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Committee 1 Task Group Report

Low-dose Extrapolation of Radiation-Related Cancer Risk

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PREFACE

Following its meeting in Oxford, UK, in 1997, Committee 1 of ICRP (the International Commission on Radiological Protection) proposed a Task Group to prepare a report on low-dose extrapolation of radiation-related cancer risk estimates based largely on higher-dose epidemiological data, and the possible implications for radiological protection. The Commission accepted this recommendation and established a Task Group, which began its work in April, 1998.

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EXECUTIVE SUMMARY

The present report, of a task group of ICRP Committee 1, considers the evidence relating to cancer risk associated with exposure to low doses of low-LET radiation, and particularly doses below current recommended limits for protection of radiation workers and the general public. The focus is on evidence regarding linearity of dose response for all cancers considered as a group, but not necessarily individually, at low doses (the so-called linear, no-threshold (LNT) hypothesis), and the possibility of a universal threshold dose below which there is no risk of radiation-related cancer. According to the LNT hypothesis, the same number of radiation-related cancers would be predicted in a population of a given size exposed to a certain small average radiation dose and in an otherwise similar population many times times larger and exposed to a proportionally smaller average dose. According to the threshold hypothesis, the radiation-related risk in the larger population would be zero if its average dose were sufficiently small.

The present document has been preceded by other, recent reports, notably those of the United Nations Scientific Committee on the Effects of Atomic Radiation and the U.S. National Council of Radiation Protection and Measurements. These reports recommended that radiation protection continue to be guided by the LNT hypothesis. The task group concurs with those recommendations.

The report is organized by scientific discipline, beginning with epidemiological studies of exposed human populations (Chapter 2). Epidemiological studies offer the most directly relevant information for risk-based radiation protection. The major scientific issues, as illustrated by the example of cancer incidence from all solid tumors combined in the Life Span Study (LSS) population of atomic bomb survivors, are (1) establishment of the existence of a dose-related risk in this population, (2) modeling radiation-related risk as a statistically uncertain parametric function of dose, modified by other factors such as sex, exposure age, attained age, and time following exposure, (3) extrapolation of estimated risk to other potentially exposed populations, with possibly different baseline cancer rates, (4) projection of the risk in the population to the end of its natural life, and (5) extrapolation of risk estimates from moderate-to-high dose levels of acute exposure, characteristic of the most informative atomic bomb survivor data, to the far more common low-dose and/or protracted exposures that occur in occupational and general settings. Consideration of each of these issues leads to more refined risk estimates but,

because information about each is uncertain, the overall uncertainty of the improved estimates is increased. There is some evidence of increased cancer risk associated with exposures on the order of 10 mGy which will be discussed in the report, and other evidence placing an upper limit on the value of any universal threshold that might exist. Also, the risk of mortality and morbidity from all solid cancers combined is proportional to radiation dose down to about 100 mGy, below which statistical variation in baseline risk, as well as small and uncontrollable biases, tend to obscure evidence concerning radiation-related risk. Extrapolation of risk estimates based on observations at moderate to high doses continues to be the primary basis for estimation of radiation-related risk at low doses and dose rates, for example at the present recommended limit for members of the public of 1mGy per year from non-medical man-made sources.

The fundamental role of radiation-induced DNA damage in the induction of mutations and chromosome aberrations and the apparent critical involvement of aberrations and mutations in the pathogenesis of cancer provides a framework for the analysis of risks at low radiation doses and low dose rate exposures (Chapter 3). A characteristic type of damage produced by ionizing radiation (IR) involves multiple lesions within close spatial proximity. Such clustered damage can be induced even by a single radiation track through a cell. Although cells have a vast array of damage response mechanisms that facilitate the repair of DNA damage and the removal of damaged cells, these mechanisms are not fool-proof, and emerging evidence suggests that closely spaced lesions can compromise the repair machinery. Also, while many of the cells containing such radiation-induced damage may be eliminated by damage response pathways involving cell cycle checkpoint control and apoptotic pathways, it is clear from analysis of cytogenetics and mutagenesis that damaged or altered cells are capable of escaping these pathways and propagating.

Cellular consequences of radiation-induced damage (Chapter 4) include chromosome aberrations and somatic cell mutations. The processing and misrepair of radiation-induced DSBs, particularly complex forms, are responsible for chromosome/gene alterations that manifest as chromosome aberrations and mutations. Current understanding of mechanisms and quantitative data on dose and time-dose relationships support a linear dose response at low doses (i.e., LNT) for total cancer risk. Considered as a whole, the emerging results with regard to radiation-related adaptive response, genomic instability, and bystander effects suggest that the risk of low level

exposure to ionizing radiation is uncertain, and a simple extrapolation from high dose effects may not be wholly justified in all instances. However, a better understanding of the mechanisms for these phenomena, the extent to which they are active *in vivo*, and how they are interrelated is needed before they can be evaluated as factors to be included in the estimation of potential risk to the human population of exposure to low levels of ionizing radiation.

Experimental approaches using animal models (Chapter 5) are well suited to precise control of radiation dose and dose rate, as well as genetic background and other possible modifiers of dose response, and can facilitate precise determination of biological outcomes. Recent studies using newly developed animal models, cellular, cytogenetic and molecular data for acute myelogenous leukemia (AML), intestinal tumors, and mammary tumors, and cytogenetic and molecular studies on the induction of AML and mammary cancer support the view that the essential radiation-associated events in the tumorigenic process are predominantly early events involving DNA losses targeting specific genomic regions harboring critical genes. As such, the response for early initiating events is likely to correspond to that for the induction of cytogenetic damage. On this basis, mechanistic arguments support a linear response in the low dose region, i.e., the process should be independent of dose rate because interactions between different electron tracks should be rare. Quantitative analyses of dose responses for tumorigenesis and for life shortening in laboratory animals also support this prediction. These studies also support a dose and dose rate effectiveness factor (DDREF), for reduction of estimated risk per unit dose based on acute, high-dose data, in the range of about 2 when data are extrapolated to low doses from effects induced by doses in the range of 2-3 Gy. Extrapolation of results from less than 1 Gy would result in lower DDREF values.

Chapter 6 presents a formal exercise in quantitative uncertainty analysis, in which the different uncertain components (as identified in Chapter 2) of estimated cancer risk associated with low-dose, low-LET radiation exposure to a non-Japanese population, in this case that represented by the U.S. National Cancer Institute's SEER registry, are combined. Attention is paid to the resulting uncertainty distribution for excess relative risk per Gy (ERR/Gy), with and without allowing for the uncertain possibility of a universal low-dose threshold, below which there would be no radiation-related risk. In the example, which involves risk from all cancers combined including leukemia, except for non-melanoma skin cancer, the major sources of uncertainty are statistical variation in the

estimated ERR at 1 Gy for the atomic bomb survivors population, subjective uncertainty (informed by experimental and epidemiological data) about the DDREF to be applied at low doses and dose rates, and the postulated uncertainty concerning the existence of a universal threshold at some dose above that for which the calculation was being made. Unless the existence of a threshold was assumed to be virtually certain, the effect of introducing the uncertain possibility of a threshold was equivalent to that of an uncertain increase in the value of DDREF, i.e., merely a variation on the result obtained by ignoring the possibility of a threshold.

The conclusions of this report are given in Chapter 7. While existence of a low-dose threshold does not seem unlikely for radiation-related cancers of certain tissues, and cannot be ruled out for all cancers as a group, the evidence as a whole does not favor the existence of a universal threshold, and there seems to be no particular reason to factor the possibility of a threshold into risk calculations for purposes of radiation protection. The LNT hypothesis, combined with an uncertain DDREF for extrapolation from high doses, remains a prudent basis for radiation protection at low doses and low dose rates.

1. INTRODUCTION

The purpose of the present report is to summarize scientific evidence relevant to the quantification of cancer risk associated with radiation exposure at (effective) doses of interest for radiation protection, particularly doses below current recommended limits for protection of radiation workers (e.g., 20 mSv per year) and the general public (e.g., 1 mSv per year). (As a rough rule of thumb, effective doses on the order of 1 Sv, 100 mSv, 10 mSv, 1 mSv, and 0.1 mSv will be called “moderately high”, “moderate”, “low”, “very low”, and “extremely low”, respectively, in this report.)

Ionizing radiation exposure is an established cancer risk factor. Compared to other common environmental carcinogens, it is relatively easy to determine organ-specific radiation dose and, as a result, radiation dose-response relationships tend to be highly quantified. Nevertheless, there can be considerable uncertainty about questions of radiation-related cancer risk as they apply to risk protection and public policy, and the interpretations of interested parties can differ radically. A major reason for disagreement is that public and regulatory concern often is focused on exposures at radiation doses far lower than those at which useful information about cancer risk can be obtained directly, that is, than can be obtained by studying populations with such exposures. Thus, risk estimates promulgated by expert committees, for example, are usually based upon epidemiological dose-response data obtained at doses ranging up to 0.2 Gy, 0.5 Gy, 1 Gy, or higher, and the resulting estimates are then extrapolated, with appropriate caveats, to lower doses. The extrapolation rules are based in part upon epidemiological observations, such as the degree of curvature of fitted linear-quadratic dose response models for leukemia and solid cancer morbidity among atomic bomb survivors, and on models derived from experimental systems.

The discussion in the present report is concerned ultimately with biological effects of ionizing radiations of low linear energy transfer (low LET), such as photons (gamma rays and X rays) and electrons (beta particles) of various energies, as contrasted with high-LET radiations such as neutrons and alpha particles. However, some biological effects that have been observed mainly in connection with high-LET exposure are clearly relevant to questions of cancer risk at low levels of low-LET radiation.

Currently, the ICRP radiation protection philosophy is based on the so-called linear, non-threshold (LNT) hypothesis, according to which, at low doses (on the order of 100 mGy or less) and dose rates (less than 6 mGy/hour averaged over the first few hours)

(UNSCEAR 1993, EPA 1999) total radiation-related cancer risk is proportional to dose. The hypothesis is not universally accepted as biological truth, but rather, because we do not actually know what level of risk is associated with very low-dose exposure, is considered by many as a prudent rule of thumb for public policy aimed at avoiding unnecessary risk from exposure.

A logical consequence of the hypothesis is that, at a sufficiently low dose D, exposure of N people to average dose D would result in the same number of radiation-related cancers as exposure of k H N people to average dose D / k, for arbitrary k > 1. This logical consequence can be used to justify the concept of "collective dose", that the product of average dose and the number of people exposed is proportional to the number of radiation-related cancers. The concept of collected dose is sometimes used to support a moral argument against widespread use of technologies or practices that would, according to the LNT hypothesis, involve individual exposures at doses so low that any associated risk, from the standpoint of the individual, would be far smaller than other risks that are casually taken in everyday life. A so-called threshold hypothesis, according to which there is no radiation-related risk associated with exposures at doses below some universal threshold dose, would obviate concern about exposures at doses below the threshold and, specifically, arguments based on the concept of collective dose. Aside from collective dose, however, it is worth emphasizing that the practical importance of the LNT vs. threshold question is associated with doses at which the associated risks, if they exist, are high enough to be of "legitimate" concern, as determined by the usual social and political processes.

Historically, the LNT vs. threshold controversy has been associated with public policy issues related to exposures that are widespread but (typically) low for individuals, such as local and worldwide exposure to radioactive fallout from above-ground nuclear test explosions carried out by different governments, mainly during the 1950s (Lewis, 1957, 1963; Caron, 2004). The threshold hypothesis, as applied to ionizing radiation and to fallout exposure in particular, drew some of its legitimacy from the field of chemical toxicology, where thresholds are the rule (Brues, 1958, 1960), whereas the LNT hypothesis is more consistent with findings from experimental radiation mutagenesis. As described by Caron (2004), the intellectual positions taken by proponents of the opposing sides during the fallout controversy of the 1950s (no compelling evidence of increased cancer risk at low radiation doses, vs. no compelling evidence against a radiation-related increased risk) are very similar to the situation at the present time. Some differences

discussed in the present report include the present general acceptance of a mutational basis for carcinogenesis, and evidence that radiation-related mutations tend to be more complex than more common mutations associated with endogenous and other causes.

The present report has been preceded by other surveys of the biological and epidemiological information that underlies our understanding of low-dose risk and its estimation by extrapolation from data obtained at higher doses, notably and recently the comprehensive reports of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR 2000, Annexes G and I) and of a committee of the U.S. National Council of Radiation Protection and Measurements, entitled “Evaluation of the Linear-Nonthreshold Dose-Response Model for Ionizing Radiation” (NCRP 2001). The existence of these reports has allowed the present ICRP Task Group to be somewhat less comprehensive in its coverage of the field than might otherwise have been necessary, and to concentrate on updated coverage of developments in areas of epidemiology, fundamental biology, experimental radiation mutagenesis and carcinogenesis, and uncertainty analysis.

Studies of cancer risk following exposure of human populations are the most obvious sources of information applicable to radiation protection policy. However, as discussed in Chapter 2, generalization of risk information, obtained from one exposed population, to other populations with different characteristics and potentially exposed to radiation from different sources, at different doses and dose rates, requires the use of dose-response models to describe the behavior of risk as a function of radiation dose, as well as possible modification of dose response by individual and environmental factors. It also requires making assumptions that are often based on uncertain information.

Chapter 3 deals with events believed to be fundamental to radiation carcinogenesis: radiation-induced DNA damage and its repair. In particular, the chapter discusses the nature of radiation-induced damage and damage response pathways including repair of DNA double-strand breaks (DSB), cell cycle checkpoint control, early sensors of DNA damage, and signal transduction after irradiation. Questions of particular relevance for the current investigation are comparability of molecular damage from radiation exposure and endogenous causes, and comparability between radiation-related damage from ionizing radiation at high cf. low doses and dose rates with respect to mechanisms, pathways, and fidelity of repair.

Cellular consequences of radiation-induced damage are discussed in Chapter 4. Rates of radiation-induced chromosome aberrations and somatic cell mutations were

among the earliest quantitative measures of the cellular effects of ionizing radiation, and studies of these outcomes have been highly informative about dose response over a wide range of doses, and about effects of dose rate and fractionation. Induction of bystander effects in cells not directly irradiated, genomic instability in the progeny of irradiated cells, and adaptive response are radiation-related phenomena that evoke questions about the generality of inferences based on cellular studies.

Considerations of statistical power, and possible bias due to unobservable and uncontrollable confounders, govern the extent to which useful epidemiological information can be obtained at exposure levels of regulatory interest, and some degree of extrapolation is unavoidable. Experimental approaches using animal models, discussed in Chapter 5, offer considerably more control of radiation exposure and dose, genetic background, and modifying factors including other exposures, and can facilitate very precise determination of biological outcomes. On the other hand, analogies between radiation-related risks in human beings on the one hand and inbred strains of experimental animals on the other are necessarily limited. Low statistical power for low-dose studies is problematic for experimental and epidemiological studies alike, but indirect approaches, based on protraction and fractionation of exposure resulting in moderate to high cumulative doses, offer insights into low-dose effects. Experimental studies can of course be replicated, to provide a firmer basis for insights into mechanisms, tissue modifying factors, and quantitative dose response.

The material of Chapters 2-5 highlights statistical variation inherent in estimates obtained by fitting parametric models to epidemiological and experimental data, but also more fundamental uncertainties about important factors that cannot be ignored, but about which there may be only limited information. The implications of these uncertainties for conventional estimates of radiation-related cancer risk, especially at low doses and/or low dose rates characteristic of exposures most commonly encountered by radiation workers and the general public, are investigated in Chapter 6. The approach taken is an exercise in quantitative uncertainty analysis similar to approaches used in a number of recent exercises by expert committees concerned with such risks. Central to the approach is recognition of the fact that radiation protection is a political process, responsive to the interests and perceptions of stakeholders with differing points of view, and relying upon a knowledge base that is extensive but also uncertain. Acceptance of this fact implies that it is important, for the benefit and information of participants and stakeholders in the radiation protection process, to identify sources of uncertainty and to quantify the

implications of such uncertainty for estimated risk. Among the questions addressed is the impact on radiation protection policy of treating the existence of a universal low-dose threshold for radiation-related cancer risk as an uncertain possibility.

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2. EPIDEMIOLOGICAL CONSIDERATIONS

2.1 Introduction

Like other areas of epidemiological research, the study of radiation-related cancer risk began with clinical observations, the earliest of which may have been the 16th century identification by the physician Georg Bauer (more often known by his Latinized name, Agricola) of a specific condition, which he called "Joachimsthal Mountain Disease", among miners in the Joachimsthal region of the present-day Czech Republic. The disease, the description of which now appears consistent with radon-related lung cancer but could also include other lung diseases such as silicosis (NAS/NRC, 1999; Toohey, 1987), was thought by Agricola to be caused by "metallic vapors" in mine atmospheres. Roentgen's discovery of x rays in 1895 and Becquerel's discovery of natural radioactivity the following year, and the subsequent use of both in science, medicine and industry, led to the recognition, documented by case reports early in its history, that radiation exposure might be harmful (Doll, 1995). The Court Brown and Doll study of mortality among British radiologists (1958; Smith and Doll, 1981; Berrington et al, 2001), which demonstrated a significantly increased risk of cancer mortality among radiologists who had registered with a radiological society before 1921 and who were therefore likely to have received higher doses than radiologists who began their practice later, is an example of an influential study in which the fact of exposure was related to risk but individual dose estimates were not available. However, experimental studies of radiation effects such as cell inactivation, mutation, and carcinogenesis have taken advantage of the experimenters' ability to regulate, with precision, radiation dose to target cells or tissues. Similarly, epidemiological investigations of exposed populations have benefited enormously from information enabling scientists to reconstruct individual, and even organ-specific, radiation doses. Benefits include the estimation of dose-response relationships and of the modification of such relationships by individual properties such as sex, age, lifestyle, and genetic inheritance. Thus, dose reconstruction is a fundamental component of the epidemiology of radiation carcinogenesis, and tends to be well worth the often considerable effort and expense required.

"Risk" is a concept in common use that is often applied to the past and future experiences of individuals, but a numerical risk value can be estimated and verified only on the basis of population rates, e.g., by comparing cancer rates, in a population exposed

to a given radiation dose, with rates in an otherwise comparable population that is either not exposed or exposed to a much lower radiation dose. Thus, when we speak of an individual's risk we are really referring to a property of a population similar to that to which the individual is assumed to belong.

The implications of risk for public policy, and for radiation protection in particular, are controversial in large part because risk estimates are uncertain and because there are legitimate interests both in avoiding radiation-related risks on the one hand and in maintaining radiation-related benefits and/or avoiding costs associated with unnecessary exposure reduction on the other. A person who may be at risk of radiation-related cancer will naturally insist on proof that the risk either does not exist or is small enough to be tolerated in view of the presumed benefit. A person whose interest is in maintaining the benefit, or avoiding costs associated with reduction of exposure, will demand proof that there is a risk that is high enough to be of concern. The problem is inherently political, and its fair resolution requires information about risk, including its uncertainty, framed so as to address the concerns of both viewpoints.

As epidemiological investigations of radiation-related cancer risk have evolved over time, emphasis has shifted from the discovery that radiation is indeed a cancer risk factor, to demonstration of radiation dose response, to identification of factors that modify dose response, to examination of assumptions inherent in the risk estimation process. Ionizing radiation exposure is a known, and well quantified, human cancer risk factor. Nevertheless, estimation of cancer risk following radiation exposure is a very uncertain process for most cases of regulatory and/or popular concern. One reason is that risk estimates are usually applied to exposed populations different from those on which the estimates are based. Another is that public and regulatory interest is usually with exposures at radiation doses far lower than those at which useful information about risk can be obtained by studying populations with such exposures.

2.1.1 Evidence regarding radiation-related transgenerational cancer risk

The current report is concerned mainly with the possibility that cancer risk may be increased following exposure to ionizing radiation. There is a great deal of information about this question. A second possibility, which is also a matter of concern, is that exposure may be associated with increased transgenerational cancer risk. Various epidemiological and laboratory studies have examined whether risks of cancer are raised in offspring, following parental radiation exposure. These studies have been reviewed in

detail elsewhere (e.g. COMARE, 2002). Cellular and animal studies indicate that the induction of cancer in the offspring of irradiated parents is possible in principle. However, the findings in mice have not been consistent. In some strains, no effect has been seen (e.g. Cattanach et al., 1995), whereas in others a raised risk has been observed that is greater than predicted by the conventional induction rate for gene mutations (e.g. Nomura, 1982).

Epidemiological studies conducted in several countries do not provide convincing evidence to suggest that occupational radiation exposure alone results in an increased incidence of childhood cancer in the offspring of male workers; data for the offspring of female radiation workers are too sparse to draw conclusions (COMARE, 2002). In the case of a cluster of childhood leukemia cases among children in the village of Sellafield, U.K., possibly associated with paternal employment at the nearby Windscale nuclear reprocessing plant (Gardner, 1990), a better case can perhaps be made in the context of the well-documented phenomenon of increased levels of childhood leukemia in so-called new towns, in which there has been an influx of residents from different areas; the postulated mechanism is an unknown viral etiology affecting previously unexposed residents (Doll et al, 1994; Doll, 1999). In addition, follow-up of about 40,000 offspring of the Japanese atomic bomb survivors has not shown any association between the incidence of cancer in children and young adults and parental dose (Izumi et al., 2003). Thus, the subject of transgenerational risk, while a legitimate subject of scientific investigation, is insufficiently developed to provide much information on risks associated with low-dose radiation. It is briefly discussed in Chapter 5 in connection with radiation-induced genomic instability, but is not pursued further in this report.

2.2 Dependence of cancer risk on radiation dose.

We have reasonably good epidemiological information on cancer risk following acute exposures in the range 0.2 Gy to 5 Gy and (for partial-body exposures) above. There are numerous epidemiological studies of populations containing “high-dose” subsets with radiation doses in this range. These populations include patients treated with radiation for benign and malignant disease, patients who received extensive diagnostic radiography over a lengthy illness, such as tuberculosis patients treated by lung collapse therapy monitored by frequent fluoroscopy examinations, persons who received substantial exposures because of their occupations, such as uranium miners exposed to

radon decay products in mine atmospheres and instrument dial painters who ingested radium contained in luminescent paint, and survivors of the atomic bombings of Hiroshima and Nagasaki, Japan. These studies, and in particular inferences based on the moderate- to high-dose component of the populations under study, form the primary epidemiological basis for estimation of radiation-related risk. Recent, comprehensive reviews of epidemiological information on radiation-related cancer risk are to be found in the UNSCEAR 2000 report (UNSCEAR 2000) and NCRP Report 136 (NCRP 2001a).

Some benchmarks of radiation exposure levels are given in Table 2.1. Yearly natural background effective doses in normal background areas are 0.4 mSv from cosmic radiation, depending upon altitude (the dose from a typical round trip between New York and Paris by commercial airline would be 0.03 mSv), 0.5 to 4 mSv from radioactivity in rocks and soil, depending on local geology, 0.25 mSv from naturally occurring radionuclides in the human body, and on the order of 2.5 mSv to the lung from inhaled radionuclides (radon, thoron, and their decay products) (UNSCEAR 2000). Common diagnostic examinations produce effective doses ranging from 0.01 mSv for x rays of a foot or hand to 4 mSv for a barium enema (Mettler and Upton, 1995), to 25 mSv for a pediatric CT scan of the abdomen if adult settings are used (Brenner et al, 2003). An astronaut may get about 2 - 3 mSv tissue-weighted effective dose on a typical 3-day space shuttle mission, and about 50 mSv on a 60-day tour in the international space station (NCRP, 2000). Estimated acute, neutron-weighted doses to the colon (weighted dose = gamma dose plus 10 times neutron dose) from the atomic bombings of Hiroshima and Nagasaki ranged from less than 1 mGy to nearly 6 Gy for survivors who were exposed within 3 km of the explosions and who were still alive in October, 1950; among survivors with estimated doses between 5 mGy and 4 Gy, the average was 200 mGy (RERF LSS mortality data set, 2003). An acute, whole-body effective dose of 5 Sv is very likely to be fatal without prompt medical attention, but radiation therapy for cancer usually involves partial-body doses an order of magnitude higher. Fractionation or protraction of exposure can allow higher doses to be tolerated in terms of acute effects. Cumulative occupational exposures among monitored radiation workers were about 20 mSv in several major studies (Gilbert, 2001) and the recommended upper limit for radiation workers is 20 mSv per year averaged over 5 years, and no greater than 50 mSv in any one year (ICRP, 1991). (However, yearly effective doses at the Mayak plutonium facility approached 1 Sv for some workers during the earlier years of production (Akleyev and Lyubchansky, 1994; Khokhryakov et al, 2000).)

2.2.1 Existence of a dose response.

Dose-response data (e.g., pertaining to cancer morbidity) can be described in a number of ways, such as by arranging observations in order of dose, grouping them into consecutive dose intervals, and plotting cancer rates by dose interval (Figure 2.1). Sophisticated modeling of dose response is not strictly necessary to establish the existence of a dose response; that can be done by a test of increasing trend, usually obtained by fitting to the data a simple model, like one of the following:

$$ERR(D) = \alpha D, \qquad (2\text{-}1)$$

$$ERR(D) = \exp\{\beta D\}-1. \qquad (2\text{-}2)$$

Here, ERR(D) is excess relative risk at radiation dose D, and α and β are unknown parameters. In testing for an increasing trend, the dose response is "statistically significant" when the statistical evidence is inconsistent with values of the parameter α or β less than or equal to zero. These simple models can be used in tests of overall tendency, or trend, and do not suffice to establish the shape of the dose response curve. In the example of Figure 2.1, in fact, neither of the fitted functions agrees particularly well with the plotted, dose-specific data points, especially at high doses, but both simple models serve to establish the existence of a dose response.

If statistical significance is not achieved by a trend test, it can be inferred that the evidence in favor of the existence of a dose response is not strong or that any dose response is too complex to be represented by such a simple parametric function. It *cannot* be inferred that there is *no* positive dose response, unless the trend is statistically significant in the negative direction; inadequate statistical power, because of inadequate sample size for the range of doses covered, can result in failure to achieve statistical significance in the presence of a positive dose response (see Section 2.4.2).

