doses.
Finally, E. Cardis reviewed briefly the data concerning the age at exposure and gene-radiation interactions. While there is no clear evidence of an age at exposure effect in the Techa River Study, compelling evidence from other populations indicate that children are more susceptible. Regarding genetic susceptibility to radiation induced cancer, E. Cardis drew the attention on a recent publication suggesting an effect of chest X-rays on the risk of breast cancer among BRCA 1/2 mutation carriers.

M.Tirmarche: *Large scale indoor radon studies: lung cancer and leukaemia risk*

M.Tirmarche reported on the joint European analysis (Darby et al) that made it possible to analyse the indoor radon risk on a total of more than 7000 cases and 14000 controls.
The results show a clear linear dose-response relationship between radon in houses and lung cancer risk. The lung cancer risk is increasing with cumulated radon exposure: RR = 1.08 for 100 Bq/m³ CI 95% = [1.03 – 1.16]
There is a *significant relationship even if limited to those exposed to ≤ 200 Bq/m³*.
There is also a significant increase in the non-smokers population, although smoking habits increases the value of the risk coefficient.
M.Tirmarche concluded that the results of this large pooled study are convincing and noted that, in parallel, Krewski and al have published in 2006 major studies from North America, with results confirming those observed on European data.
Concerning leukaemia, there is not yet enough evidence of a link between childhood leukaemia and indoor radon, but this subject should be studied in future in order to learn more on all possible risk factors in relation to childhood leukaemia.

W.-U. Müller: *Biological aspects in relation to age/gender sensitivities and discussion of potential implications*

According to W.-U. Müller, the current radiation protection system does not take sufficiently into consideration age and gender dependent differences in some contexts. “This is understandable against the background of the attempt to keep the regulations simple and pragmatic. Nevertheless, it is *worth to re-think the regulations from time to time whether it might be reasonable to improve radiation protection, because it turned out that the rules were too simple to guarantee efficient protection*. “

He underlined 4 issues for which he proposed practical solutions.

1. The concept of the „effective dose“ is not applicable to the embryo/foetus due to the impossibility to generate tissue-weighting factors for the foetus. Otherwise adult tissue-factors are used for children. A possible solution for children is the use of organ doses and of dose limits based upon organ doses (and not on effective dose).
2. The second issue is the dose constraint of 100 mSv (acute or per year) in interventions. This constraint also applies to sensitive subgroups like embryo/foetus and children.
   A possible solution is to use markedly lower dose constraints for sensitive subgroups.
3. In paragraph 81, the new draft ICRP states: „...in utero exposure should not be a specific protection case in prolonged exposure situations where the dose is well below about 100 mSv“. Such a statement may make some physicians very careless in medical exposures. Such statements should be avoided.
4. Averaging of female and male risk.
   The suggestion is that experts should stick to the pure scientific mandate and leave social decisions (like the gender question) to the policy makers.

D. Averbeck: *New biological data in relation with low dose risk*

Among the lesions induced by ionizing radiation (IR) in cellular DNA, some are rather complex such as Double Strand Breaks (DSB) and Locally Multiply Damaged Sites (LMDS). In mammalian cells, DSB are linearly induced with IR dose and are undoubtedly very deleterious lesions. LMDS are predicted to consist of local clusters of different types of lesions but still remain difficult to be properly defined and quantified. There is some evidence that LMDS are high obstacles for repair and are much more lethal than mutagenic. Thus, according to D. Averbeck, “in contrast to previous expectations (BEIR VII, ICRP), their impact on radiation-induced mutagenesis and carcinogenesis should conceivably be relatively low or even absent”.

D. Averbeck pointed to recent studies showing *differences in the responses of living cells according to the dose and dose-rate of IR.* He outlined that different gene families are induced and different types of proteins activated at high and low doses or dose-rates. According to him, at low doses and dose-rates, IR responses involve wide ranging metabolic networks, whereas “at high doses and dose rates, cellular responses are more directly channelled towards survival, genomic instability and malignant transformation or cell death”. He referred to the findings of Rothkamm and Löbrich, suggesting that, at very low doses (1 mGy) or dose rates (1.5 mGy/min) of low-LET radiation, DNA damage signalling towards the activation of DNA repair is compromised, the few damaged cells in the population being eliminated by cell death, while, at higher doses (5 mGy) and dose-rates (4 mGy/min), DNA damage signalling pathways towards cell cycle arrest and DNA repair and/or apoptosis are activated. He concluded that, owing to these results, a unique DDREF as proposed by ICRP is not applicable and that “human risks from low dose exposures are likely to be much lower than expected from LNT calculations.”