2.2.2 Estimating the dose response.

The information that can be derived from a dose-response analysis is always conditional upon assumptions about the functional relationship between radiation dose and exposure-related, excess risk. In Figure 2.1, the interval-based estimates are based on virtually no such assumptions; the different estimates are minimally correlated with each other, and that only because they share a common reference (i.e., the value for the zero-dose interval is constrained to be zero); thus, observations at any given non-zero dose

interval contribute information only towards the estimated ERR at that interval. However, for each of the two fitted models used for trend tests (the plots of which differ because their assumed functional forms are different), the corresponding dose-specific estimates are all determined by the same estimated parameter, α in *(2-1)* or β in *(2-2)*, and are therefore perfectly dependent on each other, conditionally on the estimated dose values. The confidence limits on the fitted curves are accordingly much narrower than those on estimates separately computed for individual dose intervals along the abscissa.

Once existence of a dose response has been established, it makes sense to find a parametric dose-response model that is both consistent with the epidemiological data and plausible in terms of radiobiology. Such a model provides a way to use all of the dose-response data to estimate radiation-related risk at various dose levels, and at low dose levels in particular.

Of the two models used here to test for trend, the linear model *(2-1)* is biologically plausible in the sense that the primary mechanism by which ionizing radiation exposure is thought to influence subsequent cancer risk is damage to cellular DNA from ionizing events, and the frequency of such ionizing events in a defined volume of tissue is proportional to absorbed radiation dose. The log-linear model *(2-2)* is less plausible, but is often mathematically convenient (e.g., in logistic model analyses).

An experimentally and theoretically-derived general radiation dose-response model, often cited in connection with cancer risk related to low-LET radiation (Upton, 1971; NAS/NRC, 1980) is

$$\mathrm{ERR}(D) = \alpha D \times (1 + \beta D) \times \exp(-\gamma D - \delta D^2). \qquad (2\text{-}3)$$

Here α, β, γ and δ are unknown, positive parameters. The linear term, αD, dominates at low doses (where D^2 is small) and the term $\alpha\beta D^2$ dominates at doses somewhat greater than the so-called "cross-over dose" ($D = 1/\beta$) at which the terms proportional to dose and dose-squared contribute equally to estimated risk. The exponential term,

$$\exp(-\gamma D - \delta D^2),$$

represents the competing effect of "cell killing" or cell reproductive death, observed experimentally, which would prevent a radiation-damaged cell from becoming cancerous; this term dominates at high doses, leading to a reduction in slope and eventually to a turnover and gradual decline in risk. (For present purposes, the contribution of the parameter δ is of only minor importance and we will assume $\delta = 0$ in what follows.) Like the other components of *(2-3)*, the exponential cell-killing term is modeled as a

continuous function of dose, without threshold. Thus, cell killing is considered a stochastic effect, the probability of which increases with increasing dose, and not a deterministic effect, like tissue injury, which becomes noticeable when the proportion of damaged cells exceeds some threshold level.

The general dose-response function *(2-3)* is not often used in epidemiological research, mainly because the constrained parameters β and γ produce effects opposite in curvature that may cancel each other out to some extent. While the model is used successfully with very precise and numerous experimental data, most epidemiological dose-response data lack the statistical power needed to support estimates for a model of such complexity. This observation is illustrated here using the A-bomb survivor data of Figure 2.1 for total solid cancers following a whole-body exposure, among the most statistically powerful epidemiological radiation dose-response data in existence at the time they were published (Thompson, 1994). The model fits these data reasonably well (Figure 2.2, dashed line; Table 2.2), but statistically not significantly better than the linear model of Figure 2.1 ($p = .11$). The estimated ERR per Gy at low doses (i.e., the estimated value of α), 0.52 (90% confidence limits 0.16 - 0.83), does not differ markedly from that according to the linear model, 0.57 (0.49 - 0.66); however, the confidence limits are substantially wider for the more complex model, reflecting the wide range of combinations of positive values of the parameters α, β and γ consistent with the data. The analysis offers little evidence in support of a positive value of the (dose-squared) parameter β ($p=.28$), but suggestive evidence in support of a non-zero value of the cell-killing parameter γ ($p = .07$).

Less than 1% of the members of the Life Span Study Cohort for whom dose estimates have been calculated have estimates greater than 2 Gy, and there are reasons to believe that the dose estimates above 2 Gy may be biased upward (Pierce and Preston, 2000). Restriction of the dose-response analysis to subjects with doses under 2 Gy yielded the linear-model parameter estimate $\alpha = 0.64$ (0.54- 0.74). Adding either the quadratic or the cell-killing terms to the model produced zero or minimal change whereas adding both of them yielded parameter estimates so uncertain as to be of no predictive value (Table 2.2).

In the remainder of this report, epidemiological risk estimates are based on linear dose-response analyses.

2.3 Inferences based on acute exposures in the moderate-to-high dose range

2.3.1 Modification of dose response by sex and age.

The information obtained from studies of the A-bomb survivors and other populations mentioned above is rich in detail. For many cancer sites and groups of sites, we can estimate with some precision not only dose-specific risk of radiation-related cancer, but also its variation by cancer site and by sex, age at exposure, attained age and/or time following exposure. In general (but not always), radiation-related relative risk is higher among women and following exposure at young ages. The relationship to age at exposure is marked for thyroid cancer, acute leukemia, and female breast cancer (Ron, 1995; Preston, 1994; Preston, 2002; Land, 2003). Risk decreases somewhat, in relative terms, with advancing age at observation, but increases in absolute terms because baseline cancer risk tends to increase as a power of age, and faster than dose-specific ERR decreases (Thompson, 1994; UNSCEAR, 2000; Pierce, 2002; Pierce and Vaeth, 2003).

The relative importance of exposure age and attained age as modifiers of radiation dose response is uncertain, because in any epidemiological follow-up study the two quantities are highly correlated and their effects are difficult to separate. With additional follow-up as the major exposed populations are followed to the end of life span, the importance of this question for lifetime risk will become moot because projection to the end of life will no longer be required for subgroups exposed at young ages. However, the dependence of radiation-related risk on exposure age and attained age probably will remain complicated: one consideration is the presence of secular trends in baseline risk in Japan during the period of follow-up for the atomic bomb survivors over the past half century, the reasons for which are not entirely clear (Parkin, 2002).

Statistically stable descriptions can be obtained of the dependence of dose-specific risk on sex, age, and time, for aggregations of cancer sites such as all cancers combined, all solid cancers, all leukemia types, and other groupings. This is useful because radiation protection is concerned with the totality of possible adverse consequences of exposure, but also because overall patterns of dependence may emerge from such analyses that can be incorporated into site-specific estimates, resulting in greater statistical precision (Pierce and Preston, 1993; NAS/NRC, 2000; NCI/CDC, 2003).

2.3.2 Modification by lifestyle and other individual factors.

There is a relatively small but growing amount of epidemiological information (Table 2.3) on modification of radiation-related risk by history of lifestyle factors such as tobacco smoking in the case of lung cancer (Prentice 1983; Kopecky, 1986; NAS/NRC, 1999; Lubin, 1995; Pierce, 2003), childbearing and breast feeding in the case of breast cancer (Boice, 1978; Shore, 1980; Land, 1994), ultraviolet light in the case of basal cell and squamous cell skin cancer (Shore, 2001, 2002; Ron, 1998), and disease history in the case of type C hepatitis infection and liver cancer (Sharp, 2002). Much more needs to be learned about interactions of ionizing radiation exposure with lifestyle factors and with exposures to other agents. It is not unlikely that some of our current inferences about dependence of radiation-related risk on exposure age, attained age, and sex may reflect secular changes in lifestyle, and in exposure to environmental agents, that have been associated with changes over time (and with successive birth cohorts) in both baseline and radiation-related risk.

2.3.3 Variation by population.

There does not appear to be an obvious, consistent relationship between baseline and radiation-related cancer risk, either across cancer sites within a single population or across populations for a single cancer site. In the female Japanese population generally, age standardized (world) rates per 100,000 per year are similar, at about 31 for gastric cancer and 34 for breast cancer (Parkin, 2002), whereas in the United States they are about 3 and 90, respectively. Among A-bomb survivors, the radiation-related excess relative risk at 1 Gy (ERR_{1Gy}) is 0.32 for gastric cancer and 1.6 for breast cancer (Thompson, 1994). Gastric cancer contributes a substantial proportion of total radiation-related risk, but that proportion is considerably less than the proportion of risk of *baseline* gastric cancer to total baseline cancer risk (about 22%) among A-bomb survivors (Thompson, 1994) and among Japanese generally (Parkin, 2002). In the United States, the ratio is 2% for males and 1% for females. For female breast cancer the opposite is true; the baseline rate in Japan is among the lowest in the world for developed countries whereas the total cancer rate is not much different from that in most other countries (Parkin, 2002) while, among A-bomb survivors, breast cancer contributes a disproportionately large fraction of the total radiation-related cancer burden (Thompson, 1994). In the United States, by contrast, baseline breast cancer rates are high but the

radiation-related excess risk (in absolute terms) per unit dose among medically-exposed women is similar to that among the A-bomb survivors (Preston, 2002). That is, the dose-specific, radiation-related component of total breast cancer risk is likely to be similar in absolute magnitude for exposed Japanese and western populations but, in western populations, smaller as a proportion of total breast cancer risk. For gastric cancer, on the other hand, the US baseline rate is an order of magnitude lower than that in Japan, whereas the limited information on dose-specific, radiation-related excess risk suggests that, as a multiple of baseline risk, it may be comparable to that in the A-bomb survivors (Griem, 1994; Carr, 2002).

The above information suggests that, for breast cancer, radiation-related excess *relative* risk per Gy (excess risk per Gy expressed as a multiple of the Japanese baseline risk) based on A-bomb survivor data would overestimate risk for an exposed US population while, for gastric cancer, radiation-related excess *absolute* risk (the difference between risk following exposure and the Japanese (baseline risk) would result in an overestimate for the US population. For most other cancers we have almost no information of a similar nature (Table 2.3). This is not a trivial matter, because any transfer of a risk estimate from one population to another requires making an assumption, explicit or implicit, about the relation between excess and baseline risk. Moreover, for some sites (e.g., stomach, liver, and esophagus) baseline rates can differ markedly between populations (Parkin, 2002).

It should not be surprising that the relationship between radiation-related and baseline risk in different populations is not consistent for different cancer sites. There are reasons, as yet poorly understood, why baseline breast cancer rates are high in the United States, and why baseline gastric cancer rates are high in Japan. These reasons are almost surely related to differences in lifestyle, since the descendants of immigrants to the United States, for example, have tended to develop cancer rates that are typical of the general U.S. population (Haenszel, 1968; Ziegler, 1993) and different from those of their countries of ancestral origin. The lifestyle factors affecting the rates for breast and stomach cancer are probably different, at least in part, and probably interact differently with radiation dose.

Much of environmental, nutritional, and occupational cancer epidemiology is concerned with identifying cancer risk factors that might account for some part of the variation of site-specific baseline rates among populations. While there has been much progress, the problem is vast and, as discussed in section 2.3.2, there is only limited

information on interaction between radiation dose and lifestyle factors in terms of cancer risk. Thus, it is likely that, for the foreseeable future, the most useful information relevant to transfer of radiation-related risk coefficients from one population to another will come from multinational comparisons of site-specific radiation-related risk, rather than from investigations of underlying cancer risk factors and their interactions with radiation dose.

2.3.4 Radiation quality.

Risk estimates for low-LET radiation protection purposes are based mainly on epidemiological studies of populations exposed to substantial doses of medical x ray, or to mixed gamma and neutron radiation from the Hiroshima and Nagasaki atomic bombs. According to the DS86 dose reconstruction algorithm (Roesch, 1986) as represented by public use RERF data sets (RERF, 2003), the correlation between neutron and gamma dose within each city is greater than 95%, and the proportion of total absorbed bone marrow dose contributed by neutrons is only 0.7 to 2.7% in Hiroshima and 0.3 to 0.7% in Nagasaki, depending upon shielding and exposure distance (According to the as yet unpublished DS02 dose reconstruction system, the neutron component is reduced slightly, compared to DS86, in both Hiroshima and Nagasaki. In particular, an anticipated large increase of the neutron component for low-dose survivors in Hiroshima did not materialize (Preston et al, 2004).) Because of the relatively small contribution from neutrons, there is minimal statistical power for estimating the relative biological effectiveness (RBE) of the two radiation types based on the A-bomb survivor data. Moreover, there are essentially no useful data on cancer risks in populations exposed mainly to neutron radiation (IARC, 2000) and, therefore, the relative biological effectiveness of neutron cf. gamma-ray dose can only be estimated from experimental data. Risk coefficients for gamma ray dose are obtained from the A-bomb survivor data through the use of a nominal weighting factor of 10 for the neutron component of dose (Thompson, 1994). This weighting factor has been judged appropriate at A-bomb doses of the order of 1 Gy; however, the variation in the estimated gamma-ray dose response due to uncertainty in the weighting factor is not great, with 90% uncertainty limits[1] of "7% (NCRP, 1997).

[1] Here and elsewhere in this report, “confidence limits” or “confidence bounds” are used for statistical uncertainty in the classical sense, in keeping with conventional usage. “Uncertainty limits”, “uncertainty bounds”, “probability limits”, and “probability bounds” are used interchangeably for estimates that incorporate some information for which subjective or approximate assessments of uncertainty have been employed.

Cancer risks associated with alpha radiation exposure have been studied for lung cancer among uranium miners exposed to inhaled radon decay products (NAS, 1999) and in populations exposed to lower radon levels in residential settings, for bone cancer associated with ingested ^{226}Ra and ^{228}Ra among former radium dial painters (Fry, 1998; Stebbings, 1984; Carnes, 1997) and with injected ^{224}Ra in patients treated for benign disease (Spiess and Mays, 1979; Nekolla 1999, 2000), and for cancers of the liver and other organs in patients injected with x-ray contrast media containing thorium (Travis, 2003). Thus, estimates of cancer risk associated with exposure to alpha particle radiation have a basis in direct observations, while estimation of risk associated with neutron exposure is indirect, relying on scaled estimates of risk from low-LET radiation, using experimentally-derived estimates of the effectiveness of neutrons compared to low-LET radiation.

Epidemiological risk estimates based on exposure to gamma rays (photons with energies of > 250 keV) and most medical x radiation (photons with energies in the 30-250 keV range) often are treated as interchangeable quantities (see, e.g., ICRP, 1991). However, it has long been considered, based on biophysical considerations, that medical x rays are more effective biologically than higher-energy gamma rays. This consideration has been cited as a factor that may complicate inferences based on comparisons of cancer risk associated with fractionated x-ray exposures and acute gamma ray exposures (Brenner, 1999). Kocher et al (2002; also see NCI/CDC, 2003) have estimated uncertain radiation effectiveness factors (REF), compared to gamma radiation, for 30-250 keV and soft (<30 keV) x rays, assigning subjective uncertainty distributions with mean REF values 2 and 2.7, respectively, and 95% uncertainty limits 1 – 4.7 and 1.1 – 6.4, respectively for the two x-ray energy ranges. Electrons at energies like those of secondary electron tracks induced by gamma-ray photons, i.e., above 30 keV, were assigned an REF value of 1, while lower-energy electrons were assigned an uncertain REF with mean 2.6 and 95% limits 1.2 - 5.0.

2.4 Estimation of risk at low doses and low dose rates

Except for radiation therapy, where there is a recognized benefit from the radiation dose itself, very few people are exposed to radiation effective doses of 0.2 Sv and above. Most public concern is with exposures to less than 50 mSv, the historical annual limit for radiation workers before a reduced level (20 mSv) was recommended in

ICRP Publication 60 (1991); that concern extends to effective doses well below 1 mSv, the annual limit recommended by both ICRP (1991) and NCRP (1993), as well as the annual dose from natural background radiation for most tissues other than the lung. As previously mentioned, a chest x-ray delivers about 0.1 mGy to lung tissue; the dose to breast tissue from a two-view mammography examination is about 3 mGy; and an astronaut may get about 2.4 mSv tissue-weighted effective dose on a typical 3-day space shuttle mission (NCRP, 2000).

2.4.1 Difficulties of direct estimation of low-dose risk.

Although such low-dose exposures (except, of course, the astronaut's) are very common, it is extremely difficult to estimate the associated excess cancer risks by studying populations with exposures limited to the low-dose range. This is because, at low doses, the radiation-related excess risk, which is thought to be proportional to dose or perhaps somewhat less when compared to risks at higher doses, tends to be dwarfed by statistical and other variation in the background risk level in the absence of exposure. Because of this, truly enormous sample sizes (e.g., millions) theoretically would be required to obtain a statistically stable estimate of radiation-related risk, and even then the estimate would be untrustworthy because we do not understand, and therefore cannot control or adjust for, all of the sources of variation in baseline levels of risk (Land, 1980). At higher dose levels there are fewer such problems because the excess risk tends to be large relative to statistical variation in baseline risk, and we are more likely to understand the causes of any substantial variation in baseline risk that might be confounded with radiation dose.

2.4.2 Illustrative example.

Suppose (1) that baseline cancer risk in a given population, over a period of (say) 30 years, were known to be 10%, (2) that exposure to a whole-body effective dose of 1 Sv would double risk over the same period, and (3) that excess risk were strictly proportional to radiation dose over the interval 0-1 Gy. Suppose also that it were possible to find large study populations with baseline risks known to be 10% and with uniform exposures to 1 Gy, 100 mGy, 10 mGy, or 1 mGy, and to observe them over 30 years. (This is a simplified version of a study in which observed cancer frequencies in an exposed population are compared with expected frequencies calculated on the basis of published population rates.) The estimated excess cancer rate in such a population would be the

number of cancers divided by the population size, less the known baseline rate of 10%. The estimate would be distributed approximately as a normal random variable with mean equal to effective dose *D*, in Gy, times 10%, and variance equal to (1 + *D*), times 10%, divided by the population size, *N*. The population size needed to be able to detect the excess risk associated with effective dose *D*, with probability 80% at the 5% significance level, is shown in Table 2.4. The calculation is in fact an unrealistically optimistic one since, as illustrated in a later example, we can never be that sure of the baseline rate in any exposed population.

If an enormous study population is required to detect any excess risk associated with exposure to a small radiation dose, it follows that, if we use a much smaller population and fail to detect any excess risk, the implications are unexciting. A result predictable under both of two opposing hypotheses supports neither of them against the other. Thus, for example, failure of epidemiological studies to demonstrate a statistically significant excess cancer risk associated with exposures on the order of 1 mGy does not imply that there is no risk, although it does suggest that any such risk is small relative to baseline cancer rates.

At low and very low radiation doses, statistical and other variation in baseline risk tends to be the dominant source of error in both epidemiological and experimental carcinogenesis studies, and estimates of radiation-related risk tend to be highly uncertain both because of a weak signal-to-noise ratio and because it is difficult to recognize or to control for subtle confounding factors. At such dose levels, and absent bias from uncontrolled variation in baseline rates, positive and negative estimates of radiation-related risk tend to be almost equally likely on statistical grounds, even under the LNT hypothesis. Also, by definition, statistically significant positive or negative findings can be expected in about one in twenty independent studies when the underlying true excess risk is close to zero. Thus, even under the LNT hypothesis, the smaller the dose, the more likely it is that any statistically significant finding will be a purely chance occurrence, and that it will be consistent with either beneficial effects of radiation (hormesis) or a grossly exaggerated risk (Land, 1980). Such estimates tend to be only a small fraction of the total, but when selectively presented they can give the appearance of a substantial and even overwhelming body of evidence in one direction or the other.

2.4.3 Studies of low-dose exposures

i) Medical studies.

There is, in fact, some direct epidemiological evidence of excess cancer risk associated with radiation exposures on the order of a few tens of mGy. One example is increased risk of leukemia and solid cancer, which has been observed in several studies (Stewart, 1956; MacMahon, 1962; Monson, 1984; Harvey, 1985; Bithell, 1988) among children exposed in-utero to radiation from x-ray pelvimetry. The excess absolute risk coefficient calculated in this case was 6% per Gy (Doll and Wakeford, 1997).

A less direct, but nevertheless persuasive, example is increased breast cancer risk among young women exposed to high cumulative doses from multiple thoracic fluoroscopy examinations, delivered in fractions that were, on average, on the order of 10 mGy (Boice, 1991; Doody, 2001; Howe, 1995; Davis, 1987). Successive exposures were separated by a week or more, but were repeated often enough to yield cumulative doses of hundreds or even thousands of mGy. Excess (absolute) risks per unit of total dose (about 10 excess cases per 10,000 women per year per Gy at age 50, following exposure at age 25 (Preston et al, 2002)) were comparable to those associated with acute doses among atomic bomb survivors (Boice et al, 1979; Land et al, 1980; Little, 1999; Preston et al, 2002). A similar relationship for excess risk of lung cancer, compared to estimates based on high-dose, acute exposures, was not observed among fluoroscopy patients, even though lung doses were comparable to breast doses (Howe, 1995; Davis, 1987). Although excess lung cancer risk per unit dose of acute radiation is in general less than for breast cancer (Thompson, 1994), the difference between the breast and lung cancer findings among fluoroscopy patients suggests that there may be variation among cancer sites in terms of fractionation effects. It should be remembered, however, that exposure to tobacco smoke is by far the dominant risk factor for lung cancer. Among, for example, tuberculosis patients who underwent lengthy courses of lung collapse therapy associated with high cumulative radiation dose from fluoroscopic examinations, below-average exposure to tobacco smoke might mask a radiation-related increase in lung cancer risk.

A highly significant, dose-related excess risk of thyroid cancer was observed among 10,834 Israeli patients treated as children by x-ray depilation for ringworm of the scalp (*tinea capitis*), with estimated (fractionated) dose to the thyroid gland averaging 90 mGy (range 40 – 500 mGy), cf. 16,226 non-exposed comparison subjects (Ron et al, 1995). Estimated linear model ERR/Gy was 32.5 (95% CI 14 – 57), based on 44 cases

among the exposed and 16 among the non-exposed. No significant excess was observed among 2,224 patients given similar treatment (average thyroid dose 60 mGy).in the United States, cf. 1,380 given only topical ointment treatment; 2 thyroid cancers were found in the x-ray group, consistent with general population rates, and none in the non-irradiated group. The between-study difference in risk estimates was not statistically significant, however (Shore et al, 2003).

ii) Occupational studies.

Except for (mainly historical) worker populations with fairly high levels of exposure, such as uranium miners (BEIR VI), radium dial painters (Stebbings et al, 1984), Russian plutonium workers (Gilbert, 2002), and early radiologists (Matanoski, 1975; Smith and Doll, 1982), most occupational studies can be classified as low-dose and, therefore, of low statistical power. Their main utility is to validate generally accepted estimates in the sense that they are consistent with estimated radiation-related risks among regulated radiation workers. For example, a large, combined analysis of cancer mortality among nuclear workers in the United States, the United Kingdom, and Canada found a statistically significant dose response for leukemia and a non-significant dose response for all solid cancers which, although negative, had an upper confidence limit consistent with linear extrapolation of estimates based on higher-dose data (Cardis, et al, 1995). Occupational radiation exposure and cancer mortality in the U.K. National Registry for Radiation Workers were similarly associated, and consistent with estimates based on the atomic bomb survivor studies (see below) (Muirhead et al, 1999). Patterns of cancer mortality were inversely related to year of first employment among U.S. radiological technicians, consistent with a radiation etiology given higher occupational exposures to radiation in earlier compared to more recent times (Mohan, et al, 2002, 2003).

iii) A-bomb survivor studies.

It is sometimes forgotten that the vast majority of the exposed (as distinguished from persons not present at the time of the bombings) Life Span Study (LSS) cohort of atomic bomb survivors received radiation doses under 100 mGy (Table 2.5). For solid cancer mortality between 1950 and 1997 (Preston, 2003), direct assessment of risks at low doses obtained a statistically significant dose response when the analysis was restricted to survivors with dose estimates less than about 120 mGy. The estimated ERR

per Gy over this range was 0.74 (90% CI 0.1 – 1.5). There was no indication that the slope of the fitted dose-response curve differed significantly ($p > 0.5$) from the estimate over the full dose range (ERR per Gy = 0.47), and no evidence of a threshold. As discussed below, similar result was obtained from analyses of the same epidemiological data using the DS02 dose estimates (Preston et al, 2004).

An earlier analyses of solid cancer incidence data from the LSS Tumor Registry for 1958-1994 (Pierce and Preston, 2000) was focused on persons exposed at distances under 3000 m, of whom about 10,000 had estimated neutron-weighted doses under 5 mGy and 41,000 had doses between 5 and 500 mGy. An analysis restricted to persons exposed at distances less than 3000 m found a statistically significant linear dose response which was not overestimated by linear-model risk estimates computed over the wider dose ranges 0-2 Gy or 0-4 Gy (Figure 2.3). A statistically significant estimate was obtained from an analysis restricted to the 0-120 mGy dose range; another finding was that any threshold over 60 mGy would be statistically inconsistent with the data.

When cohort members exposed beyond 3000 m were included in the analysis, the estimated slope of the fitted dose response was reduced slightly (by 3%), and the statistical significance of the fitted linear dose response in the range 0-120 mGy was reduced. Figure 2.3 shows a moving-average plot of dose-specific cancer rates over the 0-500 mGy range, with uncertainty bounds corresponding to ± one standard deviation (sd). At 100 mGy the moving average estimate of relative risk is about 3.7 sd units above one for an analysis restricted to survivors exposed at distances under 3000 m, and about 2 sd units above the redefined baseline (represented by the dotted horizontal line at about RR=1.04) using the less restricted data set.

Figure 2.4 is based on the same data as Figure 2.3, but shows linear regression estimates of the ERR per Gy over dose intervals that are progressively trimmed of high-dose data. Moving from right to left, the right-most estimate and its standard error are based on observations over the dose range 0-2 Gy, the next on 0-1.5 Gy, and so on, while the left-most one is based on data at 0-0.05 Gy. There is more variation between consecutive estimates on the left-hand side of each graph than there is on the right-hand side, and the ±SE limits become progressively wider toward the left-hand side of each panel as the dose range is further restricted at the high end (Donald Pierce, personal communication).

The reference population used in the analyses of Figures 2.3 and 2.4 is the group of “proximal” survivors (exposed within 3 km) in Hiroshima and Nagasaki with neutron-

weighted dose estimates less than 5 mGy. This choice was justified on the basis that the "distal" population exposed beyond 3 km was more rural, and may have experienced different cancer risk factors other than radiation, from those of the more urban proximal survivors. The horizontal line in Figure 2.3, corresponding to a relative risk of 1.04, represents the baseline if the distal survivors had been included in the analysis. Figure 2.5 repeats the analysis of the Figure 2.4 with the distal survivors included. While estimates of ERR per Gy based on higher-dose data are little affected by the change, the estimates at the left-hand side of Figure 2.5 are substantially lower than those at the left-hand side of Figure 2.4, with similarly wide error bounds. Comparison of Figures 2.4 and 2.5 demonstrates the sensitivity of estimates, if based only on low-dose data, to the influence of minor, and largely unknown or poorly understood, confounding factors.

The same overall patterns are seen in Figure 2.6, an analysis similar to Figure 2.5 (in that data for distal survivors contribute to the estimates) for LSS breast cancer incidence, 1950-1990 (Land, 2003). Together, Figures 2.4 - 2.6 demonstrate that regression estimates of dose-specific cancer risk for combined sites and for some single sites are highly consistent with linearity, depend substantially on excess risk observed among survivors with estimated doses under 200 mGy, and are statistically unstable when based only on data pertaining to doses under about 100 mGy. These analyses provide no strong evidence that excess risks per unit dose are substantially different at very low doses than at doses up to 4 Gy.

2.4.4 Extrapolation to low doses and dose rates.

Epidemiological data are informative about radiation-related risks at acute doses, on a logarithmic scale, in the moderately high (~ 1Gy), moderate (~100 mGy), and to some extent, low (~10 mGy) dose ranges, but not in the very low (~1 mGy) and extremely low (~0.1 mGy) ranges. Arguably the most important single problem in radiation risk protection is how to extrapolate from statistically stable, and relatively unbiased, risk estimates that pertain to higher-dose exposures, down to the lower dose levels that are of greater concern in everyday life. The analyses of Figures 2.3 - 2.6 suggest that, for the 1958-87 LSS solid cancer incidence data at least, linear extrapolation over one order of magnitude, e.g., from 2 Gy to 200 mGy, is justified. Dose-response analyses for leukemia risk, on the other hand, support a linear-quadratic dose response with approximate equivalence of the linear and dose-squared components of risk at bone marrow doses around 1 Gy (Preston, 1994). Solid cancer mortality data (all sites

combined) for 1950-1997 (Preston, 2003) suggest linearity even for doses in the 0 – 150 mGy range; however, a later analysis, using the DS02 dosimetry, found a statistically significant upward curvature over the restricted dose range 0-2 Gy, but the authors noted that linear model dose-response analyses restricted to 0 - 1 Gy, 0 – 0.5 Gy, and 0 – 0.25 Gy gave substantially higher estimates of low-dose risk and they therefore did not recommend using the linear-quadratic model to estimate low-dose risk (Preston et al, 2004).

i) Dose and dose rate effectiveness factor (DDREF).

The combined-site LSS solid cancer data support linearity of dose response down to doses of low-LET radiation on the order of 200 and even 100 mGy. They provide no evidence that linearity does not continue down to zero dose, nor do they rule out the possibility of nonlinearity in the 10-mGy and lower range. The in-utero pelvimetry studies, and the fractionated fluoroscopy study breast cancer data, suggest that radiation doses on the order of 10 mGy are associated with excess cancer risk, while leaving room for some dose-related variation in the amount (but not necessarily the existence) of excess risk per unit dose. The curvilinearity of the LSS leukemia dose response is the main epidemiological evidence in support of a reduced risk per unit dose at low and very low doses (otherwise suggested by experimental observations (NCRP, 1980)), such as the ICRP and UNSCEAR recommendation that extrapolated dose-specific risk estimates be divided by a DDREF of 2 for chronic exposures and for acute doses less than 200 mGy (ICRP, 1991; NCRP, 1993; UNSCEAR, 1993). A DDREF greater than 2 would in fact, in the context of a linear-quadratic dose-response model, be statistically inconsistent with the 1958-87 LSS solid cancer incidence data (Pierce and Preston, 2000).

An independent analysis of the 1958-87 tumor registry data by Little and Muirhead (2000) used a linear-quadratic model to assess possible overestimation of low-dose risk based on use of a linear dose-response model with these data, taking into account random errors in DS86 neutron and gamma dose estimates, and systematic errors in Hiroshima neutron dose estimates. They concluded that, for all solid tumors combined, there was some indication of upward curvature over the 0-2 Gy dose range, but felt that uncertainties in likely adjustments to the Hiroshima DS86 neutron dose estimates called for a cautious interpretation of their findings (a prescient judgement in view of the later mortality findings of Preston et al (2004) discussed above).

A DDREF would not be applied to the estimated linear-quadratic dose response for leukemia, since it is already included in the model.

ii) Site-specific differences.

The analyses of Figures 2.3 – 2.5 are based on the numerous data for all solid cancers combined, and that of Figure 2.6 is based on female breast cancer, for which the radiation-related signal-to-noise ratio is high in the sense that dose-specific, radiation-related risk tends to be high compared to the level of, and unexplained variation in, age-specific baseline breast cancer rates. Risk estimates for thyroid cancer and leukemia are based on far fewer cases, but signal-to-noise ratios tend to be high on a dose-specific basis, especially for exposures at young ages. For these three cancer types, there is evidence of radiation-related excess risk at doses below 200 mGy, and for all except leukemia there is little evidence for departure of the dose response from linearity. For most other cancer sites, however, numbers of cases and/or radiation-related signal-to-noise ratio are too low to support strong statements about low-dose risk, although it also can be said that there is little or no evidence of departure from linearity (e.g., Thompson, 1994).

The latter category of cancers includes some sites for which there is little or no epidemiological evidence that radiation exposure either is or is not associated with increased risk; examples include small intestine, prostate gland, female genital organs other than ovary, squamous cell skin cancer, and chronic lymphocytic leukemia (NCI/CDC, 2003). Rectal cancer falls into this category with respect to A-bomb exposure but has been shown to be significantly associated with high-dose, partial body exposure among patients given radiation therapy for cervical cancer (Boice, 1988). Cancer of the small intestine, which is very rare in most populations (Parkin, 2002) can be induced in experimental animals by high-dose irradiation of exteriorized intestinal loops (Osborne, 1963, as discussed by Watanabe, 1986) and the small intestine therefore is a susceptible organ. However, the small intestine appears to have characteristics that render it highly resistant to carcinogenesis at low-to-moderate levels of exposure to radiation and other environmental carcinogens (Cairns, 2002; Potten, 2002; see Section 3.2.1). Thus, inferences based on all cancers as a group, or on certain cancers for which there is substantial information about dose response and its modification by other factors, need not *necessarily* apply to all site-specific cancers, or even to all histological subtypes of cancers of any given site. Nevertheless, there is evidence of some degree of commonality,

with respect to dose effects and their modification by sex and age, for cancers of many different sites (Pierce, 1996), and it is therefore useful and informative to examine radiation-related risk for groups of cancer sites.

2.5 Thresholds cf. the linear, no-threshold hypothesis

The so-called linear, no-threshold (LNT) hypothesis (see, e.g., Brenner and Raube, 2001) is part of the current basis for risk-based radiation protection. The hypothesis assumes proportionality between radiation dose and subsequent cancer risk, usually with allowance for a DDREF to reduce risk per unit dose of low-LET radiation at dose levels below 200 mGy (ICRP, 1991). However, at doses at which the DDREF applies fully, excess risk is assumed to be proportional to dose. A consequence of the LNT hypothesis is that exposures resulting in very small average doses to very large populations are assumed to be associated with excess numbers of cancers that, although undetectable by epidemiological study, might be numerous.

The threshold hypothesis is a competing hypothesis that, if generally accepted, might make it easier to ignore possible consequences of very low dose exposures. According to the hypothesis, there is some “threshold” dose below which there is either no radiation-related health detriment or a radiation-related health benefit that outweighs any detriment. If the threshold were a universal value, for all individuals and all tissues, a consequence of the hypothesis is that, at some point, a very low dose to any number of people would have no associated risk and could be ignored. Much, of course, depends upon the value of the assumed threshold dose, since even under the LNT hypothesis there must be a level of estimated risk so low that it is not worth the trouble to avoid. If, however, thresholds existed but were known or believed to differ widely among individuals and/or tissues, the effect of this knowledge on radiation practice and philosophy might be much less, and radiation protection might be even more complex than it is under the LNT hypothesis.

One argument made against the LNT hypothesis is that there is little or no direct epidemiological evidence of excess cancer risk in populations exposed to less than 50 mGy or so. That isn’t quite true, as discussed above, but it is true that there is no direct, credible epidemiological evidence of a radiation-related risk associated with exposures on the order of 1 mGy, for example. Nevertheless, as also discussed above, the argument is a

specious one; failure to detect a risk that (if it exists) is very small is not evidence that the risk is zero.

A more subtle, and statistically more sophisticated, argument is to demonstrate that a dose-response model with a threshold, such as a linear model for dose-specific excess relative risk with a fitted negative intercept at zero dose, can fit a data set as well as a linear or linear-quadratic model constrained to have a zero intercept (Hoel and Li, 1998 with critique by Little, 1999). The approach has the potential for showing disproportionality between excess risk and dose, consistent with a threshold (and usually, but not necessarily, also consistent with a linear-quadratic dose response), and could conceivably provide more substantial evidence of a threshold. That strong support for a threshold hardly ever is found in this way is more a reflection of low statistical power in the low-dose region than of statistical evidence against the existence of a threshold. In a more recent paper, Baker and Hoel (2002) modified the then-current DS86 A-bomb doses for presumed systematic error in estimates of the neutron component of dose from the Hiroshima bomb, and a dose-dependent relative biological effectiveness (RBE) for neutrons compared to gamma rays, finding that an improved fit to morbidity data for solid cancers and leukemia was obtained by introducing a threshold. However, their assumptions about underestimation of the neutron dose for low-dose survivors of the Hiroshima bombing, on which their conclusions depended, have not been borne out by subsequent measurement data (Straume et al, 2003; Preston et al, 2004).

It is clear that epidemiological studies are very unlikely to establish the presence or absence of a threshold at some low dose level, although they can place limits upon the likely value of any possible threshold (Pierce and Preston, 2000). Radiobiological evidence presented elsewhere in this report identifies the induction of double-strand DNA breaks and more complex clustered DNA damage as probably the most important mechanism by which ionizing radiation exposure contributes to radiation carcinogenesis. Such events have been demonstrated by calculation (Brenner and Ward, 1992; Goodhead, 1994) and by experiment (Boudaiffa, 2000a, 2000b) to result from a single low-energy electron track produced by an x-ray or photon interaction. At low doses and low dose rates, the occurrence of such events is proportional to radiation dose and to the number of cells irradiated (Kellerer, 1985). Current research on development of timely assays for the presence and repair of DSBs may someday lead to findings that resolve the question of low-dose thresholds vs. the LNT hypothesis. As discussed in Section 4.5 below, the question is still very much in doubt.

2.6 Conclusions: Implications for low-dose risk

Epidemiological data from studies of human populations exposed to ionizing radiation provide direct evidence that such exposure is associated with increased risk of cancer, and reason to believe that excess risk is not confined to persons exposed to very high radiation doses. Our knowledge of radiation-related risk is highly quantified, more so than for any other common environmental carcinogen, and we have learned much about factors that modify that risk. Our understanding of risks associated with doses commonly encountered in daily life is not insignificant; we know, for example, that such risks are far lower than those observed in populations exposed to hundreds or thousands of mGy. However, the problem of quantifying risks that are so low as to be practically unobservable, and then recommending policies based on that quantification, is very difficult.

It is highly likely that there will always be uncertainty about low-dose risk, and that we will have to come to terms with that uncertainty. One way to do that is to quantify the uncertainty in a manner consistent with mainstream scientific information, and to evaluate actions and policies in terms of plausible probability distributions of risks associated these actions and policies. An example of this type of approach is given in Chapter 6 below.

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3. LOW DOSE RISK - BIOLOGY

3.1 Introduction

The fundamental role of radiation-induced DNA damage in the induction of mutations and chromosome aberrations and the apparent critical involvement of aberrations and mutations in the pathogenesis of cancer provides a framework for the analysis of risks at low radiation doses and low dose rate exposures. Several key questions are important in considering the impact of exposure to low dose and low dose rate radiation at the cell and molecular level with respect to subsequent development of chromosome aberrations, mutations, and cancer. These questions relate to the nature of radiation-induced damage, the nature of repair and damage response pathways, and their role and impact on induction of chromosome aberrations, mutations and cancer. In this regard the fundamental questions at the cell and molecular level to be considered for understanding risks at low doses are: 1) whether the damage caused by radiation is similar or distinct from endogenous damage; and 2) does damage occur at low doses/dose rates by ionizing radiation that cannot be repaired accurately; 3) is damage induced under low dose and/or low dose rate conditions repaired by distinct mechanisms from damage induced at higher doses; and 4) are the signal transduction pathways activated by low dose and/or low dose rate conditions and what impact do these pathways have in determining the propagation or elimination of radiation damage in cells and tissues. Early studies in biology related to radiation-induced cancer were largely descriptive in nature. This was mainly related to technical limitations in biological research. As such the ability to directly study low dose effects was limited. However, recent advances in techniques in cell and molecular biology are increasing the ability to directly approach these important questions.

3.2 Damage caused by radiation.

It has long been known that radiation produces a broad spectrum of DNA lesions including damage to nucleotide bases (base damage), DNA single and double-strand breaks (SSBs and DSBs). Certain types of DNA base damage such as 8-hydroxydeoxyguanosine and thymine glycols have significant biological importance, but the available data suggest that such isolated base damage by itself probably plays a minor

role in radiation mutagenesis (Ward, 1995). It is generally accepted that unrepaired or misrepaired DBSs are the principal lesions of importance in the induction of chromosomal abnormalities and gene mutations (Goodhead, 1994; Ward, 1995). However, recently it has become recognized that an important feature of radiation damage is not the presence of any of these damages individually but instead their close association creating “clustered damage”. Such clustered damage can arise from the combination of direct damage induced by the original radiation track plus damage generated from secondary reactive species arising from subsequent ionization events (indirect damage) (Nikjoo *et al*., 1999). Recent evidence has, in fact, shown that substantial yields of DSBs may result from secondary electrons, with energies below the ionization threshold, generated from the ionized nucleotides (Boudaiffa *et al*., 2000). Clustered damage may involve a SSB or DSB associated with base damage but can involve far more complex associations including multiple closely spaced DSBs. Both the frequency and complexity of clustered damage depend upon the linear energy transfer (LET) of the radiation. Using sophisticated modeling and track structure methods, it has recently been shown that nearly 30% of DSBs induced by low LET radiation are of a complex form involving two or more DSBs. This value is 70% for high-LET radiation. When breaks associated with base damage are included, then the complex proportion becomes 60% and 90% for low and high-LET radiation respectively (Nikjoo *et al*., 2002; Nikjoo *et al*., 2000; Nikjoo *et al*., 1999; Nikjoo *et al*., 2001). It is likely that as the complexity of the damage increases, the damage will become less reparable and more likely to lead to biological consequences (see below for further discussion). An important aspect in considering the impact of exposure to low doses of ionizing radiation (IR), is whether such damage is similar to that encountered endogenously. It is clear that a significant level of oxidative damage can arise in cells from the generation of reactive oxygen species (ROS) during normal cellular metabolism. In comparing ROS induced damage with that induced by IR there appears to be similarities but also important differences. One aspect of ROS and IR-induced DSBs, which can impact upon repair, is the nature of their termini. Breaks induced by restriction enzymes have 3’-hydroxyl and 5’-phosphate moieties at their termini, a prerequisite for enzymatic ligation while the majority of breaks generated by ROS and IR have ‘damaged’ termini, most frequently 3’phosphate or 3’phosphoglycolate end groups (Ward, 1998). Some 5’ termini with hydroxyl end groups are also generated. Such termini require processing prior to ligation. Excision of a damaged nucleotide will also frequently result in base loss at the break.

Recent evidence concerning the repair of such lesions will be considered below. These aspects of the breaks are similar between ROS and IR induced damage although they differ from DSBs induced during such metabolic processes as V(D)J recombination and meiosis.

The predominant forms of ROS-induced damage are base damages and SSBs. The frequency of DSBs generated by ROS depends upon the particular reactive species but typically is less that 0.5% of the damage induced. More, importantly, these DSBs are distributed relatively uniformly throughout the DNA. In contrast, due to non-homogeneous energy deposition, the damage from even low doses of IR occurs in clusters producing complex lesions. *It is unlikely that such damage will arise endogenously at any appreciable frequency*. The impact of this difference on repair will be discussed below.

UNSCEAR have explored the proposition that data on the high abundance of spontaneously arising DNA damage could be used to argue that 'a further small increment of DNA damage from low doses of radiation will not impose significant risk; that risk only becomes significant at relatively high doses when at a given level of genomic damage, DNA repair capacity is exceeded' (see UNSCEAR 2000). The principal conclusion from UNSCEAR, which generally accords with that of the Task Group, is that differences in the complexity (as discussed above) and repair characteristics (see later in this chapter) of spontaneously arising and radiation-induced DNA lesions render this proposition untenable.

3.3 Damage Response Pathways.

The cellular responses to DNA damage include pathways of DNA repair, the operation of cell cycle checkpoints and the onset of apoptosis. The latter two responses overlap significantly and utilize, at least to some extent, the same sensor molecules or complexes involved in damage recognition and signal transduction. There is mounting evidence that the damage recognition complexes that control cell cycle checkpoint arrest also influence or interact with the DNA repair machinery although the interplay between the DNA repair pathways and between DNA repair and checkpoint control/apoptosis is currently unclear. The operation of these responses serves two functions: one aim is to enhance survival and a second is to maintain genomic stability. These are not necessarily compatible outcomes. The principal evolutionary pressure for a lower organism such as

yeast is the survival of individual cells whereas in multicellular organisms a strong selective pressure is the survival of the organism. Since the propagation of genetically altered cells has the potential to kill higher organisms by tumor formation, mechanisms have developed to prevent the growth of damaged cells. However, to achieve this, the survival of individual cells may be compromised. The role of apoptosis for this purpose has been evident for some time; the function of checkpoint control in this context is just beginning to emerge. Thus, for radiation protection, it is necessary to evaluate not only the mechanisms that repair DNA damage and enhance survival but also the mechanisms that serve to limit the propagation of damaged cells. Below we consider first the processes that repair damage induced by IR and then the steps involved in the responses leading to checkpoint arrest and apoptosis. Since DNA double strand breaks (DSBs) represent the major biologically significant lesion following radiation exposure, the focus of the discussion will be on damage response mechanisms triggered by DNA DSBs.

3.3.1 DNA DSB repair

Two mechanistically distinct pathways for DNA DSB repair have been described, namely non-homologous end-joining (NHEJ) that requires little or no homology at the junctions and homologous recombination (HR) that utilizes extensive homology. A third process, single strand annealing (SSA), which utilizes short direct repeat sequences, has facets of both processes.

i) Non-homologous end joining (NHEJ)

Five core proteins that function in NHEJ have been identified in mammalian cells (Fig 1). These include the three components of the DNA-PK complex, (Ku70, Ku80 and the DNA-PK catalytic subunit (DNA-PKcs)), together with XRCC4 and DNA ligase IV (for reviews see (Jeggo, 1998; Kanaar *et al*., 1998; Lees-Miller and Meek, 2003; Lieber *et al*., 2003). Mutations in any of these core components confer dramatic radiosensitivity and an impaired ability to rejoin DNA DSBs as monitored by pulse field gel electrophoresis (PFGE). Cells lacking these NHEJ components are also impaired in their ability to carry out V(D)J recombination, a process that involves the rejoining of site specific DSBs (see below). Patients with hypomorphic mutations in DNA ligase IV display immunodeficiency and defective mice, when viable, display severe combined immunodeficiency phenotypes (SCID) ((O'Driscoll *et al*., 2001) for reviews see (Jeggo, 1998; Jeggo and Concannon, 2001; Schwarz *et al*., 2003)). Recently, a further

component, Artemis, has also been shown to function in NHEJ and V(D)J recombination (Moshous *et al*., 2001). Artemis was identified as the protein defective in a class of SCID patient. Cell lines derived from these patients are sensitive to IR but, in contrast to lines defective in the other NHEJ components, Artemis defective cell lines are proficient in DSB rejoining (Nicolas *et al*., 1998). Artemis is able to function as a single strand specific nuclease and its function in V(D)J recombination depends upon its ability to cleave a hairpin intermediate generated during this process (Ma *et al*., 2002). The role of Artemis in rejoining IR induced breaks is less clear but it has been speculated that it may function in modifying double stranded ends with additional DNA damage (Jeggo and O'Neill, 2002). Finally, analysis of cell lines from human SCID patients has provided evidence for a further factor required for NHEJ (Dai *et al*., 2003).

In yeast, a range of additional proteins appear to be required for NHEJ. Mre11, Rad50, Xrs2p are required for NHEJ in *Saccharomyces cerevisciae* but are dispensable for end-joining in *Schizosaccharomyces pombe* (for a review see (Jeggo, 1998)). In higher organisms, cell lines derived from Nijmegen Breakage Syndrome (NBS) carry mutations in Nbs1, a functional homologue of Xrs2p (Carney *et al*., 1998; Varon *et al*., 1998). NBS cell lines are proficient in their ability to carry out V(D)J recombination and do not show the characteristic DSB rejoining deficiency of NHEJ defective cell lines although they do show radiosensitivity (Yeo *et al*., 2000). Sir2p, Sir3p and Sir4p are also required for NHEJ in *S. cerevisciae* (Tsukamoto *et al*., 1996). Current evidence suggests that their role may be regulatory and recently it has been established that in *S. cerevisiae*, NHEJ is regulated in a cell-type specific manner by Nej1p/Lif2p (Frank-Vaillant and Marcand, 2001; Kegel *et al*., 2001; Valencia *et al*., 2001). Consistent with this model, Nej1p is repressed in *sir* strains. This regulation ensures that NHEJ only functions in haploid yeast cells and demonstrates that the role of the *sir* proteins in NHEJ is to regulate Nej1p. There is no data to indicate whether NHEJ is regulated in a similar manner in mammalian cells although the process clearly functions in diploid mammalian cells. An Nej1p homologue has not been identified in mammalian cells.

The heterodimeric Ku protein, consisting of 83 and 70 kDa subunits, has DNA double stranded end-binding activity and its binding to DNA ends is likely to represent an early step in the repair process. The binding of Ku to dsDNA ends serves to recruit DNA-PKcs and activate its catalytic activity. DNA-PKcs is a member of a sub-family of phosphoinositol (PI) 3-kinases, termed PI 3-K related protein kinases (PIKK), that have protein rather than lipid kinase activity (Hartley *et al*., 1995), which potentially provides

the cell with a signal transduction pathway to alert the presence of a DNA DSB. However, the function and physiological targets of DNA-PK activity is currently unclear. It does not appear to be involved in p53 activation nor for cell cycle checkpoint arrest (Jimenez *et al*., 1999). There is mounting evidence that DNA-PK may serve to auto-regulate the process of NHEJ and one clear *in vivo* substrate of DNA-PK activity is the protein Artemis, which is stimulated to cleave hairpin junctions by DNA-PK dependent phosphorylation (Ma *et al*., 2002; Merkle *et al*., 2002). Autophosphorylation of DNA-PK also appears to be essential for NHEJ (Ding *et al*., 2003).

XRCC4 and DNA ligase IV co-associate strongly and depend on each other for stability (Critchlow *et al*., 1997; Grawunder *et al*., 1997). XRCC4 has no obvious domains or motifs (Li *et al*., 1995). The crystal structure of XRCC4 reveals a globular head domain and two coiled coil tails (Sibanda *et al*., 2001). DNA ligase IV has a catalytic domain at its N terminus plus two BRCT domains at its C-terminus and interaction with XRCC4 occurs via the region between the two BRCT domains (Grawunder *et al*., 1998). It is the only mammalian ligase identified so far that can rejoin double strand DNA ends. An emerging model is that Ku serves to recruit the DNA ligase IV/XRCC4 complex to the DNA end and then translocates inwards to allow LX access to the DNA end (Kysela *et al*., 2003).

Role of NHEJ in V(D)J recombination. During B and T cell development, the V, D, and J segments become rearranged into contiguous units by a process that involves the introduction of site specific DSBs by two recombination activating genes (RAG1 and 2) (for reviews see (Fugmann *et al*., 2000; Gellert, 2002; Hesslein and Schatz, 2001). In germ line cells, each V, D or J segment, termed a coding segment, is juxtaposed to a recombination signal sequence (RSS). The DSBs are introduced at the junctions between an RSS and its adjacent coding sequence. This process involves the introduction of a single strand nick and a transesterification reaction generating a blunt ended RSS end and a hairpin coding end. Rejoining yields accurately rejoined signal junctions and coding junctions that frequently bear deletions or insertions. This rearrangement process coupled with inaccurate rejoining of coding junctions plays a significant role in enhancing the diversity of the immune response. Thus it appears that the cell utilizes the same DNA NHEJ machinery to effect rearrangements during the V(D)J recombination process and to rejoin radiation induced DNA DSBs.

The genetic requirements for signal and coding joint formation are distinct and provide insight into the nature of the rejoining process. Rejoining of the blunt ended

signal junctions requires only Ku70, Ku80, Xrcc4 and DNA ligase IV. Thus, Artemis and DNA-PKcs are largely dispensable for RSS rejoining. In contrast, all six proteins are required for coding join formation (Moshous *et al*., 2001). Recently, it has been demonstrated that Artemis is activated by DNA-PKcs, and following activation is able to cleave the hairpin coding junctions (Ma *et al*., 2002). This neatly explains the requirement of both DNA-PKcs and Artemis for coding join formation. What is the likely role of Artemis and DNA-PKcs in the rejoining of radiation induced breaks? In unphosphorylated form, Artemis has 5' to 3' exonucleolytic activity with single strand (ss) DNA specificity (Ma *et al*., 2002). Upon phosphorylation by DNA-PK, its activity changes and Artemis gains single strand specific endonucleolytic activity on both 5' and 3' overhangs as well as the ability to cleave hairpins. It is, therefore, possible that Artemis functions to modify the ends of radiation induced breaks (Jeggo and O'Neill, 2002).

ii) **Homologous Recombination (HR).**

HR is a high fidelity and efficient mechanism to repair DNA DSBs that utilizes information on the undamaged sister chromatid or homologous chromosome to retrieve information lost at the break site. In yeast, genes involved in HR belong to the Rad 52 epistasis group (Rad50, Rad51, Rad52, Rad54, Rad55, Rad57, Rad59, Mre11 and xrs-2); see (Helleday, 2003; West, 2003) for recent reviews. Homologues of some of these proteins (e.g. Rad50, 51 52, 52, 54 and Mre11) have been identified in higher organisms. The yeast proteins Rad55 and Rad57 display sequence similarity to Rad51 and in mammalian cells further members of a Rad51 family (termed Rad51-like genes) have been identified, including XRCC2, XRCC3, Rad51L1, Rad51L2 and Rad51L3 (Thacker, 1999). Steps involved in HR have been characterized in yeast and in *E. coli* and involve processing of the DNA ends, strand invasion, the formation of heteroduplex DNA and a step involving resolution of the cross-over junction (Holliday junction) (outlined in Figure 1) (Kanaar *et al*., 1998). RecA, Rad51p and human Rad51 (hRad51) polymerize on DNA to form a nucleoprotein filament that promotes ATP-dependent homologous pairing and DNA strand exchange. hRad52 stimulates homologous pairing by hRad51 suggesting that it functions in an early stage of Rad51-mediated recombination that precedes homologous pairing (Benson *et al*., 1998; New *et al*., 1998; Shinohara and Ogawa, 1998). *In vitro*, the homology searching and strand exchange reaction is facilitated by RPA, Rad55 and Rad57 although their precise roles are unknown.