D. Averbeck indicated also that non-targeted IR effects such as adaptive responses, radiation hypersensitivity, bystander effects and genomic instability are likely to influence dose-effect relationships for mutation induction and carcinogenesis of IR, particularly at low doses and dose-rates. However, *the mechanisms involved and their quantitative impact need to be clarified.*

Finally, while underlining that there is evidence for individual radiation sensitivity due to individual defaults in DNA damage signalling and repair, and while recognizing that this may modify the individual radiation risk, he judged that cases of individual radiation sensitivity are too rare for affecting risks for the general population.

9.4.2 Radiation-induced genetic risk and non-cancer diseases

K. Sankaranarayanan: *Ionizing Radiation, Genetic Risks and Radiation Protection*

K. Sankaranarayanan provided an overview of the concepts, assumptions and methods used in the estimation of genetic risks associated with the exposure of human populations to ionizing radiation and presented the estimates published by the United Nations Scientific Committee on the Effects of Atomic radiation (UNSCEAR) in 2001. These risk estimates have recently been used by the International Commission on Radiological Protection (ICRP), in the document on new recommendations, for deriving genetic risk coefficients to assess the relative contribution of genetic effects to total detriment. A major difference between genetic risk coefficients in this new ICRP document and those used in the ICRP Publication 60 (1991) is the following: in the new document, the estimated genetic risks for the first two post-radiation generations have been used for the calculations whereas in Publication 60, the 5-fold larger predicted risks at equilibrium constituted the basis. The implication of this conceptual change in the new document is that the relative contribution of genetic effects to the total detriment is now much lower than was assumed previously. The reasons for this conceptual change are discussed.

In particular, K. Sankaranarayanan commented on the concerns expressed by the Art 31 Group of Experts during the 2004 Conference (see *2.1 Background* in the present section), i.e. concerns about ICRP limiting the genetic risks estimate to 2 generations and about lack of precautionary approach.

He agreed that, when evaluated on comparable bases (risk for the first generation, for 2 generations …), the genetic risk is not reduced in the UNSCEAR 2001 Report by comparison with the previous UNSCEAR Report. He declared also very clearly that" it was never stated that the risks to generations beyond the second is zero". Having said this, he reminded the audience of ICRP's reasons for limiting attention to the estimates for the first two generations and not to those at the new equilibrium. First, he said, risk predictions at the new equilibrium imply "the totally unrealistic and untestable assumptions that (a) the circumstances (e.g., demographics, advances in medicine and health care) of human populations will remain constant over very long periods of time and (b) the estimates of the various parameters used to estimate mutation component and other quantities in the risk equation will remain unchanged over tens or hundreds of human generations." Second, "advances in molecular biology have raised strong doubts about the concept that radiation will induce genetic diseases that are similar to those that arise as a result of spontaneous mutations". According to him, among the types of damage induced by radiation, multi-gene deletions appear to be the principal ones and they are expected to manifest themselves phenotypically as multi-system developmental abnormalities. Individuals with these are expected to be severely handicapped and the chance of propagation beyond the first few generations is therefore predicted to be small.

During the 2004 Conference, Dutrillaux explained that the main problem is the radiation induction of *small* deletions leading to recessive mutations and diseases whose *phenotypes* might frequently not be *recognized* by the physicians. Such cumulative small genetic disorders may propagate in the future generations with the risk of leading to more important pathological consequences.

While recognizing that this is an important concern, K. Sankaranarayanan pointed to Lüning's

studies conducted in the late 1960s and 1970s to answer the question whether radiation-induced *recessive lethal mutations* would accumulate in mouse populations subject to radiation in every generation. Lüning went up to 15 generations of radiation but could not demonstrate accumulation of recessive lethal mutations over time. Several other population genetic studies by others focused on *fitness* in irradiated mouse populations also came up with negative results and were therefore abandoned (in contrast to studies with irradiated Drosophila populations where good evidence for accumulation of recessive lethal mutations

could be obtained) Why is this so? In part, said K. Sanka, "this could be explained by the sample sizes and techniques that could be used in the mouse for screening for recessive lethals which were not as efficient as in Drosophila. It now seems probable that this difference in part may also be related to differences in genomic architecture between Drosophila and the mouse and their response to radiations". Computer simulations conducted to examine the extent to which the incidence of recessive diseases will increase in human populations exposed to radiation in every generation, showed that one would not be able to demonstrate an increase by generation 15, and seemed thus to confirm Lüning's observations.
K. Sankaranarayanan did not address the issue of the relevance of the *pathological indicators* used in these studies, i.e. Dutrillaux's hypothesis that the real problem could be the radiation-induction of small genetic disorders or polymorphisms leading to rare and not recognized human phenotypes.