Resolution of the Holliday junction complex is carried out by RuvABC in *E. coli* and requires Rad51C and XRCC3 in mammalian cells (Liu *et al*., 2004). Mre11, Rad50 and xrs2 may play a role in early nucleolytic processing to produce ends suitable for the exchange reaction (Tauchi *et al*., 2002). There is also increasing evidence for roles of BRCA1, BRCA2 and BARD1 in homologous recombination. Specifically, BRCA2 can bind to Rad51 via its Brt domains and potentially plays a role in delivering Rad51 to single stranded DNA (Pellegrini *et al*., 2002; Yu *et al*., 2003a). BARD1 interacts with BRCA1 and loss of either prevents HR taking place (McCarthy *et al*., 2003; Westermark *et al*., 2003).

***iii)* Single strand annealing (SSA).**

A third process for DSB rejoining identified in yeast is SSA, a mechanism that uses short regions of homology, possibly to stabilize ends prior to rejoining. The analysis of sequences at the break junctions in mammalian mutants arising after radiation in higher organisms has suggested that this mechanism also functions in mammalian cells (Morris and Thacker, 1993). This mechanism is inherently inaccurate since it involves loss of sequences around the short regions of homology. This may be the mechanism utilized when HR or NHEJ fail and could thus potentially contribute to error prone DSB repair. Unfortunately, little is known about the genetic requirement for this process in mammalian cells.

***iv)* Contribution of HR and NHEJ to DNA DSB repair in mammalian cells.**

Yeast mutants defective in Rad51p, Rad52p or Rad54p are dramatically radiosensitive; yeast NHEJ null mutants display little or no sensitivity but double mutants defective in both HR and NHEJ are slightly more sensitive than single mutants defective in HR. Thus, in yeast HR is the major mechanism for DSB repair and NHEJ functions in its absence. Two factors may account for this. Firstly, Nej1p appears to regulate NHEJ in yeast resulting in repression of the process in *MAT*a/*MAT*α diploids (Frank-Vaillant and Marcand, 2001; Kegel *et al*., 2001; Ooi *et al*., 2001; Valencia *et al*., 2001). Additionally, NHEJ appears to be regulated in some additional way allowing it to function primarily in G1 phase. The situation in mammalian cells is quite different. The major radiosensitivity of NHEJ defective mammalian cells attests to the importance of NHEJ in the repair of DNA DSBs in higher organisms. However, HR does function in higher organisms and

radiosensitivity is a feature of some HR defective cell lines. Increasing evidence suggests that in higher organisms HR functions to repair breaks in late S and G2 phases and that NHEJ plays its major role in G1 phase (Fukushima *et al*., 2001; Rothkamm *et al*., 2003). In mammalian cells, HR utilizes sister chromatids rather than a homologous chromosome as the source of undamaged template. HR, therefore, plays a particular role in the repair of breaks at the replication fork and also appears to be essential for the efficient repair of breaks that arise from interstrand cross-links.

3.3.2 Cell cycle checkpoint control.

Perturbation to DNA metabolism, arising either endogenously or through exogenous DNA damaging agents causes arrest at one of several cell cycle checkpoints, collectively called DNA integrity checkpoints. Progression from one cell cycle phase to the next occurs by phosphorylation or dephosphorylation of cyclin dependent kinases (Cdks) and checkpoint arrest is effected by controlling the activity of the DSBss. In addition to checkpoint controls that operate at the boundary between cell cycle phases there is also an S phase checkpoint that presumably recognizes a stalled replication fork. These checkpoint responses have been most widely studied using *Saccharomyces cerevisiae* or *Schizosacchromyces pombe* as model systems but the operation of checkpoints is also evident in mammalian cells and homologues of most of the yeast checkpoint proteins have now been identified. The checkpoint responses involve three stages; damage recognition, signal transduction and effector proteins. A brief overview of the process in yeast will be given first followed by a discussion of the available knowledge in mammalian cells.

***i)* DNA integrity checkpoints in yeast.**

In yeast there are several points where cell cycle delay or arrest can occur: (a) G1/S that serves to prevent replication of damaged chromosomes, (b) intra-S phase which slows down or delays replication, and (c) G2/M which prevents transition from G2 into M. In addition, there is a distinct response that monitors the replication status of the DNA and prevents mitosis if replication has not been completed. SpRad3 (*Schizosaccharomyces pombe* Rad3) or ScMec1 (*Saccharomyces cerevisciae* Mec1) are the phosphatidyl inositol 3-kinase-like kinases (PIKKs) that initiate the signal transduction process by phosphorylating key proteins involved in cell cycle regulation (see (Furuya and Carr, 2003; Osborn *et al*., 2002; Rouse and Jackson, 2002). Both kinases

have partner proteins, SpRad26p and ScLcd1p/ScDdc2p, which most likely function to target the kinase to the site of damage with recent evidence indicating that recruitment of the proteins to the break site requires initial binding of RPA to single stranded regions of DNA (Cortez *et al*., 2001; Zou and Elledge, 2003). Activation of the kinases, however, requires additional complexes. One is an RFC-like protein or protein complex represented by ScRad24p and SpRad17p. The second complex contains PCNA-like proteins (ScRad17p/ScDdc1p/ScMec3p and SpRad1p/SpRad9/SpHus1). The RFC-like proteins can target damaged sites independently of the PIKKs and are required to load the PCNA-like proteins. Downstream phosphorylation of transducer proteins in cell cycle checkpoint control, such as the Chk1p and Rad53/Cds1 kinases, requires all the proteins described above. Through effector proteins that include the Wee1 kinase, Cdc25 phosphatase and Mik1 kinases, key Cdks that control cell cycle progression are activated or deactivated. These include the mitosis-inducing kinase Cdc2.

ii) **Checkpoint responses in mammalian cells.**

Although the steps are less well understood in mammalian cells, the checkpoint responses are clearly conserved between organisms (for reviews see (Durocher and Jackson, 2001; Rouse and Jackson, 2002)). However, whereas in yeast, nearly all checkpoint signaling is carried out by the ScMec1/SpRad3 kinases, which respond to a range of different DNA damages, in mammalian cells there appears to be some divergence of function with two PIKK kinases, ATM (ataxia telangiectasia mutated protein) and ATR (ataxia telangiectasia and Rad3-related protein), both contributing to damage-dependent phosphorylation events (Abraham, 2001; Bradbury and Jackson, 2003; Shiloh, 2001). ATM appears to respond primarily to DNA DSBs and, therefore, is the PIKK activated by IR. ATR, in contrast, appears to be activated by single stranded regions of DNA arising during at stalled replication forks or during processing of bulky lesions (Zou and Elledge, 2003). A further significant difference in higher organisms is the role of p53 in the signal transduction process, for which there is no functional homologue in yeast. Mounting evidence suggests that recognition complexes similar to those found in yeast, sense damage and by phosphorylation initiate signal transduction pathways (Rouse and Jackson, 2002). In mammalian cells, these pathways also target p53. The result of this is that in mammalian cells checkpoint activation, in addition to inducing transient delays at cell cycle transitions, can also mediate permanent cell cycle arrest or apoptosis (outlined in Figure 2).

3.3.3 Early sensors of DNA damage.

i) Role of ATM

Ataxia-telangiectasia mutated (ATM) is the protein defective in ataxia-telangiectasia (A-T), a multi-system disorder associated with diverse characteristics that include cancer predisposition and clinical radiosensitivity (Taylor *et al*., 1996). A-T cell lines are defective in a range of damage responses following IR including an inability to arrest at the G1/S, S and G2/M cell cycle checkpoints (Goodarzi *et al*., 2003; Shiloh, 2001; Shiloh, 2003). Significantly, p53 levels are not elevated following radiation in A-T cell lines suggesting that ATM functions upstream of p53 potentially as part of an early damage sensor mechanisms (Kastan *et al*., 1992; Lu and Lane, 1993). ATM is a member of the PIKK family with homology to SpRad3 and ScMec1 although the yeast homologue of ATM is Tel1 (Savitsky *et al*., 1995). ATM can function as a ser-thr protein kinase both *in vivo* and *in vitro* and specifically can phosphorylate the serine 15 residue of p53. (Banin *et al*., 1998; Canman *et al*., 1998; Khanna *et al*., 1998). This residue of p53 fails to become phosphorylated in irradiated A-T cells demonstrating that ATM functions as the major, if not the only, kinase phosphorylating this residue of p53 after irradiation. This was initially thought to provide the explanation underlying p53 induction following irradiation. However, this is clearly an over-simplification; firstly phosphorylation of this residue does not appear to be a key factor controlling p53 stability, secondly ATM can phosphorylate other sites on p53, and thirdly it can phosphorylate other kinases such as Chk1 and Chk2, which themselves phosphorylate p53 on serine 20, which is required to stabilize p53 (see section on p53 below). Furthermore ATM can also phosphorylate MDM2, an event that could itself influence p53 stability. Added to this complex picture, other kinases including DNA-PK and ATR, can, at least *in vitro,* phosphorylate the S15 residue of p53. Thus, a complex picture of p53 regulation by phosphorylation emerges in which ATM clearly plays an important role either directly or indirectly. Taken together, these data suggest that ATM plays a key role in sensing DNA DSBs and by phosphorylation initiating signal transduction pathways that control cell cycle arrest. ATR probably serves the same role for UV induced lesions and stalled replication forks and overlaps to some degree with ATM for DNA DSBs.

ii) Role of Nbs1, hMre11 and hRad50.

Nijmegen Breakage Syndrome (NBS) is another syndrome associated with cancer predisposition and radiosensitivity that is distinct from, but overlaps with A-T (International Nijmegen Breakage Syndrome Study Group, 2000; Shiloh, 1997). In contrast to their somewhat distinct clinical characteristics, cell lines derived from A-T and NBS display similar phenotypes including radiosensitivity, cell cycle checkpoint defects and decreased ability to stabilize p53. The gene defective in NBS has been shown to encode a protein, Nbs1 or p95 (Carney *et al*., 1998; Varon *et al*., 1998). Nbs1 interacts strongly with hMre11 and hRad50. In yeast Mre11 and hRad 50 interact with a third protein, Xrs-2p, and mutants defective in any of these proteins share identical phenotypes (Johzuka and Ogawa, 1995). Nbs1 appears to be a functional homologue of Xrs-2p although the two proteins share only limited sequence homology. Like other DNA repair proteins, Nbs1 has a fork-head associated (FHA) domain and a BRCT domain, which appears important for function (Cerosaletti and Concannon, 2003). The link between A-T and NBS has been even further strengthened recently by the finding that a milder variant form of A-T called A-T like disorder (ATLD) has mutations in hMre11 (Stewart *et al*., 1999). hMre11 and hRad50 null mice show embryonic lethality and the mutations in hMre11 in ATLD impair but do not inactivate hMre11 function, a feature consistent with the milder clinical features of this variant class of A-T. hMre11, hRad50 and p95 (called the MRN complex) co-localise in nuclear foci which form at the sites of DSBs (Kobayashi *et al*., 2002)]. The precise role of the MRN complex is still hotly debated. In yeast and vertebrates, there is evidence that MRX functions in both HR and NHEJ (Tauchi *et al*., 2002). In mammalian cells it is not an essential component of the NHEJ machinery, however (O'Driscoll *et al*., 2001). Importantly, current evidence also shows that MRN is required either directly for ATM activation or to aid ATM-dependent phosphorylation events (Girard *et al*., 2002; Uziel *et al*., 2003). Taken together the findings suggest that MRN acts in concert with ATM in an early sensor complex that activates by phosphorylation a number of damage response mechanisms that include p53-dependent and independent processes.

iii) BRCA1 and BRCA2.

Germline mutations in these genes confer a high risk of breast and ovarian tumors and both have been identified as genes defective in familial breast cancer patients (Miki *et al*., 1994; Wooster *et al*., 1995). Recent evidence points to the involvement of both gene

products in damage response mechanisms and cells carrying mutations in either protein show pronounced genomic instability (see (Venkitaraman, 2002) for a review). *BRCA1* has an N-terminal RING finger domain that mediates protein-protein interactions and a tandem BRCT motif at its C-terminus, which appears to represent a phospho-protein binding module (Manke *et al*., 2003; Yu *et al*., 2003b). BRCA1 defective cells show marked genomic instability and impaired checkpoint responses including impaired S and G2/M checkpoint arrest (Xu *et al*., 2001). BRCA1 is also localised to H2AX foci after DNA damage and thus co-localises with MRN, 53BP1and MDC1 (Paull *et al*., 2000). BRCA1 is phosphorylated after DNA damage and emerging evidence suggests that it is required to facilitate at least some ATM-dependent phosphorylation events, a feature also displayed by other proteins that localise to the H2AX foci (Foray *et al*., 2003; Lee *et al*., 2000). However, following irradiation BRCA1 also co-localizes with Rad51 to nuclear foci, which are distinct from the H2AX foci (Zhong *et al*., 1999). Consistent with this finding, BRCA1 defective cells are impaired in HR (Moynahan *et al*., 1999). Taken together, these results suggest that BRCA1 may have two independent functions, one in checkpoint signaling and another in promoting HR. Thus, like p53, BRCA1 has a "caretaker" role.

BRCA2 defective cells do not appear to show cell cycle checkpoint defects but they are impaired in homologous recombination (Moynahan *et al*., 2001). Rad51 foci do not form in BRCA2 defective cells and it has been suggested that BRCA2 is required for the delivery of Rad51 to the sites of single stranded DNA (Pellegrini *et al*., 2002; Yang *et al*., 2002). The link with DNA repair has been further strengthened by the surprising recent finding that *FANCD1*, a gene involved in cross-link repair and defective in some patients with Fanconi anaemia, is in fact *BRCA2* (Howlett *et al*., 2002).

iv) Role of H2AX.

H2AX is a variant form of the histone H2A, which becomes phosphorylated in response to DNA damage and plays a critical role in the retention of repair factors at the site of double strand breaks (Celeste *et al*., 2003; Paull *et al*., 2000). Mice lacking H2AX are viable but show genomic instability and radiosensitivity (Celeste *et al*., 2002). H2AX phosphorylation is a rapid response following the introduction of DSBs and phosphorylation rapidly extends to H2AX molecules located up to 3 megabase pairs within the region of the DSB (Rogakou *et al*., 1999). Using phosphospecific antibodies,

phosphorylated H2AX (termed γ-H2AX) can be observed as discrete foci and current evidence suggests that all DSBs are marked by the presence of such foci (Rothkamm and Lobrich, 2003). The analysis of such foci is promising as a tool to monitor the formation and repair of DSBs (see also section 3.5).

v) MDC1, 53BP1 and SMC1.

Recent data have led to the identification of additional proteins that accumulate at the site of (-H2AX foci and are required for an efficient checkpoint response. Lack of these proteins confers at least some level of radiosensitivity. 53BP1 was originally identified through its ability to bind to p53 via C-terminal BRCT repeats present in 53BP1 (Mochan *et al*., 2003; Wang *et al*., 2002). MDC1 was identified simultaneously by several laboratories, one of which identified it as a binding partner of the Mre11 complex (Goldberg *et al*., 2003; Lou *et al*., 2003; Stewart *et al*., 2003). Both proteins form foci that co-localise with H2AX and MRN foci after irradiation (Abraham, 2002; Fernandez-Capetillo *et al*., 2002; Goldberg *et al*., 2003; Lou *et al*., 2003; Stewart *et al*., 2003). SMC1 is also required for normal cell cycle checkpoint arrest and for radioresistance (Kim *et al*., 2002; Yazdi *et al*., 2002). SMC1 also localises at H2AX foci after DNA damage.

3.3.4 Signal transduction after irradiation.

i) Role of p53.

An early response of mammalian cells that occurs within minutes of a cell sustaining DNA damage is an increase in the levels of p53 (Kastan *et al*., 1991). In addition to changes in p53 levels, its ability to function as a transcriptional activator may also be increased (see (Ashcroft *et al*., 1999; Lakin and Jackson, 1999) for reviews). In combination, these changes in p53 result in the transcription of key proteins involved in a number of distinct damage response mechanisms (see below). The role of p53 in the response to radiation damage is complex since it affects some aspects of DNA repair, cell cycle checkpoint arrest and the onset of apoptosis (see (Fei and El-Deiry, 2003) for a review). The importance of p53 and the significance of the damage response mechanisms it controls is underscored by the dramatically elevated cancer predisposition in patients with mutations in p53 (Li-Fraumeni syndrome patients), and in p53 knock-out mice

(Donehower *et al*., 1992; Malkin *et al*., 1990; Srivastava *et al*., 1990). Additionally, mutations in p53 are found in around 40% of tumors covering all the cancer types.

Since p53 is so critical to the cell and to the whole organism, it is not surprising that it is subjected to stringent regulation, the complexity of which is ever increasing (see (Ashcroft *et al*., 1999; Deb, 2003; Lakin and Jackson, 1999) for reviews). A key protein controlling p53 is Mdm2 (Deb, 2003). Mdm2 binds to the amino-terminus of p53 and targets it for ubiquitination and subsequent degradation by ubuiquitin controlled proteosomes (Kubbutat *et al*., 1998). Thus, in undamaged cells p53 is maintained at low levels via Mdm2 binding and ubiquitin-dependent degradation. Following radiation exposure, changes to p53 and/or Mdm2 decrease their binding potential with consequent increase in the half life of p53. Additionally, however, Mdm2 binding represses the ability of p53 to act as a transcription activator (Momand *et al*., 1992). Thus, Mdm2 negatively regulates both stabilization of p53 and its function. Knock-out mice for Mdm2 are embryonic lethal due to high endogenous levels of p53 but double mutant p53/Mdm2 knock out mice are viable. More significantly, mutations in Mdm2 are frequently found in tumors, particularly those tumors without p53 mutations. Mdm2 is also itself subject to controlling mechanisms which include multi-site phosphorylation and sumoylation (Meek and Knippschild, 2003). Another factor influencing Mdm2 function is the tumor suppressor protein called $p19^{ARF}$ in humans, which is derived from an alternative reading frame of INK4a. $p19^{ARF}$ binds directly to Mdm2 in a region distinct from the p53 binding domain. It does not inhibit p53/Mdm2 binding but does inhibit p53 degradation probably by sequestering Mdm2 into the nucleolus. The major mechanism regulating MDM2 bind to p53 is phosphorylation, both of p53 and MDM2 itself. As discussed above, ATM plays a role in both of these events.

ii) G1/S arrest.

Careful analysis has demonstrated that two types of G1/S arrest can occur in mammalian cells: prolonged arrest which is a p53-dependent response, and a more transient response (Di Leonardo *et al*., 1994; Little, 1968) . The latter appears to be similar to the G1/S response observed in yeast. The major p53 response protein required for G1/S arrest is p21 (Wahl and Carr, 2001). Whilst p21 is transcriptionally regulated by p53, there is also recent evidence that p53 regulates the stability of p21 via another p53 protein, p53RPF (Ng *et al*., 2003). p21 is an inhibitor of cyclin dependent kinases and plays its major role in G1/S arrest by binding to the cyclin D/Cdk6 complex and

inhibiting its ability to phosphorylate pRb, which in turn inhibits the release of pRb from E2F, an essential step that triggers S phase progression (see Ko and Prives (1996) for a review). Consistent with this model, neither p53 nor ATM null cells show prolonged radiation induced G1/S arrest. A-T cells are, however, capable of arresting at the G1/S boundary following UV irradiation, demonstrating the specificity of the upstream signal transduction mechanism. However, the operation of this checkpoint does not necessarily serve to elevate survival to IR since transformed fibroblasts (which normally lack this response due to p53 inactivation) as well as p53 null cell lines display elevated radioresistance compared to primary or $p53^{+/+}$ cells (Lee and Bernstein, 1993).

iii) S phase arrest.

Replication in mammalian cells is also inhibited following irradiation, which can be observed by decreased ability of replicating cells to incorporate radioactive precursors into DNA. Cells from ATM and NBS display a phenotype called radioresistant DNA synthesis (RDS), which is believed to be due to a failure to undergo S-phase delay (Jackson, 2002). Current evidence suggests that early S phase arrest after irradiation is ATM-dependent but at later times S phase arrest is mediated via ATR (Zhou *et al*., 2002). Chk2 and possibly Chk1 represent strong candidate proteins involved in mediating S phase arrest via Cdc25A degradation (Iliakis *et al*., 2003; Xiao *et al*., 2003). S phase arrest encompasses inhibition of ongoing replication forks, stabilisation of replication forks and the inhibition of late firing replicons (Feijoo *et al*., 2001; Tercero *et al*., 2003).

iv) G2/M arrest.

Progression from G2 to M is controlled largely by the DSBs-cyclin B complexes. Activation or inhibition of these complexes is controlled by opposing kinases and dephosphatases affecting the phosphorylation status of the Thr14 and Tyr15 residues of Cdk. Currently, the prevailing evidence suggests that ATM phosphorylates Cds1 and/or Chk1, which in turn phosphorylates and inactivates Cdc25, the event that prevents dephosphorylation and activation of Cdc2-cyclin B. G2/M arrest after γ-irradiation, though ATM dependent is p53 independent. In earlier studies, confusion arose concerning the G2/M checkpoint due to the ability of cells to arrest in two distinct ways in G2. Normal cells in G2 at the time of irradiation show a delay in entry into mitosis, which represents the operation of a G2/M checkpoint. A-T cells in G2 at the time of irradiation

show a reduced delay compared to normal cells showing that this arrest is at least partially ATM dependent (Beamish and Lavin, 1994). However, following higher doses asynchronous A-T and control cells can show a permanent arrest at G2/M which has recently been shown to be ATR-dependent (Wang *et al*., 2003). The contribution of G2/M arrest to survival following radiation exposure is unclear, although the prevailing view is that arrest enhances survival and reduces the probability of genomic alterations.

v) Apoptosis.

Apoptosis is a process utilized to balance cell proliferation and cell death. It is crucial to certain developmental processes and is for example used during immune development to remove cells that have failed to undergo productive rearrangements (Sohn *et al*., 2003). It is also utilized to remove cells damaged by exogenous DNA damaging agents. The onset of apoptosis in normal cells by radiation is p53-dependent although p53-independent routes to apoptosis have also been described (Adams, 2003). Additionally, there are significant differences between cells lineages in their propensity to undergo apoptosis following irradiation.

The signaling processes leading from p53 induction to apoptosis are complex and diverse. All the pathways, however, converge in the activation of proteases termed caspases (Adams, 2003; Thornberry and Lazebnik, 1998). Caspases exist as pro-enzymes that require activation and finally effect apoptosis by protein degradation that results in disassembly of cell structures such as nuclear lamin, degradation of DNA repair proteins such as PARP, ATM and DNA-PKcs, and by enhancing DNA fragmentation via the cleavage of I^{CAD}, an inhibitor of a nuclease capable of fragmenting DNA. One pathway leading to apoptosis, and probably the pathway that plays the major role following IR involves the bax/bcl2 family (Adams, 2003), of which at least 15 members have been described. Bcl2 itself, first identified by its presence at a chromosomal translocation break site in B-cell lymphomas, is an anti-apoptotic protein whilst, in converse, Bax, with which it can dimerise is a pro-apoptotic protein. Bcl2 family members together regulate the release of cytochrome C from the mitochondria, which serves to activate caspases through an interaction with Apaf1 (Cory and Adams, 2002). Other routes to apoptosis involve death receptor proteins that activate death ligands, which in turn activate caspases (Ashkenazi, 2002).

3.4 Fidelity of DSB repair.

A crucial consideration for radiation protection is the level of fidelity with which DSBs breaks are rejoined and the impact of error-prone rejoining. In this context, several factors are important: a) the inherent fidelity achievable by the distinct DSB rejoining mechanisms, b) the fate of unrejoined and misrejoined breaks and c) the ability of radiation damage to undergo accurate repair compared to other forms of DNA damage, particularly endogenous damage. These three issues are discussed below.

3.4.1 The fidelity achievable by HR and NHEJ.

HR is clearly a high fidelity process utilizing sequence information from an undamaged template to repair coding information lost at a break site. The level of fidelity achievable by NHEJ, for either simple breaks or complex breaks, is still an open question. One difficulty in evaluating the studies on fidelity is that frequently restriction enzymes are used to induce DSBs, and these may be repaired with different fidelity to radiation induced DSBs. Studies in *S. cerevisciae* have examined the fidelity of rejoining simple restriction-enzyme induced breaks in the presence and absence of the individual NHEJ components. From these studies it has been concluded that Ku-dependent NHEJ is an accurate process that can act as a barrier to an alternative error prone end-joining mechanism (Boulton and Jackson, 1996). Recently, a study examining repair of a transposase-induced DSB in mammalian cells also concluded that NHEJ was normally accurate (van Heemst *et al*., 2004).

As discussed previously the NHEJ pathway is also used during V(D)J recombination. The rejoining of these V(D)J breaks can also provide information on the accuracy of the process in mammalian cells. Although the coding joints generated during V(D)J recombination are rejoined inaccurately due to specific processing unique to lymphoid cells, the signal junctions are rejoined accurately (Gellert, 2002). In cell lines lacking components of the NHEJ machinery, both the frequency and fidelity of signal joint formation is dramatically reduced (O'Driscoll *et al*., 2001; Riballo *et al*., 2001; Taccioli *et al*., 1993). This suggests that for these types of breaks, if rejoining is compromised, then the ends are subjected to nuclease digestion and repair is inaccurate. This suggests that NHEJ has the ability to rejoin a blunt ended break accurately and indeed, does so predominantly.