J. Angulo *New data on genetic risk: Mutagenesis of Repeated DNA regions to assess the risk of chemical and physic genotoxic agents*

Angulo reminded first the audience to Dubrova's work, who detected increased germline mutagenesis in various human populations exposed to ionizing radiation, by genotyping minisatellite loci. Molecularly, "minisatellites" are composed of tandemly repeated DNA sequences with a high spontaneous mutagenic rate. He noted that, initially, repetitive DNA sequences were considered to lack any functional significance, and for this reason were called "junk DNA". However, today several diseases are known to be associated with changes in these DNA arrays. In the particular case of microsatellites, there are more than 40 neuropathies associated with contraction or expansion.

In mouse, repeated loci called ESTR are used to monitor germline mutagenesis. Chemical and physical genotoxic agents induce a similar increase in the mutation rate and the mutation rate increases linearly with radiation dose. Moreover, *the detected increase is similar when comparing the coding and noncoding regions* of the genome, indicating that this is not a process exclusively linked to repetitive sequences. Angulo concluded that genotyping of DNA using repeated loci is a robust and convenient method to monitor mouse germline mutagenesis and raises the possibility of testing low doses of chemical and physical genotoxic agents that were impossible to analyse before.
It should be noted that similar number of germline mutations are induced by acute and chronic exposures. Current estimates of the genetic hazards of low-dose radiation have been extrapolated from data obtained using high doses of radiation, and assuming that chronic radiation exposure induces 25-33% as many germline mutations as an acute dose. According to Angulo, "the data presented here on the similar mutagenicity of chronic and acute low-LET exposure indicate that this extrapolation does not hold true". The risk factors for chronic and acute irradiation for this dose range should be regarded as similar. This observation may be particularly important for the estimation of the genetic hazards to the human germline.

Angulo presented also new data indicating that *germline mutagenesis depends on the integrity of DNA repair systems.* This was possible by using genetically modified mice mimicking well-known cancer-prone human diseases. His data support the idea that a delay in repair of DNA damage in mice may slow down replication fork progression, which, in turn, affects ESTR mutation rate. The increased instability in the germline of genetically modified mice allowed the identification of two genes coding for proteins that recognise single- and double-strand breaks as being involved in the enhanced ESTR mutagenesis induced by ionising radiation.
In conclusion, according to Angulo, ESTR mutagenesis studies allow the direct characterisation of recessive mutations and modification of noncoding DNA regions. This contrasts with most of the approaches used until now, in which recessive mutation induction

is estimated by extrapolation from dominant mutations. The *possibility that small DNA modifications induced by IR destabilise replication and produce a "cascade effect" leading to a general enhanced mutagenesis* can now be tested using specific mutants. "This will certainly help to improve germline risk estimates".

N. Kleiman: *Radiation-induced cataracts: new evidence*

Present ocular guidelines are predicated on the view that cataractogenesis is a deterministic event and requires a **threshold** radiation dose before lens opacities will develop (NCRP, 1993, 2000). Yet, in populations exposed to far lower doses of radiation, including those undergoing CAT scans (Klein, 1993), radiotherapy (Wilde, 1997; Hall, 1999), the astronaut pool (Cucinotta, 2001; Rastegar, 2002), atomic bomb survivors (Minamoto, 2004; Nakashima, 2006), residents of contaminated buildings (Chen, 2001) and the Chernobyl accident "liquidators" (Worgul, 2003, 2007), dose-related lens opacification at exposures significantly lower than 2 Gy was reported. As an example, in the Ukrainian American Chernobyl Ocular Study (UACOS) established in 1996 to monitor the effects of radiation exposure on the eyes of Chernobyl clean-up workers, the data indicate that the threshold for radiation cataract is far lower than current guidelines imply. Even though most study subjects had not yet progressed to stage 2 cataract or greater, with accompanying visual loss, the evidence to date already points to a dose threshold no greater than 700 mGy with ongoing study likely to lower the threshold response in future years (Worgul, 2007). For stage I posterior subcapsular radiation cataracts, the maximum likelihood estimate of a dose threshold was 350 mGy. These findings strongly indicate that the current ICRP guidelines following fractionated or prolonged exposures of a 5 Gy threshold for detecting opacities and 8 Gy for visual impairment (ICRP, 1991) are much too high. Even current ICRP occupational guidelines for exposure limits to the lens, currently 150 mGy/year, may need to be re-evaluated in light of the present findings and any continued follow-up and presumed cataract progression in the Liquidator population. "If, indeed, there is a threshold for cataracts, it may be as much as an order of magnitude lower than current guidelines permit."
This conclusion is further supported by re-analysis of the atomic bomb cataract data 56 years after exposure (Nakashima, 2006) which, at its lower limit for statistical significance, reported a zero dose threshold for cataract development. The findings in the Liquidator population are also in agreement with those from experimental low-dose radiation cataract animal studies.
Recent findings demonstrate dose-related significant lens opacification after exposure of rats to as little as 100 mGy X-rays.