However, the repair of radiation induced breaks may be more demanding than the repair of the breaks discussed above. Many of the radiation induced breaks may represent non-ligatable ends or ends that require additional processing prior to ligation. One approach that has been used to assess the fidelity of radiation-induced breaks during NHEJ is a technique that monitors the mis-repair of DSBs by pulse field gel electrophoresis (PFGE), a procedure that separates large DNA fragments (of the order of 10^6 base pairs) on the basis of size (Rothkamm *et al*., 2001). To assess the fidelity of rejoining the gels were probed using a large unique DNA fragment generated by digestion of genomic DNA using a rare-cutting restriction enzyme. Following radiation exposure, the unique restriction fragment became a smear of smaller size due to the presence of DSBs within it. Following incubation to allow repair, the band was recovered representing accurate repair. It was argued that whilst fragments smaller than the anticipated size could arise from either inaccurate rejoining or lack of rejoining, fragments larger than the anticipated size could only arise by mis-rejoining. When the experiment was carried out following exposure to a high dose (80 Gy), significant mis-repair could be seen. Although a limitation of this technique is that it necessarily involves the use of high doses, the results, nevertheless, demonstrate that, under such conditions, NHEJ has the potential to rejoin breaks inaccurately. Studies with NHEJ deficient cells further suggest that the observed mis-repair is, in fact, mediated by the NHEJ pathway. Using the same technique, mis-repair was also examined following 80 Gy delivered at a low dose rate where radiation induced DSBs would be less likely to be in close proximity to one another in both space and time. Under these conditions, there was much less detectable mis-repair observed. Taken together, these findings suggest that the accuracy of NHEJ may be influenced by the presence of neighboring breaks and suggest that the process has the potential to be of higher fidelity when few breaks are present in any one cell but that its fidelity may be compromised when many breaks arise independently. Interestingly, similar experiments carried out following exposure to alpha particles showed that there was no reduction in mis repair with increasing fractionation (Kuhne *et al*., 2002). These data are consistent with dose and dose rate data for the induction of chromosome alterations following exposure to IR where effects are significantly reduced for low dose rate exposures. It is important to note, however, that these data show that misrepair can occur at high dose rates. They leave open still the question of whether misrepair can also occur at low dose rates and doses. Exposure of mammalian cells to IR causes a linear dose dependent increase in chromosome breaks, gaps and rearrangements

at relatively low doses and low dose rates. Making the reasonable assumption that chromosomal rearrangements represent erroneous DSB rejoining events, such data would argue for mis-repair mediated via the NHEJ machinery even under conditions where the distribution of radiation induced DSBs are not in close proximity in space and time. This argument, while strong, cannot be directly tested experimentally at this time.

Cells lacking components of the NHEJ machinery (for example xrs-6 cells) show elevated radiation-induced chromosomal aberrations relative to normal cells (Darroudi and Natarajan, 1989; Kemp and Jeggo, 1986). This suggests that in the absence of Ku, a lower fidelity rejoining process takes place. Although this finding does not directly address NHEJ fidelity, it does strongly suggest that there is elevated infidelity in the absence of Ku. In other words, Ku serves to promote accurate rejoining.

Finally, recent studies have also provided evidence for a process of rejoining DSBs that involves rejoining of the breaks to dysfunctional telomeres (Bailey *et al*., 2004; Latre *et al*., 2003). These studies thus open a new pathway for misrepair that represents DSB-telomere fusions and could represent an important cause of genomic instability induced by radiation (Urushibara *et al*., 2004).

3.4.2 The fate of unrejoined and misrejoined breaks.

The role of cell cycle checkpoints is to prevent the proliferation of damaged cells. It has been argued that a single unrejoined DSB is lethal to a cell. From the perspective of a multi-cell organism this may not be unduly harmful. A misrejoined break, may not, however, be recognized by a cell and therefore may pose a bigger threat as a potential oncogenic lesion. Failure of cell cycle checkpoint control coupled with impaired DSB repair will, however, pose a particular risk. In this light, patients such as A-T and NBS patients, where the defects impair both DNA repair mechanisms as well as cell cycle checkpoint control display significant cancer predisposition. Similarly, the combination of p53 mutations with mutations in essential DNA repair genes (such as DNA ligase IV) promote survival at the expense of elevated tumor predisposition (Zhu *et al*., 2002). In this light, the ability of low doses of radiation to affect cell cycle checkpoint control is particularly important to evaluate.

3.4.3 The impact of the nature of DNA damage on repair.

As described above (section 3.2), the damage induced by IR is distinct from endogenous ROS induced damage in its complexity. Single strand breaks (SSBs) are

repaired accurately and rapidly, and there are an array of glycosylases that recognize and initiate the excision of specific damaged bases (for review see (Scharer and Jiricny, 2001: Slupphaug, 2003). It is important to point out, however, that although ROS induced damage may not directly induce DSBs, it is likely that DSBs do arise endogenously potentially through the processing or replication of other lesions. It is also likely that such breaks will have ends that require processing prior to rejoining. The major evidence suggesting that DSBs arise spontaneously, is that cells lacking either NHEJ or HR components display elevated instability (Difilippantonio *et al*., 2000; Karanjawala *et al*., 1999).

The repair of complex lesions induced uniquely by IR, may, however, pose a problem for the DNA repair machinery. Studies are now emerging on how one type of damage influences the repair of another. The current evidence suggests that the ability of glycosylases to recognize and remove a damage base is impeded by the presence of a nearby nick on the opposite strand (David-Cordonnier *et al*., 2000; David-Cordonnier *et al*., 2001). Since clustered base damage arises frequently after irradiation, a considerable number of additional DSBs could arise if clustering of base damage inhibits repair (Gulston *et al*., 2004). How the presence of a nick or damaged base affects DSB repair is entirely unknown.

Classical analysis of post-irradiation cell survival has also provided evidence that the highly complex lesions induced by high LET radiation are less reparable than low LET radiation. Most specifically, cells lacking Ku (e.g. xrs-6), are relatively more sensitive when compared to wild type cells to low LET radiation than to high LET radiation, consistent with the notion that high LET radiation has a higher non-reparable component (Thacker and Stretch, 1985). These studies have been discussed in detail in previous ICRP and UNSCEAR reports and will not be discussed further here.

As mentioned above, ROS-induced damage as well as the damaged induced by IR frequently have damaged termini, precluding their repair by direct ligation. Recently, polynucleotide kinase (PNK), a protein that has both DNA kinase and DNA phosphatase activities has been found associated with Xrcc1, one of the proteins involved in SSB repair (Whitehouse, 2001). Furthermore, Xrcc1 can stimulate the activity of PNK. Thus, the damaged 3’ end, which cannot be subjected to direct ligation, is first processed by PNK in the presence of Xrcc1, which then coordinates gap-filling, if necessary, by an interaction with DNA polymerase β followed by subsequent ligation (Caldecott, 2002).

This important finding demonstrates how cells use their resources to coordinate repair involving several distinct steps. However, these damaged termini arise frequently endogenously which has likely provided a strong selective pressure to drive the evolution of this co-coordinated repair process. This may not be the case for other, more complex, lesions unique to IR.

3.5 Impact of defects in DNA repair, checkpoint control and apoptosis.

Disruption of the NHEJ components in mice result in varied phenotypes; loss of XRCC4 and DNA ligase IV causes embryonic lethality, Ku defective mice senesce prematurely whereas DNA-PKcs defective mice grow and develop normally although manifesting severe combined immunodeficiency (Bosma *et al*., 1983; Frank *et al*., 1998; Gu *et al*., 1997; Nussenzweig *et al*., 1996). Significantly, however, DNA-PKcs defective mice have only a small elevated spontaneous cancer incidence. The situation with Ku is exceptionally unclear; Ku80 defective mice display no elevated tumour incidence whereas Ku70 defective mice develop a high incidence of lymphomas. Thus the impact of the loss of NHEJ on tumor incidence in mice remains to be resolved. A defect in DNA ligase IV has been identified in a leukemia patient who was normal until the onset of leukemia at age 14 (Riballo *et al*., 1999). The mutation identified in this patient conferred significantly decreased but not ablated ligation activity. This suggests firstly that impairment of NHEJ can be compatible with life, and confers significant radiosensitivity without overt immunodeficiency. More importantly, the defect may confer leukemia predisposition. The fact that this patient has decreased activity rather than totally ablated activity may be significant.

Haploinsufficiency of ligase IV has been shown to result in an increased incidence of sarcoma in ink4a/arf-/- mice (Ferguson et al., 2000; Sharpless et al., 2001). Decreased but not ablated DNA PKcs activity has also been associated with increased sensitivity to radiation induced lymphomas (Mori et al., 2001) and mammary tumors (Yu et al., 2001) in mice and lung and colon cancer in humans (Auckley et al., 2001; Rigas et al., 2001). It has been hypothesized that because of the importance of the NHEJ pathway, complete loss of function of one of the components in this pathway may result in a low frequency of tumors because of significant problems with genomic integrity and stability. Cells with such significant problems would manifest substantial genomic damage and would likely be eliminated by the cell cycle and apoptotic response pathways before having the

opportunity to progress to become tumors. On the other hand, with less severe defects in this pathway the cellular effects would be less severe and it would be more likely that cells with less severe forms of damage could escape elimination (Ferguson et al., 2000).

Recent evidence suggests that defects in checkpoint control or apoptosis confer a very different phenotype with significantly elevated cancer predisposition. Mice defective in p53 display elevated spontaneous tumor formation both in the homozygous and heterozygous state. Recently CHK2 was identified as the germ line tumor suppressor loci of a small number of Li-Fraumeni families that did not have TP53 mutations. Both A-T and NBS patients display significantly elevated tumor incidence. BRCA1 and BRCA2 defects are associated with cancer predisposition. Taken together, this suggests that whilst lack of repair may simply enhance sensitivity, failure to arrest at a cell cycle checkpoint or failure to undergo apoptosis may result in elevated carcinogenesis. The impact of these processes for radiation protection is two fold. Firstly, although the effect of low dose irradiation on DNA repair has been investigated almost nothing is known about the impact of low doses on cell cycle checkpoint arrest. Secondly, the variation in these responses between individuals is not known.

It has been proposed recently that a low-dose threshold could result, not from the absence of DSBs and complex lesions at very low doses, but from the absence of repair; i.e., affected cells are unable to replicate, and therefore do not contribute to carcinogenesis. That is, the affected organism, or tissue, might be genetically programmed to tolerate a certain amount of cell loss as a means of minimizing the risk of mutation and cancer due to DNA misrepair. A recent study by Rothkamm and Lobrich (Bonner, 2003; Rothkamm and Lobrich, 2003) involved the irradiation of cultures of nondividing primary human lung fibroblasts with 90 kV x rays at doses ranging from 2 Gy down to 0.1 mGy. Numbers of DSBs formed were measured by immunofluorescence of foci of the phosphorylated histone, (-H2AX). The investigators found that the number of DSBs formed was linear with radiation dose, but that DSBs induced at 1.2 mGy (0.1 foci per cell cf. 0.05 per cell among controls) remained unrepaired for many days, in contrast to efficient DSB repair following exposure at higher doses (0.66 and 0.22 per cell at 20 mGy and 5 mGy, declining to 0.1 per cell after 24 hours). However, there is some question about the extent to which the assay can be relied upon to quantify DSB frequency following radiation exposure. For example, in this study the assay indicates a surprisingly high frequency, and persistence over time, of DSBs in control cells, and a

high persistence of radiation-related DSBs following high-dose exposure compared with findings from split dose experiments. It has been demonstrated that those proteins involved in DSB rejoining, including H2AX, translocate substantial distances along the DNA from the break, implicating other functions for these proteins (Rogakou *et al*., 1999). Also, Petrini and Stracker (2003) note that, although late foci of DSB repair proteins and γ-H2AX appear to be genuine reflections of DSB metabolism, it is problematic to use them to draw inferences about recruitment to DSB sites because the vast majority of DSBs are repaired by 90 minutes after their induction. There is also some question whether the DSBs examined by Rothkamm and Lobrich were direct or indirect effects of radiation exposure (Seymour and Mothersill, 2004). Thus, the implications of this intriguing study for low-dose risk are at present unclear.

3.6 Conclusions.

Ionizing radiation is able to produce a unique type of damage in which multiple lesions are encountered within close spatial proximity. Even a single track of IR through a cell is likely to induce these unique clustered damages. This type of damage is unlikely to be generated frequently endogenously or by other exogenous agents, and thus, there may not have been a strong selective pressure driving efficient repair. Although cells have a vast array of damage response mechanisms that facilitate the repair of DNA damage and the removal of damaged cells, these mechanisms are not fool proof. Moreover, clustered radiation–induced lesions pose a particular problem and current emerging evidence suggests that closely spaced lesions can compromise the repair machinery. On this basis, there is not any strong evidence for a radiation dose below which all radiation-induced damage can be repaired with fidelity. Whilst many of the cells containing such radiation-induced damage may be eliminated by damage response pathways involving cell cycle checkpoint control and apoptotic pathways, it is clear from analysis of cytogenetics and mutagenesis that damaged or altered cells are capable of escaping these pathways and propagating. This further argues against the likely possibility of a threshold for radiation-induced cellular effects.

3.7 References

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4. CELLULAR CONSEQUENCES OF RADIATION-INDUCED DAMAGE

The mis-repair of radiation-induced DNA double-strand breaks and other lesions is believed to be the principal pathway for the induction of chromosome and gene alterations responsible for the killing, mutagenic, and carcinogenic effects of ionizing radiation. Studies focusing on cytogenetic damage and mutagenesis were among the earliest quantitative measures of the cellular effects of ionizing radiation (Sax, 1938). On a practical level such studies have provided considerable information on dose response relationships over a wide range of doses and on the effects of dose rate and fractionation (NCRP, 1980). On a more fundamental level these studies have provided a substantial amount of information relevant to DNA damage after radiation, repair kinetics and on underlying mechanisms. Because of the close mechanistic relationship between chromosome aberrations, mutations, and cancer (UNSCEAR, 2000) such studies also have particular relevance to radiation risks and the question of risks at low doses.

4.1 Radiation-induced chromosome aberrations.

The first documented account of the cytogenetic effects of x rays described the production of dicentrics, centric rings and deletions in plant microspores irradiated in the extended G_1 phase (Sax, 1938). It was very difficult using the standard staining technique to observe reciprocal translocations and inversions; these aberrations are the most frequently present in tumors of different types. This latter fact is the consequence of reciprocal translocations and inversion being transmissible from cell generation to generation, whereas dicentrics, centric rings and deletions are cell lethal as the result of the loss of genetic material at cell division. The ability to analyze all types of chromosome aberrations has been greatly enhanced by the use of Fluorescence In Situ Hybridization (FISH) that is discussed in more detail below.

The early studies by Sax and his colleagues also demonstrated that the dose response curve for dicentric aberrations fit a linear-quadratic model ($Y = \alpha D + \beta D^2$), suggesting that some dicentic exchanges were produced by one ionization track (αD) and some by two independent tracks (βD^2). Neutrons induced the same types of chromosome aberrations, but in contrast the dose-response curve for dicentrics was linear indicating a one-track mechanism of formation. The prediction, based on the proposed mode of formation of aberrations, was that chronic exposures to x rays would produce all types of

aberrations linearly with dose, and that split doses would lead to lower aberrations frequencies than the same dose given as a single exposure. These predictions were borne out in experiments with Tradescantia microspores (Sax et al, 1955) and have, of course, been subsequently confirmed in an expansive range of studies covering many cell types and species. Some of the most comprehensive studies examining low doses of radiation were those of Lloyd and co-workers (1992). A further prediction from these studies is that over a low dose range (and for low dose rates) the dose-response curve for chromosome aberrations is linear (αD) and time (i.e., dose rate) independent because the one-track mechanism dominates the response. Thus, the linear slope for low dose and low dose rate exposures in this dose range would be the same. This has been borne out in careful studies of the induction of chromosome aberrations over a range of dose rates by Cornforth et al. to specifically test the prediction of a limiting slope at low doses and dose rates (Cornforth et al, 2002).

While details of mechanisms involved in the formation of chromosome aberrations remain under investigation, the current view is that the majority of radiation-induced chromosome aberrations are produced by the misrepair of DNA double-strand breaks (DSB) quite possibly those involved in complex DNA lesions (multiply damaged sites). The observations presented above with respect to low dose linearity would support this view. The repair of DSBs (described in Chapter 3) is performed by nonhomologous end-joining and homologous recombination; the former is the prevalent mechanism in mammalian cells. In some cases the pairs of DSB required for the formation of chromosome aberrations by misrepair are produced by one or more electron tracks from a single photon and in others by two or more tracks from different photons. While DSB are generally presumed to be produced linearly with dose for low LET radiations, the probability of conversion into chromosome aberrations is not established. The probability of conversion will depend upon the probability of misrepair and the overall kinetics of DSB repair, and it is likely to be linear with dose given the predictable one-track/two-track nature of the dose-response curve for chromosome aberrations.

The development of FISH techniques has allowed for the assessment of the nonlethal reciprocal chromosomal events, i.e. reciprocal translocations and pericentric inversions, as well as complex events involving multiple chromosomal exchanges that would not typically be identified by conventional staining. The dose response for reciprocal translocations is quite similar to that for dicentrics, discussed above, and involves a one-track and a two-track process (Camporoto et al., 2003). Thus, the effects

of dose rate and dose fractionation are also similar to those described above for dicentrics. Low dose linearity is observed for acute and chronic low-LET exposures.

The “complex exchanges” observed with FISH are often considerably more complex than previously thought. These complex exchanges can involve multiple interactions among several chromosomes. Such complex aberrations constitute a large fraction of aberrations observed after exposure to high LET radiations and that fraction does not appear to vary with dose. For low LET radiation, the fraction of aberrations that are complex is more dose dependent. At relatively high doses (2 to 4 Gy) the fraction is high but at low doses the fraction of aberrations that are complex is substantially lower but still present. More precise data on dose response and dose rate effects for these complex aberrations at low doses will be forthcoming from ongoing studies over the next several years. The mechanisms underlying these complex aberrations are not clear at present and are under investigation. They do appear to involve interactions between sites of complex DNA damage of the type particularly prevalent after exposure to high LET radiation. Such damage is much less prevalent as a result of low LET irradiation, but is still present even at low doses. The significance of these complex exchanges in mutagenesis and carcinogenesis is also unclear. Many of them are probably lethal and therefore not likely to impact such endpoints. However, certain complex aberrations are potentially transmissible and could have a significant impact on mutagenesis, carcinogenesis and the initiation of genomic instability. As a result, the understanding of the mechanisms involved in the development of these complex aberrations may provide important information relevant to low dose risks.

Thus, the prevailing view is that chromosome aberrations of all types result from the interactions of pairs (or greater number) of DNA lesions. These lesions can be induced by a single track or by combinations of two or more tracks. However, there is a possible exception to this general rule. Griffin et al. (1996) assessed the efficiency of 1.5 keV aluminum x rays at inducing complex chromosome aberrations (requiring three or more interacting lesions for their formation). Based upon the rather high efficiency of this process, the authors suggested that damaged DNA could interact with undamaged DNA to produce some of the aberrations. A proposed mechanism, similar to the production of recombinations during meiosis (Szostak et al., 1983), is not supported by data developed by Cornforth (1990) who concluded that a one-hit exchange probably did not occur, although it could not be ruled out at low doses. The impact of a one-hit exchange process on the shape of the dose-response curve at high doses and the exchange

yields at low doses is readily apparent; a steeper slope than that described by the αD component of the linear-quadratic equation would be predicted. The question of its likelihood requires further study.

Additional details of the mechanisms of formation of chromosome aberrations and the relevance of their distribution among and within cells to low dose responses can be found in NCRP Report No. 136 (2001). These data and those presented above support the conclusion that at low doses of high- or low-LET radiations the dose-response curves for chromosomal aberrations are linear. Predictions can be made for threshold responses, but the existing data do not support or refute them. The same conclusion applies for supralinear low-dose responses.

4.2 Radiation-Induced Somatic Cell Mutations

Radiation is capable of inducing a wide spectrum of mutations, from point mutations in single genes to deletions that encompass several physically linked genes (UNSCEAR, 2000). The nature of mutation assays limits the ability to detect large deletions in certain genes because of their close linkage with sequences that are essential for survival of the cell. With this complicating factor in mind, most molecular evidence indicates that DNA deletions resulting in gene loss are the primary events responsible for the mutagenic effects of ionizing radiation (UNSCEAR, 2000). It is also important to note, in this regard, that when data are available, a close relationship between radiation-induced mutations and chromosome aberrations has been found (UNSCEAR, 2000). This spectrum differs from spontaneous mutations, mutations induced by ultraviolet light and many chemical mutagens where the majority of mutations are a result of point mutations (UNSCEAR, 2000). Interestingly, radiation-induced point mutations tend to occur randomly throughout a gene while spontaneous mutations tend to be clustered at specific sites ((Grosovsky et al., 1988;Nelson et al, 1994). The data indicating a predominance of deletion type mutations, and the distribution of point mutations, suggest differences between underlying damage induced by ionizing radiation compared with that from endogenous processes.

Mutagenesis is essentially a result of the attempts of the cell to repair damage and analyses of induced mutations can provide clues about mechanisms involved. Sequence analyses of radiation-induced deletion-type mutations have revealed that, as in the case of radiation-induced chromosome aberrations, the mutations are much more complex than

originally thought. Deletions often include inversions and insertion of genetic sequences from other chromosomes and frequently involved short direct or inverted DNA repeat sequences ((Morris and Thacker, 1993;Morris et al. 1993;Thacker, 1986). Overall, these analyses support double strand breaks as an important initiating lesion in the pathogenesis of the large deletions characteristic of ionizing radiation and the involvement of DNA DSB repair pathways in the mutagenic process (UNSCEAR, 2000). The presence of repeat sequences suggest that illegitimate recombination associated with double strand breaks is often responsible for the mutagenic process when large deletions are involved. Limited studies with cells defective in specific repair pathways also suggest an important role for DNA DSB repair in the mutagenic effects of ionizing radiation (UNSCEAR, 2000). While double strand breaks are more difficult to repair with fidelity than base damage, radiation-induced base damage is also important. It is clear that base damage can often lead to base substitutions (point mutations) and that certain repair pathways involved in base damage repair can be mutagenic.

Quantitative studies on dose response relationships for the induction of mutagenesis can be more complicated than studies of chromosome aberrations with considerable variation depending upon the nature of the mutations that can be assayed in each system, genetic background, tolerance for large genetic changes such as deletions, and sensitivity of the system. In systems that have sufficient sensitivity to examine effects at relatively low doses, either linear or linear quadratic dose responses have been reported when a wide dose range has been examined (UNSCEAR, 2000). In either case, in the low dose region, data are consistent with a linear dose response. This linear response is consistent with current models of mechanisms of mutagenesis involving DNA damage and its processing. Such a linear dose response has been observed down to ~200mGy by Thacker et al (1992).

Studies of dose rate effects are more complex. In most systems, the effectiveness of low-LET radiation at doses greater than 1 Gy is reduced, at low dose rates, by a factor of 2-4; however there are data in which the effectiveness has remained the same or even increased after low dose rate exposures (Thacker et al , 1992). For example, no dose rate effect or even an inverse dose rate effect is observed in TK6 and other DNA-repair deficient human cells and in many rodent cell lines at very low dose rates (Amundson and Chen 1995, 1996; Vilenchik and Knudson 2000). These dose rate data are consistent with expectations when repair plays a major role in mutagenesis. Cells defective in DNA repair capacity are likely to have little dose rate effect and in fact inverse dose rate effects

might be anticipated in cells with defects in damage response pathways at low dose rates (UNSCEAR, 2000; Thacker et al, 1992). A systematic study of this hypothesis would be important in clarifying mutagenic risks following protracted exposures.

4.2.1 Summary

The processing and mis-repair of radiation-induced DSBs, particularly complex forms, are principally responsible for chromosome/gene alterations that manifest as chromosome aberrations and mutations. Current understanding of mechanisms and quantitative data on dose and time-dose relationships support a linear dose response at low doses with no compelling evidence for the existence of a threshold dose below which there would be no effect.

4.3 Bystander Effects, Genomic Instability and Adaptive Responses

Recently, studies on the induction of so called “bystander effects” in cells not directly irradiated, and the development of genomic instability in the non-irradiated progeny of irradiated cells many generations after exposure, have served to challenge the conventional view that only those cells directly traversed by radiation are targets for cellular effects of radiation including cell killing, and the induction of chromosomal aberrations and mutations. In addition, the assumption that multiple exposures at low doses are additive has come into question as a result of studies demonstrating an adaptive response in certain cells following low dose radiation exposures. The concept of additivity is a result of the view that, following repair, a cell will respond similarly to a second exposure as it did to the first. Studies demonstrating an adaptive response, however, suggest that this may not always be the case; the induction and/or activation of genes likely involved in damage response pathways can influence, positively or negatively, the response to subsequent exposures. If these three phenomena occur *in vivo,* they could impact in particular on the shape of the dose-response curve for low dose, low dose-rate exposures in human populations.

4.3.1 Adaptive Response

The adaptive response was first described for chromosomal aberrations (Olivieri et al, 1984). It was observed that pre-exposing cells to a low “priming” dose of radiation appeared to protect these cells from the effects of a second, larger “challenging” dose.

This effect was demonstrated most clearly in human lymphocytes, where a decrease of up to 50% in the frequency of aberrations induced by the challenging dose has been observed in cells pretreated with a small priming dose (Wolff, 1996; Sugahara *et al*, 1992). Since the appearance of the initial report over 20 years ago, literally hundreds of reports have been published describing this phenomenon in various experimental systems and for various endpoints including micronucleus formation, mutations and neoplastic transformation; many of these were reviewed in 1994 by the UNSCEAR Committee (UNSCEAR Report, 1994). Despite all of this research, the mechanisms for this phenomenon remain unclear, in contradistinction to the adaptive response to alkylation damage (Lindahl et al, 1988). The effect is not consistently seen in all cell types, and there has been considerable donor variation in studies with human lymphocytes.

In the earlier studies of the adaptive response to chromosomal aberrations in lymphocytes, low dose-rate exposure from tritiated thymidine was used as a priming dose, though it was later shown that an acute exposure to x-rays would also trigger the effect (Shadley and Wiencke, 1989). Priming doses of 5-100 mGy are generally required to induce the protective effect (Shadley and Wiencke, 1989; Sasaki, 1995). These doses are high enough to produce significant damage in all cells irradiated. Adaptation takes place within 3-6 hours when the cells become resistant to the larger challenge dose, usually 1 Gy or higher. Gap junction mediated intercellular communication has been implicated in this process (Ishii and Watanabe, 1996). The magnitude of the effect depends on many factors including dose, dose-rate, cell and tissue type and the endpoint measured.

The mechanisms for the effect remain unclear. It is now known that low doses of radiation can modulate the expression of a variety of genes (e.g., Hallahan *et al*, 1991; Leskov *et al*, 2001; Sasaki *et al*, 2002) . Sasaki *et al* (2002) found that p53 appeared to play a key role in the adaptive response while the DNA-PKcs, ATM and FANCA genes were not involved. They proposed that the adaptive response and apoptosis constitute a complementary defense mechanism. It has also been reported that the induction of heat shock proteins may be involved in the adaptive response (Lee et al, 2002; Kang et al, 2002).

While it has been hypothesized that the phenomenon reflects the induction of some type of DNA repair process that requires a certain level of damage in the cell, no such inducible DNA repair mechanism for DNA strand breaks has been clearly demonstrated in mammalian cells. Restriction enzymes that produce DNA double strand

breaks will induce adaptation in human lymphocytes (Wolff, 1996), and the rate of repair has been reported to be more efficient in adapted cells (Ikushima *et al,* 1996). Evidence has been presented to suggest the involvement of DNA repair in the adaptive response in yeast (Dolling *et al,* 2000), and Haber and colleagues (personal communication) have shown that when a single DSB is introduced in budding yeast cells synchronized in G_1, the cells become significantly resistant to a challenge dose of MMS applied during the discrete period approximately 6 hours later when repair is taking place. It is of interest in this context that the inducible repair of thymine glycols by the base excision repair process has been described (Le *et al,* 1998). Generally, however, DNA base damage is not thought to be the principal mechanism for the induction of mutations and chromosomal aberrations by ionizing radiation. It has also been proposed that the priming dose may lead to persistent free radical activation as part of the post-irradiation cellular stress response that includes the up-regulation of genes associated with signal transduction and cell cycle control (Bravard *et al,* 1999).

A number of reports have presented evidence for an adaptive response for the induction of specific gene mutations (Sanderson and Morley, 1986; Kelsey *et al,* 1991; Zhou *et al,* 1994; Rigaud *et al,* 1995). In general, the mutation frequencies induced by relatively high radiation doses have been shown to be decreased by approximately 50% if the exposure is preceded by a priming dose of approximately 10 mGy 5 to 24 hours previously. These experiments have been carried out in various different systems, though generally but not exclusively with cells of lymphoid origin (lymphocytes, established lymphoblastoid cell lines and a human T-cell leukemia cell line). The adaptive exposure to radiation may also decrease the frequency of neoplastic transformation either arising spontaneously or induced by a subsequent high radiation dose (Azzam *et al,* 1994; Redpath and Antoniono, 1998; Redpath *et al,* 2001; 2003). Adaptive responses have been described in human tumor cells with irradiation protocols closely resembling clinical applications (Smith and Raaphorst, 2003).

Evidence is emerging for the occurrence of adaptive phenomena *in vivo*. These include the induction of leukemia and lymphoma (Ishii *et al,* 1996; Bhattacharjee and Ito, 2001; Mitchel *et al,* 1999; 2003), as well as teratogenic effects and the development of heritable germline mutations (Somers *et al,* 2002). In one study (Bhattacharjee, 1996), pre-irradiating mice with five repeated exposures of 10 mGy a day appeared to reduce significantly the incidence of thymic lymphoma induced by a challenge dose of 2 Gy. It has been reported that short-term low dose occupational exposures may act as an *in vivo*

adaptive dose for the induction of micronuclei by *in vitro* irradiation of lymphocytes (Thierens *et al*, 2002).

The adaptive response shares some similarities with the phenomenon of "low dose hypersensitivity" described by Joiner and his colleagues (Joiner *et al*, 1996) based on the multiphasic shape of the single dose survival curve for some mammalian cell lines. They have observed a steep decline in cell survival in the low dose range, followed by a plateau which they hypothesize represents induced radioresistance. In a recent study (Short *et al*, 2001), cells displaying a strong hypersensitivity response showed increased killing following multiple low dose exposures. Similar to the adaptive response, it has been proposed that the phenomenon may represent the manifestation of inducible processes facilitating the repair of DNA damage (Joiner *et al*, 2001; Marples and Joiner, 2000). In two quite different experimental systems for the study of malignant transformation *in vitro*, evidence has been presented that the spontaneous transformation frequency is actually reduced by very small doses of radiation (doses as low as 1 mGy) (Azzam *et al*, 1996; Redpath *et al*, 2001; 2003). The frequency of transformation rose rapidly at higher doses.

Despite such provocative findings, there are still many questions concerning the adaptive response (Wolff, 1998; Stecca and Gerba, 1998). The response for chromosomal damage has been shown to vary with the donor, some individuals being unresponsive and others showing a synergistic effect (Bosi and Olivieri, 1989). The same is true for different cellular systems and for other biological endpoints such as cell survival (Boothman *et al*, 1996; Short *et al*, 1999; Sorensen *et al*, 2002). In the absence of firm knowledge of molecular mechanisms, it is difficult to evaluate the potential significance of the adaptive response for the risk from exposure to ionizing radiation in human populations. Clearly, the phenomenon appears to be a real one in many cellular systems, one that could influence the response to protracted radiation exposure. It will be important, however, to determine the extent to which it is active *in vivo* at relevant dose and dose-rate levels for human exposures before it can be considered as a factor in risk estimation.

Adaptive responses including those in relation to radiation induced cancer and stimulatory effects on the immune system were comprehensively reviewed by UNSCEAR in 1994 (UNSCEAR 1994) and some aspects were revised in UNSCEAR (2000). The general conclusion from these reports was that there was insufficient information on the role and mechanisms of adaptive responses to influence judgments on low dose cancer

risk. Recent animal carcinogenesis studies relating to adaptive responses (Mitchel et al 1999, 2003) raise the possibility that adaptive-like responses may increase tumour latency whilst not affecting life-time risk. These data are of scientific interest but remain of rather uncertain relevance to radiological protection.

4.3.2 Radiation Induced Genomic Instability

The term radiation-induced genomic instability refers to a phenomenon observed in a number of different cellular systems whereby radiation exposure appears to induce a type of instability in individual cells that is transmitted to their progeny, leading to a persistent enhancement in the rate at which genetic changes arise in the descendants of the irradiated cell after many generations of replication. The genetic endpoints studied have included malignant transformation, chromosomal aberrations, specific gene mutations, and cell survival. Typically, this phenomenon has been studied by examining the occurrence of such genetic effects in clonal populations derived from single cells surviving radiation exposure (Little, 2003), although some studies have relied upon the post-irradiation analysis of cells in mass culture rather than clonal isolates..

Early evidence for the existence of such a phenomenon was derived from an examination of the kinetics of radiation-induced malignant transformation of cells *in vitro* (Sinclair, 1964; Kennedy *et al*, 1980; Kennedy and Little, 1984). These results suggested that transformed foci did not arise from a single, radiation-damaged cell. Rather, radiation appeared to induce a type of instability in 20-30% of the irradiated cell population; this instability enhanced the probability of the occurrence of a second, neoplastic transforming event. This second event was a rare one, occurring with the frequency of approximately 10^{-6}, and involved the actual transformation of one or more of the progeny of the original irradiated cells after many rounds of cell division. This transforming event occurred with the constant frequency per cell per generation, and had the characteristics of a mutagenic event (Kennedy *et al,* 1984). Thus, neoplastic transformed foci did not appear to arise from the original irradiated cell but rather from one or more of its progeny. These findings were consistent with the hypothesis that radiation induces genetic instability in cells that enhances the rate at which malignant transformation or other genetic events occur in descendants of irradiated cells after many generations of cell replication.

This hypothesis has subsequently been confirmed in a number of experiment systems for various genetic endpoints (Morgan *et al,* 1996; Little, 1998; Baverstock,

2000; Romney *et al,* 2001a; Morgan, 2003a). In terms of mutagenesis, approximately 10% of clonal populations derived from single cells surviving radiation exposure showed a significant elevation in the frequency of spontaneously arising mutations as compared with clonal populations derived from non-irradiated cells (Chang and Little, 1992; Little *et al,* 1997). This increased mutation rate persisted for approximately 30 generations post-irradiation then gradually subsided. Interestingly, the molecular structural spectrum of these late-arising mutants resembles those of spontaneous mutations in that the majority of them are point mutations (Grosovsky *et al,* 1996; Little *et al,* 1997), indicating that they arise by a different mechanism from that of direct x-ray-induced mutations which involve primarily deletions. An enhancement of both minisatellite (Li *et al,* 1992) and microsatellite (Romney *et al,* 2001b) instability has also been observed in the progeny of irradiated cells selected for mutations at the *thymidine kinase* locus, further evidence that a subpopulation of genetically unstable cells arises in irradiated populations. It is of interest that instability as measured in minisatellite sequences of x-ray-transformed mouse 10T½ cells was markedly enhanced when the cells were grown *in vivo* as compared to prolonged cultivation *in vitro* (Paquette and Little, 1994*).*

An enhanced frequency of non-clonal chromosomal aberrations was reported in clonal descendants of mouse hematopoietic stem cells examined 12-14 generations after exposure to alpha radiation (Kadhim *et al,* 1992). Persistent radiation-induced chromosomal instability has since been demonstrated in a number of other cellular systems (Sabatier *et al,* 1992; Holmberg *et al,* 1993; Marder and Morgan, 1993; Kadhim *et al,* 1995; Little *et al,* 1997; Ponnaiya *et al,* 1997; McIlrath *et al,* 2003). Susceptibility to radiation-induced chromosomal instability differs significantly among cells from different strains of mice (Watson *et al,* 1996a; Ponnaiya *et al,* 1997), and similar differences in genetic susceptibility to radiation-induced chromosomal instability have been observed in primary human fibroblasts (Kadhim et al, 1998).

It is now clear that genomic instability, both chromosomal and mutational instability, can be induced by high or low LET radiation (Little *et al,* 1997; Belyakov *et al,* 1999; Limoli *et al,* 2000; Evans *et al,* 2001), and in most normal and transformed human and rodent cases as described above. The fact that Dugan and Bedford (2003) found no evidence for induced chromosomal instability in a normal human diploid fibroblast strain may be related to genetic factors as described by Kadhim *et al* (1998), who observed variability in the response of different strains of human diploid fibroblasts. Furthermore, delayed reactivation of p53 and a persistent induction of reactive oxygen

species has been reported in normal human fibroblasts (Rugo *et al,* 2003) as well as in human fibrosarcoma cells (Suzuki *et al,* 2003). Long-term instability can be induced by irradiation of cells with single alpha particles from a focused microbeam (Kadhim *et al,* 2001), supporting earlier observations that the instability phenotype can be activated by low radiation doses, becoming saturated at higher doses (Kadhim *et al,* 1995; Grosovsky *et al,* 1996; Little *et al,* 1997).

Finally, a persistently increased rate of cell death has been shown to occur in cell populations many generations after irradiation (Seymour *et al,* 1986; Chang and Little, 1991; Belyakov *et al,* 1999). This phenomenon has been variously referred to as occurring as a result of "lethal mutations" or "delayed reproductive failure", but has been measured as a reduction in the ability of cells to attach and form macroscopic colonies in a classic clonogenic survival assay. In some cellular systems, an increased rate of apoptotic cell death has been shown to accompany this phenomenon (Jamali and Trott, 1996; Limoli *et al,* 1998; Belyakov *et al,* 1999). Persistent reproductive failure has been linked to chromosomal instability (Limoli *et al,* 1998) and malignant transformation (Lewis *et al,* 2001; Redpath and Gutierrez, 2001), and evidence presented to suggest that DNA is at least one of the critical targets in the initiation of this phenomenon (Limoli *et al,* 1999). Instability was attenuated by treating the irradiated cells with free radical scavengers or allowing potentially lethal damage to be repaired by confluent holding prior to analyzing the subsequent development of chromosomal instability (Limoli *et al,* 2001). It has been proposed that oxidative stress perhaps consequent to enhanced, p53-independent apoptosis may contribute to the perpetuation of the instability phenotype in these populations (Limoli *et al,* 1998; Redpath and Gutierrez, 2001).

Of importance in terms of radioprotection is whether this phenomenon occurs *in vivo* and thus may be related to the induction of cancer. A number of mouse models for genetic instability have been described (Reliene and Schiestl, 2003). The transmission of chromosomal instability *in vivo* has been reported in several distinct experimental models (Pampfer and Streffer, 1989; Watson *et al,* 1996b; Watson *et al,* 2001; Ullrich and Davis, 1999), though not in others (Bouffler *et al,* 2001a), and *in vivo* aspects of transmissible instability are addressed in detail later in this report. Evidence for transmissible instability in irradiated human populations is inconsistent (Nakanishi *et al,* 2001; Whitehouse and Tawn, 2001). While it has been suggested that instability induced in X-irradiated mouse hematopoietic stem cells may be related to the occurrence of the non-specific genetic damage found in radiation-induced leukemias in these mice (MacDonald

et al, 2001), other work from the same laboratory indicates that susceptibility to radiation-induced leukemia/lymphoma is generally separable from sensitivity to induced genomic instability (Boulton *et al,* 2001).

In the case of murine mammary tumors induced by radiation, the link between genomic instability and early events in mammary cancer development appears stronger (Ullrich and Davis, 1999; Okayasu et al., 2000). In this instance, the instability appears to be directly related to a defect in the function of the DNA repair enzyme DNA-PKcs.

4.3.3 The Bystander Effect in Irradiated Cell Populations

The bystander effect of radiation refers to the evidence that damage signals may be transmitted from irradiated to non-irradiated cells in a population, leading to the occurrence of biological effects in cells that receive no radiation exposure. The use of this term has been interpreted broadly, however, as is evidenced by the experimental protocols employed to study such effects *in vitro*. The first protocol employs monolayer cultures of mammalian cells in which a small fraction of the cells in the population are irradiated, generally by alpha particles, and the biological effect examined in the non-irradiated, neighboring cells. A corollary protocol involves mixing experiments in which irradiated cells are mixed with non-irradiated cells and the biologic effect subsequently measured in the non-irradiated cohort of the population. The second protocol involves the harvesting of conditioned medium from irradiated cultures and incubating this with non-irradiated cells; the bystander cells are thus not in physical proximity to the irradiated cells. Both mixing and medium transfer techniques permit the examination of effects with low LET as well as high LET radiations.

The experimental model employed in many of these studies has involved the exposure of monolayer cultures of mammalian cells, often confluent or sub-confluent, to very low fluences of alpha particles, fluences whereby only a very small fraction of the nuclei in a cell population will actually be traversed by an alpha particle. This may be accomplished by irradiation from an external source of alpha particles (Metting *et al,* 1995) or by use of precision microbeam irradiators whereby specific cells can be targeted (Hei *et al,* 1997; Prise *et al,* 1998; Prise *et al,* 2000; Folkard *et al,* 2001; Shao et al (2003a). A grid arrangement has also been employed to protect many cells in a population exposed to relatively high fluences of alpha particles (Lorimore *et al,* 1998).

The first evidence for this phenomenon was derived from studies of the induction of sister chromatid exchanges (SCE) by very low fluences of alpha particles from an external source (Nagasawa and Little, 1992). It was observed that an enhanced frequency of SCE occurred in 20-40% of the cells exposed to fluences whereby only about 1/1000 to 1/100 cell nuclei were actually traversed by an alpha particle. This finding was later confirmed and evidence presented to suggest that the phenomenon involved secretion of cytokines or other factors by irradiated cells leading to the up-regulation of oxidative metabolism in bystander cells (Deshpande *et al,* 1996; Narayanan *et al,* 1997; Lehnert and Goodwin, 1997; Narayanan *et al,* 1999). It has since been shown that an enhanced frequency of specific gene mutations occurs in bystander cells in populations exposed to very low fluences of alpha particles (Nagasawa and Little, 1999). As a result, the induced mutation frequency per alpha particle track increases at low fluences where bystander as well as directly irradiated cells are at risk for the induction of mutations. This leads to a dose-response curve in which the slope is initially steeper than it is at higher doses. Studies with microbeam irradiation have provided evidence for an enhanced frequency of micronucleus formation, cell killing and apoptosis in bystander cells (Prise *et al,* 1998; Prise *et al,* 2000; Belyakov *et al,* 2001; Schettino *et al*, 2003; Shao *et al*, 2003a), as well as an enhanced frequency of mutations (Zhou *et al,* 2000; Zhou *et al*, 2001) and malignant transformation (Sawant *et al*, 2001a).

It has also been shown that changes in gene expression occur in bystander cells in monolayer cultures; the expression levels of p53, $p21^{Waf1}$, CDC2, cyclin-B1 and rad51 were significantly modulated in non-irradiated cells in confluent human diploid cell populations exposed to very low fluences of alpha particles (Azzam *et al,* 1998). These experiments were carried out by western blotting and *in situ* immunofluorescence staining techniques utilizing convocal microscopy; although only about 1-2% of the cell nuclei were actually traversed by an alpha particle, clusters of cells showed enhanced expression of $p21^{Waf1}$. This phenomenon involved cell-to-cell communication via gap junctions (Azzam *et al*, 1998; 2001), as has also been shown for micronucleus formation (Shao *et al,* 2003b) and mutations (Zhou *et al,* 2001). It appears that radiation exposure itself can enhance intercellular communication as evidenced by an up-regulation of Connexin 43 (Azzam *et al,* 2003a). Evidence for DNA damage in bystander cells was provided by examining micronucleus formation, a surrogate measure of DNA damage; that the up-regulation of the p53 damage response pathway in bystander cells was a consequence of this DNA damage is supported by the observation that p53 was phosphorylated on serine

15 (Azzam *et al*, 2001). Interestingly, it has been hypothesized that the apparent persistence of DNA double strand breaks after very low dose x-ray exposure might be the result of such a bystander effect (Rothkamm and Lobrich, 2003).

DNA damage in bystander cells, however, appears to differ from that occurring in directly irradiated cells; whereas the mutations induced in directly irradiated cells were primarily partial and total gene deletions, over 90% of those arising in bystander cells were point mutations (Huo *et al*, 2001). This would be consistent with the evidence that oxidative metabolism is up-regulated in bystander cells (Narayanan *et al*, 1997; Azzam *et al*, 2002), and has led to the hypothesis that the point mutations are a result of oxidative base damage occurring in bystander cells (Huo *et al*, 2001). A similar mechanism has been proposed for the observation that localized cytoplasmic exposure from a microbeam irradiator led to a significant increase in the frequency of point mutations which appeared to involve the generation of reactive oxygen species (Wu *et al*, 1999; Shao et al, 2004)). Bystander cells defective in the non-homologous end joining pathway including mouse knockout cell lines for Ku80, Ku70 and DNA-PKcs are extremely sensitive to the induction of mutations and chromosomal aberrations (Nagasawa *et al*, 2003; Little *et al*, 2003). Interestingly, the mutations in these repair deficient bystander cells were primarily the result of partial and total gene deletions (Nagasawa *et al*, 2003), whereas those in wild type bystander cells were predominantly point mutations. The marked sensitization of repair-deficient bystander cells to the induction of mutations and chromosomal aberrations may be a consequence of unrejoined DNA double strand breaks occurring as a result of clustered damage arising from opposed oxidative lesions and single strand breaks. Mutations in wild-type cells arise primarily from oxidative base damage.

In earlier studies, it was reported that alpha particle irradiation could induce the intracellular generation of reactive oxygen species (ROS) including the superoxide anion and hydrogen peroxide (Narayanan *et al*, 1997). This ROS response did not require direct nuclear irradiation, as an ROS response was induced in non-irradiated cells with conditioned medium from alpha irradiated cells. The various studies examining the role of oxidative metabolism and gap junction mediated intercellular communication have been summarized by Azzam *et al* (2003b). The role of oxidative stress in modulating signal transduction and micronucleus formation in bystander cells was examined in confluent monolayer populations of human diploid cells exposed to low fluences of alpha particles (Azzam *et al*, 2002). The results support the hypothesis that superoxide and hydrogen peroxide produced by flavin containing oxidase enzymes mediate the activation

of several stress inducible signaling pathways as well as micronucleus formation in bystander cells. These include the p53 damage response pathway as well as the MAP kinase family of signaling pathways. It has also been reported that nitric oxide may initiate intercellular signal transduction pathways influencing the bystander response to radiation (Matsumoto *et al*, 2001; Shao *et al*, 2002). It thus appears that ROS may be the primary mediators of the bystander effect (Szumiel, 2003).

Interestingly, this up-regulation of oxidative stress in bystander cells is reminiscent of the effect associated with radiation-induced genomic instability (Redpath and Gutierrez, 2001; Limoli *et al*, 2001), and it has been proposed that the bystander effect may be related to the induction of an inflammatory-type response *in vivo* (Lorimore *et al*, 2001). The activation of MAP K proteins and their downstream effectors in bystander cells (Azzam *et al*, 2002) is of particular interest in terms of the recent observation that membrane signaling pathways are involved in the bystander effect in monolayer cultures (Nagasawa *et al*, 2002; Shao et al, 2004).

Bishayee *et al* (1999) and Howell and Bishayee (2002) developed a three-dimensional tissue culture model which utilized Chinese hamster V79 cells to study bystander effects caused by non-uniform distributions of radioactivity. Cells labeled with ^{125}IdUrd were mixed with unlabelled cells and multicellular clusters formed by centrifugation. A decrease in clonogenic survival occurred among the unlabelled cells which, based on inhibitor studies, appeared to depend upon gap junction mediated intercellular communication (Bishayee *et al*, 2001). On the other hand, when cells irradiated with carbon beams were co-cultured with non-irradiated cells, cloning efficiency and proliferation of the non-irradiated recipient cells was increased (Shao et al, 2003c), reminiscent of the well known feeder layer effect. When a mixture of ^{125}I-labeled and unlabeled human tumor cells were injected into nude mice, a distinct inhibitory effect on the growth of the unlabeled cells was observed (Xue *et al*, 2002). Belyakov et al (2003) have presented evidence for a bystander effect in a primary tissue explant model. Watson *et al* (2000) transplanted a mixture of irradiated and non-irradiated bone marrow cells in a mouse system that allowed the discrimination between irradiated donor stem cell-derived cells and non-irradiated stem-cell derived cells *in vivo*. They were able to demonstrate chromosomal instability in the progeny of the non-irradiated hematopoietic stem cells, providing a link between a bystander effect of ionizing radiation and the induction of genomic instability *in vivo*.

There is a long history of the apparent induction of clastogenic factors by radiation, primarily as measured in the plasma of irradiated individuals. These studies are reviewed in detail by Mothersill and Seymour (2001). These workers have reported that the exposure of cells in culture or explants of tissue to gamma radiation doses as low as 10 mGy can lead to the release of factors into the medium by the irradiated cells; when this conditioned medium is transferred to non-irradiated cells, their cloning efficiency is reduced associated with increased levels of apoptotic cell death (Mothersill and Seymour, 1998). This phenomenon has been associated with early changes in mitochrondrial membrane permeability and the induction of reactive oxygen species (ROS) (Lyng et al, 2001).

Overall, however, a clear picture has yet to emerge from the experience with medium transfer experiments. There is convincing evidence that factors are released into the medium by irradiated cells that can lead to changes in the viability of non-irradiated cells incubated with such conditioned medium. The results from different laboratories, however, are not entirely consistent. Some workers report that incubation with conditioned medium harvested from irradiated cultures leads to a reduction in cloning efficiency of the recipient cells (Lyng *et al,* 2002; Sawant *et al,* 2002), while others find it is enhanced (Iyer and Lehnert, 2002; 2002) or dependent on cell type (Mothersill and Seymour, 1997). The effect of medium irradiation alone is particularly controversial (Lehnert and Goodwin, 1997; Belyakov *et al*, 2001; Zhou *et al,* 2002). In terms of genetic effects, one laboratory describes a bystander effect for sister chromatid exchanges in conditioned medium transfer experiments (Lehnert and Goodwin, 1997), whereas another finds little or no evidence for a bystander mutagenic effect under similar conditions (Zhou *et al,* 2002). The effect appears likely to be mediated by cytokines or reactive oxygen species, but the exact nature of the factor or factors responsible for the biological effects in the non-irradiated, bystander cells remains to be elucidated.

In sum, the results of these studies of bystander effects indicate clearly that damage signals can be transmitted from irradiated to non-irradiated cells. In confluent monolayer cultures, this phenomenon involves gap junction mediated cell to cell communication, and appears to involve both the induction of reactive oxygen species and the activation of extra-nuclear signal transduction pathways. Preliminary evidence suggests a role for membrane signaling. Multiple biological effects may occur in bystander cells including cell killing, the induction of mutations and chromosomal aberrations, and the modulation of gene expression. Some evidence suggests that

regulation of the p53 damage response pathway may be central to this phenomenon. Damage signals may in addition be transmitted through the extracellular medium, also appearing to involve the production of reactive oxygen species. Finally, preliminary studies with tissue explant models and a mouse bone marrow stem cell transplant system suggests that the effect may occur *in vivo*.

4.4 Conclusions: Implications for Risk Assessment

There is increasing evidence that the development of invasive metastatic cancer involves a series of distinct genetic events some of which can be associated with specific stages in the carcinogenic process (Fearson and Vogelstein, 1990). A question that arises is how as many as six to eight such genetic events may accumulate in a single cell lineage, given that the prevalence of most mutations is about 10^{-5}. Loeb *et al* (2003) and others have postulated that early in the process of carcinogenesis a mutation may arise in a gene that is important in maintaining genomic stability, yielding a cell lineage with a mutator phenotype. This phenotype would enhance the frequency with which spontaneous mutations arise in these cells, and thus facilitate the accumulation of the requisite number of genetic events to produce a cancer. One such example is hereditary non-polyposis colon cancer which is associated with a germline defect in DNA mismatch repair. While genomic instability is a hallmark of tumor cells, most types of cancer have not been associated with specific DNA repair defects.