Moreover, new observations even are consistent with the absence of a dose threshold**.** Although the mechanism of radiation induced cataracts is not known precisely, genomic damage resulting in altered cell division, transcription and/or abnormal lens fibre cell differentiation is now considered to be the salient injury, rather than cell killing. For this reason, the classification of cataracts as a deterministic effect must be called into question. Several lines of evidence from experimental and epidemiologic studies strongly suggest a **stochastic** basis for radiation cataracts. Animal studies have shown that individuals that are haplo-insufficient for multiple genes involved in DNA damage repair and/or cell cycle checkpoint control may be more susceptible to the cataractogenic effects of ionizing radiation than wild-types. Atm, Brca1 and Rad9 heterozygotes demonstrate enhanced sensitivity to radiation-induced cataract formation (Kleiman, 2007). The roles of Atm, Rad9 and Brca1 in the cell cycle and during DNA repair are consistent with a genotoxic basis for radiation cataractogenesis. These findings may have important implications for radiosensitive subsets of the human population and for the astronaut core.

Globally, new data from animal models and from exposed human populations suggest that lens opacities occur at doses far lower than those generally assumed to be cataractogenic and these observations are even consistent with the possible absence of a dose threshold.

Given that all national and international risk standards for ocular exposure are predicated on a relatively high threshold, *current risk guidelines for ocular radiation safety require reassessment.*

9.5 Conclusions and Recommendations

During the meeting of the Article 31 Group of Experts on 18 October 2007, the data that had been presented and discussed during the Seminar have been thoroughly debated, particularly as regards their potential impact on the future EU and international BSS. The following key points were agreed upon by the Article 31 Group of Experts, which proposed to the EU Commission to convey them to ICRP. Note that concerns related to equitable management of inter-individual differences (age, sex, genetic susceptibility) have been again expressed and amply debated, but no common stand could be obtained on how to take this into account.

Key points agreed by the EURATOM Article 31 Group of experts:

- **Cataracts**
  - **Current threshold concept and current yearly dose limit for lens are challenged by new evidence**
  - **Revisiting the relationship between the dose to the lens and the occurrence of cataracts should not be postponed**
  - **Revisiting the protection system (for instance introducing lens dose constraints based on good practice) should not be postponed**

- **Pregnancy : the 100 mSv figure**
  - **Due to the uncertainties and to childhood cancer risk, formulations which could be interpreted as 100 mSv during pregnancy being the « limit of concern » in medical exposures and prolonged exposure situations would be unacceptable**
  - **There is a real danger that such a 100 mSv figure would be considered as a general threshold : ICRP should warn against this misinterpretation**

- **Genetic risk**
  - **There was agreement that genetic risk is not limited to two generations and that there are still uncertainties related to the following generations that should be clarified**
  - **This should be explicitly recognized**

- **DDREF (Dose and Dose Rate Effectiveness Factor)**
  - **A central estimate of DDREF that is lower than 2 is indicated by the currently available evidence**
  - **This is reflected by the central estimate of the DDREF of 1.5 adopted by the BEIR VII Report**
  - **From a Radiation Protection point of view, adopting the position of BEIR VII regarding DDREF seems to be more justified.**

- **Radon**
  - **New epidemiological data show statistically significant effects at radon concentrations lower than 200 Bq m^{-3}**
  - **From a public health perspective, taking action for radon concentrations as low as 100 Bq m^{-3} for dwelling might be justified,**

depending on national situations

- **The constraints (reference levels) proposed by ICRP (identical to the old higher action levels: 600 Bq m^{-3} for dwelling and 1500 Bq m^{-3} for work places) should be reassessed in the light of the new evidence.**

These key points were presented by the Chairman of the RIHSS WP, on behalf of the EU Commission, to the international community during the NEA/ICRP Forum meeting in Prague on 24-25 October 2006 (The Future Policy for Radiological Protection: a stakeholder dialogue on the implications of the ICRP proposals). These concerns will be addressed further by the Article 31 Group of experts in their discussions of the next Basic Safety Standards.