The finding that radiation itself may induce an instability phenotype has thus attracted considerable interest. It would suggest that the initial radiation-induced event may be a frequent one involving as many as 10-20% of the population, rather than a rare mutagenic event. This increased level of instability which is transmissible over many generations of cell replication would thus enhance the rate at which multiple genetic events important to the development of cancer would arise in the cell population. It is not yet clear, however, the extent to which this radiation-induced phenomenon may be of importance in carcinogenesis. The fact that it appears to saturate at fairly low doses (of the order of 100-500 mGy) implies that it could influence the extrapolation to low dose effects. On the other hand, as it may not represent an irreversible carcinogenic event such as mutation, it might be susceptible to modulation by external factors. Clearly, additional research is needed to determine the mechanisms involved in radiation-induced genomic instability, in terms of both the initiating event and how the effect is transmissible for

many generations of cell replication, before its implications for the assessment of the carcinogenic risk of low dose, low dose-rate exposure to ionizing radiation can be clarified.

Another area where this phenomenon could well be of significance involves potential transgenerational effects of irradiation. The sum of the available evidence suggests that such instability is induced in the germ cells of irradiated parents and in the offspring born to them (Niwa, 2003). If exposure to low levels of ionizing radiation thus induces the instability phenotype in germ cells of the offspring of irradiated parents, it is entirely feasible that this instability could increase their susceptibility to cancer or other genetic effects. For example, Pils *et al* (1999) reported that genomic instability manifested by lethal and teratogenic effects may be passed on to two successive generations of offspring in mice after irradiation of the zygote, while Niwa and Kominami (2001) and Dubrova and his colleagues (Dubrova *et al,* 1998; Dubrova and Plumb, 2002) presented evidence for transmissible germline instability at mouse minisatellite loci. There is preliminary experimental evidence to suggest that such transmissible instability might lead to increased susceptibility to the induction of tumors in the offspring of irradiated mice (Nomura, 1982; Lord *et al*, 1998). The question of radiation-related transgenerational cancer risk in experimental animals and human populations is discussed above, in Section 2.1.1; the induction of transmissible genomic instability by radiation in germ cells would provide a mechanism for such transgenerational effects.

The bystander effect has clear implications in terms of human exposures to very low fluences of high LET particulate radiation, such as alpha particles from environmental radon or densely-ionizing galactic cosmic rays in space (Brenner and Elliston, 2001). In the case of radon, for example, only a small fraction of a person's bronchial epithelial cells, the presumed target for lung cancer, will be hit each year by an alpha particle arising from residential radon exposure. In the past, the genetic or carcinogenic risk has been assumed to be related directly to the number of cell nuclei actually traversed by an alpha particle, thus yielding a linear dose response relationship. The evidence that irradiated cells may transmit damage signals to neighboring non-irradiated cells that result in genetic alterations in these "bystander" cells would invalidate this assumption. Rather, it would suggest that the dose-response curve may be non-linear at low mean doses yielding a greater effect than that predicted on the basis of the dose received by individual cells at low alpha particle fluences. Preliminary data, based

primarily on cell mixing experiments, are emerging to suggest that a bystander response also occurs with low-LET radiation. However, these preliminary data are at present insufficient to draw any conclusions concerning the significance of this effect at low radiation doses, particularly at levels such that the track fluence is less than the number of cells in the radiation field (J. B. Little, personal communication).

Evidence for the convergence of the three phenomena (adaptive response, genomic instability, and bystander effects) is also of interest (Lorimore and Wright, 2003; Morgan, 2003b). Several different studies involving both *in vitro* and *in vivo* assays have shown, for example, that transmissible genomic instability may arise in bystander cells (Lorimore *et al*, 1998; Watson *et al*, 2000), and that the bystander effect may be modulated by the adaptive response (Iyer and Lehnert, 2002; Mothersill *et al*, 2002; Sawant *et al*, 2002b; Zhou *et al*, 2003). Defects in the non-homologous end joining DNA repair pathway have been associated with both radiation-induced genomic instability (Okayasu *et al*, 2000) and the bystander effect (Little *et al*, 2003). It has been reported that conditioned medium from certain (but not all) unstable clones harvested many cell generations post-irradiation is highly cytotoxic to unirradiated cells (Nagar *et al*, 2003). Finally, oxidative stress manifested by enhanced levels of reactive oxygen species has been implicated in all three phenomena.

When considered as a whole, the emerging results with these three phenomena raise the possibility that the dose response at low doses of ionizing radiation is uncertain, and a simple extrapolation from high dose effects may not be appropriate. In some cases, such as the induction of mutations by exposure to very low fluences of high LET particles, or as reported for the cytotoxic effects of very low doses of x-rays, the effect may be greater than predicted from a linear extrapolation. On the other hand, certain studies of malignant transformation have revealed a reduced effect for very low doses. Overall, however, these findings imply that the biological effects of radiation in cell populations may not be restricted to the response of individual cells to the DNA damage they receive, but rather that tissues respond as a whole. These three phenomena are of importance as they may influence in particular the nature of the dose response relationship at low doses and low dose-rates. However, a better understanding of the mechanisms for these phenomena, the extent to which they are active *in vivo*, and how they are interrelated is needed before they can be confirmed as factors to be included in the estimation of potential risk to the human population of exposure to low levels of ionizing radiation.

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5. CARCINOGENIC EFFECTS OF IONIZING RADIATION

5.1 Mechanisms of Radiation-Induced Cancer

Studies on the cellular and molecular mechanisms of carcinogenesis over the last several years have provided substantial insight with respect to the complex multi-step nature of the process of neoplastic development (Hanahan 2000; UNSCEAR 2000). Such studies have identified a number of specific target genes and gene pathways and also important variations among different tumor types. From such studies tumor development is now generally viewed as a multi-step clonal process of cellular evolution and selection. The conversion of a normal somatic cell into a cell with neoplastic potential is generally referred to as initiation (UNSCEAR 2000; Knudson 2001) . Subsequent to initiation, the process of neoplastic development continues via the progression phase. This phase includes clonal selection and the development of additional mutational events. As such, this stage may be viewed ***as the early developmental and evolutional phases of an initiated cell during neoplastic progression.*** Factors such as cell-cell communication, mitogenic stimulation, cellular differentiating factors, mutational processes and cell-tissue interactions all play a role in determining the probability of progression of initiated cells. Specific genetic changes involved in progression often differ among tissue types, although key related pathways are generally involved (Hanahan 2000; UNSCEAR 2000). The end phase in tumor progression is the conversion of a cell or cells to the malignant phenotype. Because of the high degree of instability associated with such cells, progression and evolution within a population of malignant cells will continue indefinitely (Loeb 1991). Overall, it is clear that only a small fraction of cells that enter the pathway of neoplastic development as initiated cells will complete the full sequence of events leading to malignancy, a process that can require years in the human being.

Although radiation-induced tumorigenesis in experimental animals and in humans has been the subject of intense study for many years, until recently direct evidence with respect to underlying mechanisms of radiation carcinogenesis has been lacking and models have relied heavily on indirect inferential data. For example, it has been suggested for many years that the primary effect of radiation is principally on early events, i.e. the primary effect of radiation is as a tumor initiating agent. This is based on several observations. First, animals and human beings are generally more sensitive to the tumorigenic effects of ionizing radiation at young compared to older ages. This suggests that radiation effects have more to do with tumor initiation than with promotional effects

that accelerate the development of pre-existing neoplasms (UNSCEAR 2000; Clifton 1986; Fry 1977; Fry 1987; Fry 1992). Second, experimental animal data from studies of skin cancer development, specifically designed to examine the influence of radiation on different stages of tumorigenesis, show that radiation only weakly promotes the development and progression of chemically-initiated tumors but has significant initiating activity (Jaffe 1987). Finally, it is observed in humans and animals that single acute doses of low-LET radiation are sufficient to produce a dose-dependent increase in cancer risk and that, in quantitative animal studies, dose protraction decreases that risk. The last observation also supports the inference that the major effect of radiation is on early events in the carcinogenic process (IARC 2000; Hanahan 2000). While this inference appears to be logically based, until recently there has been no direct evidence in support of it.

Advances in cell biology, cytogenetics, molecular biology and mouse genetics over the past several years have enabled more direct investigation of events in the tumorigenic process following radiation exposure. *Such studies, by linking specific cell and molecular effects directly to the tumorigenic process, provide valuable insights into mechanisms as well as a better understanding of potential radiation-related risks.* Of particular importance in this regard have been animal studies using newly developed models, both in inbred strains of mouse and rat and in genetically engineered rodents. Quantitative studies using mouse and rat models for radiation-induced mammary cancer and for thyroid cancer in rats have now provided direct evidence to indicate that the principle effects of ionizing radiation are on early events (Adams 1987; Bouffler 1996; Bouffler 1997a; Ethier 1984; Jaffe 1987; Ullrich 1996; Domann 1994; Gould 1987; Watanabe 1986; Mulcahy 1984). Cellular, cytogenetic and molecular data for AML, intestinal tumors, and mammary tumors also provide evidence for *clonal* development of radiation-induced pre-neoplasms, implying an initial, single-cell target (Bouffler 1997b; Haines 2000; Ullrich 1996). Recent cytogenetic and molecular studies on the induction of AML and mammary tumors in inbred mouse strains, and of a variety of tumors in transgenic mouse models, have provided more specific information on the potential nature of these early events (Bouffler 1996; Selvanayagam 1995a; Silver 1999; Haines 2000; Kemp 1994; Pazzaglia 2002). These studies provide direct support for the view that the critical radiation-associated events in the tumorigenic process are predominantly early events involving DNA losses targeting specific genomic regions harboring critical genes. Since many of the radiation-associated DNA loss events in these tumorigenesis models involve large chromosomal regions within the genome, mechanisms for radiation-induced

chromosome aberration induction appear to be of particular relevance to the understanding of radiation effects at low doses. The predominant importance of DNA DSB induction and post-irradiation error-prone NHEJ repair for the induction of aberrations, and the apparently critical role for radiation-induced aberrations in the pathogenesis of cancer in these experimental models, would tend to argue against the proposition of a low dose threshold in the dose-response for the initiation of carcinogenesis.

More recently, experimental studies have questioned whether the initiating events produced by radiation are direct chromosomal or mutational effects or whether the mutations and chromosomal rearrangements result indirectly as a consequence of genomic instability induced by the radiation exposure (Little 1997; Little 1990; Morgan 1996; Selvanayagam 1995b; Yu 2001).

It is well known that the development of tumors is frequently accompanied by the acquisition of genomic instability phenotypes that serve to promote the mutational evolution involved in neoplastic progression. This form of genomic instability is increasingly well understood and many of the responsible tumor gene mutations have been identified (Loeb 2001). This instability, however, differs from radiation-induced genomic instability described during the last decade (Selvanayagam 1995b). Evidence has accumulated that, under certain experimental conditions, the progeny of cells surviving radiation appear to express new chromosomal and gene mutations over many post-irradiation cell generations. The details of radiation-induced genomic instability have been discussed in detail earlier in this report. What may be unique about radiation-induced instability with respect to its potential role in tumorigenesis is that, because of the high frequencies of instability observed following radiation exposure (10-50% of irradiated cells), such instability would not appear to be a result of radiation-induced mutations in a specific gene or family of genes (Kadim 1991; Selvanayagam 1995b; Wright 1995). On the basis of data discussed earlier on radiation-induced genomic instability, and the previously reported high frequency of neoplastic cell transformation (Kennedy 1980; Selvanayagam 1995b), it has been suggested that such events can serve to de-stabilize the genomes of a substantial fraction of the progeny of irradiated cells, and that it is the elevated post-irradiation mutation rates in cell progeny rather than gene-specific initial mutations that act to drive radiation tumorigenesis (Selvanayagam 1995b). The question then arises as to the impact of this type of mechanism on assumptions with respect to low dose risks.

Instability associated with telomere dysfunction appears to be of particular relevance to tumorigenesis (Mills 2003; Lo 2002a; Lo 2002b; Desmaze 1999b; Ducray 1999; Bouffler 2001; Morgan 1996). Such dysfunction can be manifest in several forms. Telomeric repeat sequences $(TTAGGG)_n$ cap the ends of mammalian chromosomes and serve to protect against replicative erosion and chromosomal fusion; in normal human cells in culture, telomere shortening and instability is a natural feature of replicative cell senescence. Telomeric repeats are also found in subtelomeric and interstitial chromosomal locations and there is some evidence that these loci may act as sites at which radiation-induced and other forms of genomic damage are preferentially resolved. There is also good evidence that telomeric instability is a recurrent feature of tumorigenic development. Of particular relevance to the question of unstable translocation junctions are the so-called segmental jumping translocations which have been well-characterized in spontaneously arising human leukemias. With respect to radiation-induced leukemia, detailed cytogenetic analyses suggest an excess of complex aberrations and segmental jumping translocations in leukemias arising at old ages in high-dose A-bomb survivors (Nakanishi 1999). Telomeric instability at radiation-associated deletion/translocation breakpoints in mouse myeloid leukemia has also been reported but it is not a general characteristic of such tumor-associated events. Interestingly, excess spontaneous telomeric instability is often found to be associated with deficiencies in DNA repair or damage response (Mills 2003).

Evidence for the involvement of telomeric sequences in the pathogenesis of at least some forms of radiation-induced instability comes from several laboratories. Early studies on the post-irradiation development of chromosomal instability in *in vitro* passaged human diploid fibroblasts were among the first to suggest a link between telomeres and instability. Initial studies using this in-vitro model were suggestive of instability effects in a high proportion of irradiated cells (Sabatier 1989; Sabatier 1992). Subsequent studies by the same research group have served to address issues related both to the pathogenesis of instability as well as its frequency(Desmaze 1999a; Ducray 1999; Lo 2002a; Lo2002b). Detailed cytogenetic analyses suggested that passage-dependent instability in cultured human fibroblasts primarily represented telomeric events expressing in cell clones naturally selected by growth rate during passage. Overall the data obtained may be interpreted as evidence that initial radiation exposure brings forward in time the natural process of clonal telomeric instability associated with cell senescence and telomere shortening. Equally important is the suggestion that selection

processes lead to an overestimate with respect to the frequency of induction of instability by radiation. Whether selection processes impact estimates of the frequency of instability in other systems remains to be addressed

A different form of post-irradiation telomere-associated instability is expressed in a hamster-human hybrid cell system where in some clones chromosomal instability is persistently expressed at translocations that have telomeric sequences at their junction (Morgan 1996). Similar unstable structures have been observed in non-irradiated hamster cells undergoing gene amplification. Such data suggest that radiation induces genomic structures that enhance the natural expression of instability. A number of other reports have also suggested that radiation-associated chromosomal exchange can lead to the formation of unstable junctions that undergo secondary change, leading to the formation of complex chromosomal aberrations (Desmaze 1999b; Desmaze 2003; Lo 2002a; Lo 2002b; Morgan 1996).

The mechanistic role of instability in radiation tumorigenesis is not clear and the two model systems used to study this question have yielded differing results. Radiation-induced genomic instability in hematopoietic cells was first demonstrated in studies showing a persistent excess of chromatid type aberrations in the progeny of mouse bone marrow cells irradiated *in vitro* with alpha particles and subsequently grown in culture (Kadim 1991). Alpha particles are considered to be substantially more effective than low-LET radiation in inducing this form of genomic instability, which has also been reported in the progeny of cells which had not sustained an alpha track traversal; i.e. induced instability may occur as a bystander effect (Lorimore 1998). *In vivo* post-transplantation growth of *in vitro* irradiated bone marrow cells was also reported to result in excess chromatid aberrations. On the basis of these observations it was proposed that such instability had a major role in radiation-induced murine acute myelogenous leukemia (AML). More recent data have not supported this hypothesis, and in fact suggest that radiation-induced instability is not involved in the initiating events in murine AML (Bouffler 2001). Of particular importance in this regard were studies demonstrating that susceptibility to radiation-induced instability in hematopoietic cells, and susceptibility to radiation-induced AML, are not genetically linked phenotypes (Boulton 2001).

In contrast to these studies are data on instability and radiation-induced mammary cancer. Differences in radiosensitivity and susceptibility to induced tumorigenesis among inbred mouse strains are well recognized and there is good evidence that the BALB/c

mouse is unusually sensitive to the induction of tissue injury and mammary tumors, while the C57BL/6 mouse falls into the radio-resistant category (Hanson 1987). Initial cytogenetic studies showed that mammary epithelial cells cultured from irradiated BALB/c mice persistently expressed substantially more chromatid aberrations during passage than those derived from irradiated C57BL/6 animals (Ponnaiya 1997). In follow-up investigations the chromatid instability phenotype of BALB/c was shown to be associated with a partial deficiency in the NHEJ repair protein DNA PKcs together with compromised post-irradiation DNA DSB repair (8 2000; Yu 2001). This study, which included an intercomparison of inbred mouse strains, showed the deficiency of DNA PKcs and DNA DSB repair to be restricted to BALB/c, suggesting genetic associations with persistent genomic instability and with mammary tumor susceptibility. Molecular genetic analyses showed that BALB/c mice carry a rare variant form of the gene (*Prkdc*) encoding DNA PKcs. Subsequent analysis of recombinant mice provided strong evidence that variant *Prkdc* directly determined DNA PKcs deficiency and post-irradiation chromatid instability in mammary epithelial cells (Yu 2001). On the basis of these data it was proposed that induced genomic instability and mammary tumor susceptibility were genetically co-determined. Importantly these investigations provide genetic evidence that a deficiency in the repair of DNA DSB is likely to determine persistent instability. Interestingly, recent observations have suggested a link between DNA PKcs function, telomeric integrity, and genomic instability. The question as to whether such instability is a primary causal element in mammary tumorigenesis remains to be resolved (Bailey 1999; Bailey 2001).

While the role of radiation-induced genomic instability in radiation-induced cancer is still a matter of investigation, there are several observations that provide a framework for its potential role in cancer development following radiation exposure. In the case of radiation-associated, persistent telomeric rearrangement and unstable chromosome translocation junctions, a strong case may be made that a certain fraction of misrepaired genomic damage after radiation may be prone to ongoing secondary change in clonal progeny. Since there is evidence that such secondary genomic rearrangement can be a normal component of tumor development, it is reasonable to assume that instability of this type would be involved in the pathogenesis of some radiation-associated tumors. It is unclear whether it plays a major role and, if so, for which tumor types. The genetic evidence from mouse mammary studies, which implies that post-irradiation instability can associate with mammary tumor development, supports a role for genomic

instability in this system. Thus in certain genetic settings, such as individuals harboring specific types of DNA repair deficiencies, a role for post-irradiation instability in tumorigenesis appears reasonable.

Interestingly, recent data in the SCID and in the BALB/c mouse strains, both of which have defects in DNA-PKcs, suggest that telomeric instability may be the underlying mechanism for the induction of instability, and that the resulting cytogenetic instability plays an important role in early carcinogenic events in the mouse mammary carcinogenesis model discussed above. In particular, it appears that dysfunctional telomeres may tend to interact with sites of radiation induced DSBs, increasing the probability of misrepair (Bailey 1999; Bailey 2001; Mills 2003). It would be predicted that mechanisms involving DNA DSB and telomeric sequence interactions would be particularly important at low doses where DNA DSBs are in relatively low abundance. This appears to be consistent with observations that instability is induced in a dose-dependent manner at radiation doses below 0.5 Gy, whereas no dose dependence is observed at higher doses, at which the response appears to plateau. Importantly, the emerging evidence suggests a role for radiation-induced DSBs in the induction of instability and provides a mechanistic link between DSBs, chromosome aberrations, and cancer not unlike that for more directly induced effects. This linkage would also suggest that predictions of effects at low doses will be unaffected by the underlying mechanism whether that mechanism involves direct effects of radiation or is mediated by radiation-induced instability.

Observations of microsatellite instability in acute AML among A-bomb survivors (Nakanishi 2001) appear to provide only weak evidence of involvement of this phenomenon in radiation leukemogenesis, with significantly more instability among exposed cf. non-exposed cases but with little evidence of a dose response among the exposed, or of greater involvement in cases in which radiation exposure was more likely to have played a causal role (Little 2002; Cox 2002; Plumb 2003; Little et al, 2003). The question remains open, however, and studies with greater statistical power may some day resolve the issue.

Microsatellite instability, observed in radiation-related pediatric thyroid cancers associated with human population exposure to radioactive fallout from the 1986 Chernobyl accident, was significantly greater for tumors diagnosed within 6-8 years after the accident compared to those with later onsets (9-11 years); however, without

individual radiation dose estimates it was not possible to evaluate the effects of dose on instability (Nikiforov 1998, Richter 1999, Lohrer 2001).

5.2 Tissue Modifying factors

It is well known that the probability that individual initiated cells will progress to become tumors can be modulated by interactions with surrounding cell and tissue components as well as systemic host factors (Bissell 2001). Studies have also provided evidence that radiation can influence these cell-cell, cell-tissue, and host factor interactions (Barcellos-Hoff 1998; Barcellos-Hoff 2001; Barcellos-Hoff 2001b; Bissell 2001; Park 2003). There has been renewed interest in these effects as a result of recent studies that have begun to identify potential underlying mechanisms involved in modulation of tumorigenic progression and expression (Barcellos-Hoff 1996; Barcellos-Hoff 2000; Barcellos-Hoff 2001b; Bissell 2001). Research in this area will be extremely important in understanding the overall processes involved in neoplastic development but a clear understanding of their potential impact on radiation-induced cancer remains to be determined.

Two key points tend to support the view that factors involved in modulation of tumor progression and expression are not likely to play a major role in determining low dose risks. It has been demonstrated in a number of instances that an important early and ongoing events in the process of neoplastic development is the acquisition of genomic instability (Selvanayagam 1995a). This instability increases the rate of mutational and chromosomal changes in the cells and increases the probability for mutations that will allow initiated cells to escape from the inhibitory effects of cell, tissue, and host modifying factors. Further, it is also known that with age, there are changes in the tissue microenvironment which also tend to reduce inhibition by normal cells and tissues of the ability of initiated cells to express their neoplastic potential. Over time and with increasing age, therefore, it is highly likely that mutations in initiated cells and alterations in tissue microenvironment will result in the emergence of a cell or population of cells capable of escaping or overcoming these cell, tissue and host modulating factors. Because of this, it seems prudent to focus on early initiating cell and molecular events as the major determinant of risks at low doses.

Studies on in vivo tumor induction in mice and rats also suggest that early cell and molecular events represent the principle determinant of radiation-related cancer risk in tissues. In this regard, fractionation studies are particularly relevant. Comparisons of the

carcinogenic effects of fractionated exposures to effects of acute radiation exposures of rat skin (Burns 1977; Burns 1975; Vanderlaan 1975) and mouse lung (Ullrich 1980; Ullrich 1984; Ullrich 1987) have clearly demonstrated that the greatest reduction in the carcinogenic effect is for fractions separated by times of 24 hours or less. Such time periods are compatible with repair of initial damage. Longer times of up to 30 days between fractions, which would allow for tissue effects to impact cancer risk, have not been found to result in further reduction in risk.

5.2.1 Target cells

In hierarchical-type tissues, where less-differentiated precursor cells produce well differentiated and mature functional cells, cancers are generally considered to originate from tissue stem cells, which possess unlimited division capacity. These tissue stem cells are transformed by carcinogenic agents, altering their differentiation patterns so that cell renewal predominates over differentiation, leading to growth of the abnormal cell population. Stem cells have been well characterized in haemopoietic, epithelial and spermatogenic tissues (Potten, 1983, 1997). They have renewal and location characteristics that are specific to a particular tissue. They renew themselves more slowly than their dividing and differentiating daughter cells, and hence, in protracted irradiation scenarios, receive more ionisations per cell cycle. Stem cells are often located at the static end of a polarized system of cell production, for example near the bottom of intestinal crypts, in the basal layer of epithelia, and more centrally in red bone marrow. These locations can provide some protection against exposure from short-range radionuclides deposited on (for example) epithelial surfaces or lumenally.

In the case of the colon, it has been suggested that tumours may originate in cells on the intercryptal plate rather than, or in addition to, stem cells at the base of the crypt (Shih et al., 2001). This study indicated that most early neoplastic lesions of the colon contain dysplastic cells only at the orifices of crypts and on the luminal surface between crypts. Analysis showed loss of the *APC* gene and high expression of β-catenin in such dysplastic cells but not in cells with normal appearance within the crypts. Mutations in the *APC* gene are the earliest genetic alterations in the genesis of colorectal tumours and appear to be required to initiate clonal evolution, involving over-expression of β-catenin (Fodde et al., 2001). This suggestion of target cells on the luminal surface is contentious (Wright and Poulsom, 2002; Preston et al 2003). In normal tissue, differentiated

epithelial cells on the intercryptal surface would have a very limited life-span of a few days, and would be destined to be lost into the intestinal lumen in the normal process of cell renewal. To develop into a tumour, these dysplastic cells would need to escape this process completely to allow time for progression to malignancy, involving a number of mutational events (Vogelstein et al., 1988; Goyette et al., 1992). Although this scenario seems highly unlikely, the possibility cannot be excluded that daughter cells of the stem cells, situated at higher cell positions in the crypt, are also target cells, perhaps to a lesser degree. *For the purposes of the ICRP report on the Human Alimentary Tract (reference to be added), doses are calculated to the estimated position of the stem cells.* However, in considering uncertainties, the possibility that cells higher in the crypts may also be targets has been addressed, including the extreme case of target cells on the intercryptal luminal surface.

There are other protective mechanisms in stem cell systems, such as the selective retention of template DNA strands in stem cells, providing protection of the stem cell genome (Cairns 1975, 2002). An example of this is the stem cells in the crypts of the small intestinal mucosa, which divide about a thousand times during the lifespan of a laboratory mouse. Yet these cells show little evidence of any decline in proliferative potential and rarely produce overt tissue abnormalities, suggesting that their genome is extremely well protected. Protection against DNA replication-induced errors can be achieved by the selective sorting of old (template) and new DNA strands with all template strands retained in the stem cell line. Experiments have shown that the template strands in the stem cells can be labeled during development or during tissue regeneration using tritiated thymidine (3HTdR) (Potten et al 2002). Labeling newly synthesized strands with a different marker (bromodeoxyuridine, BrdUrd) allowed segregation of the two markers to be studied. It was shown that template strand label was retained (3HTdR), whereas label in the newly synthesized strands (BrdUrd) was lost following the second division of the stem cell. Random errors may still occur in the template strands owing to environmental agents.

Another protective mechanism is apoptosis. Apoptosis is the non-inflammatory and 'altruistic' cell suicide that involves characteristic molecular and cytological features. It occurs naturally at a low level in many hierarchical tissues in the stem cell zone, and the frequency is enhanced by irradiation. This type of cell death is very radiosensitive. Hypotheses for the low rate of cancer in the small intestine have been proposed, based on

apoptosis which deletes mutated stem cells (Potten et al., 1992). These hypotheses suggest that radiation-induced TP53-dependent apoptosis in the stem cell zone in the small intestine prevents the propagation of mutated dividing progenitor cells. This is consistent with the increased frequency of cancer in *Tp53*-null mice compared to wild-type mice. Experiments in mice show that the level of apoptosis saturates after acute doses above 100 mGy, there is no detectable dose-rate effect (Hendry et al 1982), and the incidence of apoptosis is repeatable after each dose in a series of small radiation exposures. This provides a potential mechanism in this tissue for the often purported presence of a threshold dose for carcinogenesis. Higher doses indeed are capable of inducing tumours, as found in rats given irradiation to a temporarily exteriorized loop of small intestine (Osborne et al 1963). In the large intestine there is also natural and radiation induced apoptosis. However, *Tp53* is not expressed in the stem cell zone, and *bcl-2* expression promotes cell survival and allows the development of mutated progenitor cells (Merritt et al., 1995). Hence this potential protective mechanism does not operate in the colon. Also, carcinogenesis in the colon may be exacerbated by the longer presence of fecal contents containing carcinogens.

In other organ systems such as lung and thyroid, cell renewal is very slow and a much greater proportion of the total cell population may be target cells. In these cases the above mechanisms are very unlikely to apply, and the long-lived target cells would accumulate multiple mutations in the conventionally-described multistage process of carcinogenesis (Vogelstein et al., 1988; Goyette et al., 1992).

An important question in with respect to protective mechanisms in target cells and the removal of damaged cells via apoptosis is the persistence of radiation-initiated cells once the initial damage has been produced. Hoshino and Tanooka examined the persistence of latent carcinogenic damage in irradiated mouse skin (Hoshino and Tanooka 1975) and found that radiation initiated cells could persist as latent carcinogenic damage for up to 400 days. Yokoro and his co-workers, in studies examining the interaction of radiation and hormones in breast cancer development, also found that latent radiation-initiated cells persisted for a substantial portion of the rats' lifetimes (Yokoro and others, 1977).

5.3 Radiation induced Cancer in Animals

On the basis of the discussion of ***cellular and molecular mechanisms*** above, it can be predicted that the dose response and time-dose relationships for radiation-induced cancer would be similar to those for radiation-induced chromosomal aberrations. Specifically, at low doses a linear dose response would be anticipated. There are, however, relatively few studies on animal carcinogenesis where the data are sufficient to address the issue of dose response relationships or the issue of dose rate effects, protraction, and/or fractionation effects and rigorously test these predictions. Those studies where such analyses are possible are mainly limited to rodent studies, principally studies in mice. A further caveat is the applicability of animal data to human risks. The pathogenesis of certain tumors in experimental animals ***appears*** to involve unique mechanisms for induction that do not appear to be compatible with known mechanisms of cancer development in humans. This section will describe the available data and its applicability to understanding of low dose risks and risks following low dose rate or protracted exposures. This is not meant to be a comprehensive review but is limited to those data sets which focus on effects at low doses (< 0.5 Gy) and low dose rate exposures following external irradiation. Data from studies using internal emitters are not included because of the dosimetric issues that complicate interpretation. Likewise, studies with low statistical power in the low dose range have also been excluded.

At first glance an examination of available animal data suggests a high degree of complexity in that a variety of dose responses have been observed ranging from threshold responses to linear or linear quadratic responses. However, a more systematic examination of the data with a view toward the underlying biology involved in the pathogenesis of individual tumor types reveals a clearer picture. In this regard it is useful to first separate the discussion of the data into that for induction of leukemias and solid tumors.

5.3.1 Leukemia

The induction of leukemia and lymphoma has been examined in two murine systems, thymic lymphoma and acute myelogenous leukemia. The dose response for induction of thymic lymphoma is complex and reducing the dose rate results in a large reduction in the effectiveness for radiation-induced thymic lymphoma (Ullrich 1979a). The applicability of these data to human risk estimates is unclear. The development of thymic lymphoma in mice following irradiation is an extremely complex process largely

mediated through indirect mechanisms (Kaplan 1964; Kaplan 1967). Importantly in this regard, expression of thymic lymphoma can be substantially reduced or eliminated by protection of a small fraction of bone marrow stem cells from radiation-induced cell killing. The complex nature of the pathogenesis of murine thymic lymphoma involving substantial bone marrow cell killing, and the lack of a comparable counterpart in humans argues against thymic lymphoma as an appropriate model for the understanding of dose response and time-dose relationships in humans.

In contrast, data on the biology and pathogenesis of murine acute myelogenous leukemia (AML) suggest strong similarities between mouse and human. Such data support its applicability to radiation-induced leukemognesis in humans with respect to studies of mechanisms and potential low dose risks (Rithidech 1999; Rithidech 2002; Silver 1999; Tenen 2003). For murine AML the most comprehensive data on dose response and dose rate or fractionation pertain to radiation-induced myeloid leukemia in CBA mice and RFM mice (Mole 1983; Mole 1983; Ullrich 1987; Upton 1970). The CBA mouse has also been used to dissect underlying radiation-induced molecular events described previously (Bouffler 1996a; Bouffler 1996b; Bouffler 1997). Over the 0 to 3 Gy dose range (the lowest dose used was 250 mGy), the dose response for both strains could be described by a pure quadratic dose response relationship, although linear-quadratic and simple linear dose responses also provided an adequate fit to the data sets. After fractionation or protraction of the dose there was a reduction in the leukemogenic effects of radiation at doses of 1.5 Gy and higher resulting in a linear dose response over a wide range of doses in both strains. Barendsen has analyzed the RFM data set including acute high dose rate, fractionated and low-dose rate exposures and concluded that a linear-quadratic model derived from the high dose rate data adequately predicted the low dose rate and fractionation effects (Barendsen 1975). Importantly, these data and the analysis by Barendsen are fully compatible with predictions based upon the known role for aberrations/deletions in chromosome 2 in the pathogenesis of murine AML and predictions based upon data for induction of chromosome aberrations by radiation.

5.3.2 Solid Tumors

Data from experimental studies examining dose response relationships following whole body external exposures are also available for a limited number of solid cancers. The tumor types for which sufficient data are available include Harderian gland, pituitary, and ovarian tumors in female RFM mice (Ullrich 1979a; Ullrich 1979b), and lung and breast cancers in female BALB/c mice (Ullrich 1983; Ullrich 1987). Data are also

available in female Sprague-Dawley rats for mammary tumors (Burns 1975; Burns 1977; Finkel 1968; Hulse 1969; Shellabarger 1980) and for skin in mice and rats and bone tumors in mice . The data for skin and bone tumors involve localized exposures since the induction of these tumors generally requires radiation doses that are too high to be well tolerated when given as whole body exposures.

The observation that high radiation doses are required for induction of skin and bone tumors supports the view that a threshold might exist for induction of these tumors. However, this does not imply that low doses of radiation cannot and do not result in the initiation of skin and bone cells. Studies in mouse skin clearly demonstrate that low doses of radiation can initiate cells that have the potential to progress to become tumor cells (Jaffe 1987). Rather, these data suggest that for these tissues, factors influencing tumor progression play an important role in determining whether or not initiated cells progress and ultimately express their tumorigenic potential. The high doses required suggest an important role for radiation-induced cell killing resulting in disruption of cell-cell and cell-tissue interactions as well as the recruitment of growth factors all of which may participate in the progression of initiated cells in these systems. It is important to note that skin and bone are also not considered highly sensitive to radiation-induced cancer in humans as well. By far the greatest contribution to estimates of radiation risk comes from tissues that are more sensitive to tumor induction and for which risks at low doses are of more concern.

The apparent lack of sensitivity of bone and skin at low doses does not mean that risks can be ignored. Exposure to ultraviolet light has been shown to be an effective promoting agent following exposure of the skin to ionizing radiation (Shore 1984). Such exposure allows the expression of initiated cells that would not be expressed otherwise. As a result the relationship between the dose of ionizing radiation and skin tumor development shifts from one with an apparent threshold to a much more linear response. This effect underscores the argument made previously in this section that it is important to focus on early initiating cell and molecular events as the major determinant of risks. An apparent threshold cannot be assumed to indicate that there is no increased risk to an individual who might be exposed to other agents with promoting effects or for whom intrinisic risk factors could exist which could allow expression of initiated cells that would normally not be expected.

Data from the studies using RFM and BALB/c mice and Sprague-Dawley rats are most applicable with respect to low dose and low dose rate effects because of the

sensitivity of these tissues to radiation-induced cancer and the dose range over which data has been obtained. Again, caution must be exercised in the application of data derived from all tumor types without regard to the underlying biology involved in tumorigenesis. The most dramatic example is that for ovarian cancer in mice. Ovarian cancer in the mouse following whole body irradiation appears to be a result of an indirect mechanism involving oocyte cell killing, and subsequent alterations in the pituitary-ovarian hormonal interactions leading to ovarian tumorigenesis (Foulds 1975). Because of the close association between cell killing and ovarian cancer in mice and because mouse oocytes are uniquely sensitive to the killing effects of radiation (LD50) for oocyte killing is approximately 50 mGy, ovarian tumors at high frequencies are observed following very low doses. Consistent with an indirect mechanism mediated by cell killing, a threshold dose response has been observed for the induction of ovarian tumors. Lowering the dose rate increased the threshold dose from approximately 110 mGy to 700 mGy (Ullrich 1979c; Ullrich 1979b). There is no evidence for similar indirect mechanisms for radiation-induce cancer at any site in human studies and, therefore, radiation-induced ovarian tumorigenesis will not be included in further discussions below.

Data for the induction of Harderian gland and pituitary tumors in female RFM mice and lung and mammary cancer in female BALB/c mice generally support the linear-quadratic model over a dose range from 0.1 - 2Gy (Ullrich 1979a; Ullrich 1979b; Ullrich 1987) while the induction of mammary tumors in Sprague-Dawley rats tend to be more linear over this dose range (Shellabarger 1980). For these tumor types it has also been found that reducing the dose rate or fractionating the dose into small fractions reduces the risk for development of radiation induced cancer in the manner predicted by the linear-quadratic model. At high doses (>1 Gy) the risk of cancer development is reduced primarily as a result of the diminution of the quadratic portion of the dose response resulting in a limiting linear slope over a wide dose range that is equivalent to the linear slope of the high dose rate dose response in the low dose range. At lower total doses radiation effects are time independent and therefore the incidence of tumors increase in a linear fashion with dose.

Overall, relevant animal tumor data tend to support a linear response with no threshold at low doses.

5.4 Life shortening

A large number of studies in mice and dogs have been conducted using life-span shortening as a means to quantify late radiation effects (NCRP 1980; Carnes 1989; Carnes 2002; Carnes 2003; Storer 1979; Storer 1982; Storer 1983; Thompson and Grahn 1988, 1989; Thompson et al 1981a, 1981b, 1983, 1985, 1986; Sacher et al 1958, 1976; Lesher et al 1960, 1965; Grahn and Hamilton 1964; Grahn and Sacher 1957, 1958; Grahn et al 1963). While it has been argued that life shortening can serve as an integrated measure of the deleterious effects of radiation, the interpretation of these studies is not straightforward. A large variation in life shortening is observed as a function of strain, species, gender, and physiological status of the animals. This variation is largely a result of differences in the spectra of spontaneous and induced disease, and the age distribution of disease occurrence. For example, a high degree of life shortening is observed in animals susceptible to the induction of radiation-induced cancers that tend to occur early in life, such as thymic lymphoma or myelogenous leukemia. Studies using animals that are not susceptible to such typically early-developing neoplasms but, rather, tend to develop late-occurring solid tumors following radiation exposure have observed considerably less life shortening at the same radiation dose. Regardless of the degree of life shortening observed, however, analyses of experimental studies indicate that at low doses of radiation and for radiation delivered at low dose rates, radiation-induced life shortening is due almost entirely to radiation-induced cancer (NCRP 1980; Carnes 2002; Storer 1979; Storer 1982; Lesher et al 1960). Life shortening attributable to non-neoplastic effects has only been observed at single acute doses in the range of 500 mGy and higher and no such effects have been observed following low dose rate or protracted exposures to low LET radiation(Carnes 2002; Storer 1979; Storer 1982).

Experiments designed to address questions of risk following low dose rate or protracted exposures have also been performed. With few exceptions dose response relationships derived from data following single acute radiation doses, fractionated exposures and terminated low dose rate exposures all suggest linear dose responses over a wide range of doses (NCRP 1980; Carnes 2003; Storer 1979; Thomson and Grahn 1988, 1989; Thomson et al 1981a, 1981b, 1983, 1985, 1986; Tanaka et al 2003). This is not surprising since the dose response for life shortening represents the integrated dose responses for a variety of tumor types whose individual dose responses may vary widely. The primary effect of fractionating the radiation dose or reducing the dose rate at which

the dose is delivered is to reduce the slope of the linear response. Importantly, experiments using multiple low dose-rate, terminated exposures suggest a limiting linear slope in all cases. Once this limiting linear response is reached, no further reduction in effect is seen if dose rate is reduced further. Protracting exposures over the entire life-span can result in a further reduction in life shortening per unit dose. There are two confounding factors in protraction studies that must be considered. First, in such studies the radiation injury induced very late in life often does not have sufficient time to be expressed. Second, it is difficult to determine the dose at which specific effects have been induced because the exposure continues even after the processes involved have been initiated. Both factors tend to result in an overestimation of the dose required to produce a specific degree of observed life shortening (NCRP 1980). This overestimation of the dose reduces the slope of the dose effect relationship beyond the limiting slope obtained following terminated exposures.

5.5 Summary

Studies on the cellular and molecular mechanisms of carcinogenesis over the last several years have provided substantial insight with respect to the complex multi-step nature of the process of neoplastic development and on radiation-induced cancer. These studies provide direct support for the view that the critical radiation-associated events in the tumorigenic process are predominantly early events involving DNA losses targeting specific genomic regions harboring critical genes. Since many of the radiation-associated DNA loss events in these tumorigenesis models involve large chromosomal regions within the genome, mechanisms for radiation-induced chromosome aberrations appear to be of particular significance. The predominant importance of DNA DSB induction and post-irradiation error-prone NHEJ repair for the induction of aberrations, and the apparently critical role for radiation-induced aberrations in the pathogenesis of cancer in these experimental models, would tend to argue against the proposition of a low dose threshold in the dose-response.

More recently, experimental studies have questioned whether the initiating events produced by radiation are direct chromosomal or mutational effects or whether the mutations and chromosomal rearrangements result indirectly as a consequence of genomic instability induced by the radiation exposure. However, at this point the mechanistic role of instability in radiation tumorigenesis is not clear. Data thus far suggests that in certain genetic settings, such as individuals harboring specific types of

DNA repair deficiencies, a role for post-irradiation instability in tumorigenesis appears reasonable but its general applicability and its impact on low dose risks remains a matter of investigation.

Factors that modify the progression and persistence of initiated cells must also be considered when addressing low dose risks. It is well known that the probability that individual initiated cells will progress to become tumors can be modulated by interactions with surrounding cell and tissue components as well as systemic host factors. Data to date, however, suggest that such factors are not likely to play a major role in determining low dose risks. Another important question is the persistence of radiation-initiated cells once the initial damage has been produced. It has been hypothesized, for example, that apoptosis could be a protective mechanism which removes potentially neoplastic cells and could in effect result in a threshold at low radiation doses. Two studies using different experimental systems (skin and mammary gland) have addressed this issue and found that latent radiation initiated cells could persist for a substantial portion of the rats' lifetimes. At present, therefore, it seems prudent to focus on early initiating cell and molecular events as the major determinant of risks at low doses.

On the basis of the discussion of cellular and molecular mechanisms in this chapter, it can be predicted that the dose response and time-dose (i.e., fractionation and protraction) relationships for radiation-induced cancer would be similar to those for radiation-induced chromosomal aberrations. Specifically, at low doses a linear dose response would be anticipated. There are, however, relatively few studies on animal carcinogenesis where the data are sufficient to address the issue of dose response relationships or the issue of dose rate effects, protraction, and/or fractionation effects and rigorously test these predictions. Those studies where such analyses are possible are mainly limited to rodent studies, principally studies in mice. Overall, these animal tumor data tend to support a linear response at low doses and dose rates with no threshold.

A large number of studies in mice and dogs have been conducted using life-span shortening as a means to quantify late radiation effects and it has been argued that life shortening can serve as an integrated measure of the deleterious effects of radiation. Support for this argument comes from the observation that, regardless of the degree of life shortening observed, radiation-induced life shortening is due almost entirely to radiation-induced cancer. Life shortening experiments have examined risks following low dose, low dose rate or protracted exposures. The primary effect of fractionating the radiation dose or reducing the dose rate at which the dose is delivered is to reduce the

slope of the linear response. Importantly, experiments using multiple low dose rate terminated exposures suggest a limiting linear slope in all cases adding further support for the view that effects at low doses are consistent with a linear no threshold model.

5.6 Conclusions: Implications for radiation-related cancer at low doses

Models of radiation action as well as a wide range of molecular, cellular and animal data have been used to argue that data on radiation-induced cancer in human populations derived from studies following acute radiation exposures tend to overestimate radiation risks at low doses and dose rates (NCRP 1980; ICRP 1991). In this regard, a number of advisory groups have used a similar approach to quantify the degree to which extrapolation of acute high dose data might tend to overestimate risks at low doses and low dose rates. Essentially, the effectiveness per unit dose for acute exposures has been determined using a linear interpolation of data between the 2-3 Gy dose range and control data at 0 Gy. Effects per unit dose following low dose rate exposures were derived by calculating the slope of the entire dose response (not just in the 2-3 Gy dose range). By dividing the tumorigenic effectiveness per unit dose of acute exposures using the high dose data and low dose rate exposures, an effectiveness ratio was obtained. This ratio has been termed the Dose and Dose Rate Effectiveness Factor. The rationale for using only the high dose data and not data at lower doses was based on the assumption that this would simulate analyses of risks from epidemiological studies where most of the available data were for single acute exposures at relatively high doses. Since the actual dose response for most radiation-induced tumors following single acute exposures has generally been found to be linear quadratic (see discussion above), this procedure would tend to overestimate effects for low single acute radiation doses (in the dose range where the response is predominantly linear) and for low dose rate exposures over a wide range of total doses.

In spite of its apparent simplicity, the derivation and application of dose and dose rate effectiveness factors (DDREF) must be performed with caution. Tumors for which there is evidence (from knowledge of their mechanisms), that they are unlikely to be applicable to radiation carcinogenesis in human populations, should not be considered. This leaves a limited data set upon which to base DDREF calculations. These data sets include myeloid leukemia, and a few solid tumors including Harderian gland (for which there is no comparable tissue in humans), lung adenocarcinomas, and mammary tumors.

All the data sets for myeloid leukemia support a reduced carcinogenic effect when comparing high and low dose rate exposures over the 0 to 3 Gy dose range. Calculation of DDREF values using the procedures described above yield estimates on the order of 2 to 6 with most values in the range of 4-5. For lung adenocarcinomas and for Harderian gland tumors DDREF values of approximately three have been calculated over the 0 to 2 Gy dose range. For mammary tumors all of the data suggest a DDREF value of less than 2 and more nearly close to a value of 1 when effects of high dose rate and low dose rate exposures are compared in this 0-2 Gy dose range. Thus, it appears that myeloid leukemia is probably more sensitive to dose rate effects than are solid tumors.

It should emphasized that these values are based upon extrapolation of data from acute doses of 2-3 Gy, and may represent maximum DDREF values (NCRP 1980). Total dose-dependent dose-rate effects have also been reported and quantified for cytogenetic endpoints by Sorensen and co workers (Sorensen et al 2000). The impact of dose range must be considered when applying DDREF factors to human risk estimates for which there are now reliable data at and below 1 Gy.

It has been argued that life shortening data may be a more appropriate measure of overall risk, and therefore, the use of these data is a better approach to the derivation of a single DDREF value. The complications of life shortening data have been described in an earlier section including changes in disease spectrum as a function of dose and dose rate, and complications associated with terminated versus life-time exposures. These complications notwithstanding, DDREF values determined from terminated radiation experiments indicate maximum DDREF values following extrapolation of acute effects in the 2 Gy dose range on the order of 2. Protraction of the radiation exposure over a significant portion of an animal's life-time tends to reduce the effectiveness of the exposure more than that observed following a simple reduction of dose rate to specific total doses. However, as discussed earlier this experimental approach makes the determination of true effects per unit dose difficult if not impossible. Because of this, the application of these large (i.e., >2) protraction factors to human risks is problematic.

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6. QUANTITATIVE UNCERTAINTY ANALYSIS

6.1 Overview

Chapter 2 described the epidemiological basis for estimation of radiation-related cancer risk in exposed populations, including various uncertain factors that must be considered when applying epidemiological risk estimates from one population to another, especially when the base data are as yet incomplete and must be projected forward to end of lifetime of the study population. The discussion was focused on uncertain biases introduced by random dose-reconstruction error in the first population, population differences in baseline cancer rates, and extrapolation of estimates, derived largely from moderate-to-high dose data, to situations of low-dose and very-low-dose exposure. The topic of the present chapter is quantitative uncertainty analysis of estimated cancer risk associated with low-dose, low-LET radiation exposure, illustrated in terms of the application of atomic bomb survivor risk coefficients to the population of the United States.

Quantitative uncertainty analysis (QUA) was developed in a decision-theoretic framework and has been extensively applied to nuclear reactor safety (U.S. Nuclear Regulatory Commission 1974, 1990) and ecological risk assessment (IAEA 1989; Warren-Hicks and Moore 1998; Gilbert 1994). It involves the application of Bayesian probability methods to estimates and decision rules based on uncertain statistical and subjective information. As stated by Warren-Hicks and Moore (1998), benefits of quantitative uncertainty analysis include improved transparency and credibility, avoidance of worst-case assumptions, focus on critical areas of uncertainty that might benefit from further data collection, and improved decision support. Limitations of the method include the practical inability to consider all possible sources of uncertainty, the possibility that the method may be used incorrectly, and lack of universal awareness and acceptance of the methodology.

The approach (i.e., QUA) is used here, not to reach a particular decision, but to illustrate the implications for radiation protection of the various types of (mostly uncertain) information that contribute to our estimates of radiation-related risk. The emphasis on uncertainty is appropriate because the need for radiation protection is driven by the likelihood and magnitude of exposure-related risks, because estimates based on statistical data and realistic assumptions are uncertain, and because radiation protection is

a political process which must take account of the diverse interests and viewpoints of individuals and population subgroups affected by implementation of radiation protection policies. The development of such policies, to be successful, requires accommodation and consensus. It must be seen to be done fairly and openly, on the basis of facts and assumptions accessible to and challengeable by all of those affected by implementation. An important aspect of the information relevant to the political process of radiation protection is the uncertainty of estimates of radiation-related risk derived from a combination of statistical and largely subjective information sources.

Different people have different points of view about risk. For example, a risk-averse person may tend to focus on how high the risk from exposure might reasonably be (e.g., on its upper 90% uncertainty limit), while a person who is primarily averse to the costs of exposure reduction may tend to demand proof that the risk is high enough to worry about, e.g., may focus on its lower uncertainty limits. A complete uncertainty distribution for estimated risk summarizes all the uncertainty information inherent in the statistical data used and in the consensus estimates of crucial assumptions needed to apply the statistical data to the matter at hand. That summary is highly relevant to both of these points of view and to others as well.

Radiation-related cancer risk is among the subjects most suitable for QUA. It is highly quantified, and a number of major sources of uncertainty have been explored (NIH 1985; CIRRPC 1988; Sinclair 1994; NCRP 1996, 1997; EPA 1999; NCI/CDC 2003). Knowledge of uncertainty is highly relevant to radiation protection philosophy and practice, and it can be at least as important as knowing the value of a single-valued “best estimate”. For example, a point estimate of one lifetime excess cancer death per thousand, with 90% probability (uncertainty) limits 0.5-2.0 per thousand, has different implications for, say, a risk-benefit analysis than the same point estimate with probability limits 0.1-10 per thousand. In the second case, assuming a lognormal uncertainty distribution, the likelihood that the risk per thousand is between 0.5 and 2.0 is only 38% and the likelihood that it is greater than 2.0 is 31%.

Statistical analyses of epidemiological or experimental observations on radiation carcinogenesis are usually concerned with quantifying risk in the context of a particular study. Applications of the original risk estimates in other contexts, without adjustment, may be misleading for a number of reasons discussed earlier in this chapter. Adjustment requires other steps and assumptions, about which the original study may not be

informative. The incorporation from other sources of additional information, which may be uncertain, may modify the resultant risk estimate and its uncertainty.

Uncertainty analysis is concerned with such changes and their implications for the ultimate application of (in the present case) risk estimates. The approach has been extensively applied in assessments of environmental contamination (NCRP 1996). The 1985 NIH radioepidemiological tables report (NIH 1985) was possibly the first formal application to radiation-related cancer risk. The approach was subsequently taken a step farther, at the request of the United States Department of Veterans Affairs (VA), by the Committee on Interagency Radiation Research and Policy Coordination (CIRRPC 1988). The following discussion is based primarily on the following sources: NCRP Commentary 14 (1996) discusses uncertainty analysis applications to assessment of dose and risk related to environmental contamination; NCRP Report 126 (1997) was derived in part from Sinclair (1994) and is specifically concerned with applications of radiation-related mortality risk estimates to low-LET radiation protection; an Environmental Protection Agency report (1999) deals with the same subject; and a recent revision of the 1985 NIH radio-epidemiological tables report (NCI/CDC 2003) is concerned with applications to adjudication of compensation claims for radiation-related cancer morbidity.

When we estimate the radiation-related cancer risk associated with a particular low-dose exposure history, what is it we are estimating? Some possibilities:

a) **An increase in lifetime cancer rate**, e.g., from r to $r' = r \times (1 + x)$, for a particular population specified by age, sex, lifestyle, etc. Note that this increase theoretically can be verified by observation of cancer rates among exposed and non-exposed members of the population. Estimation requires information on:

i) Dose-related risk in some population (or group of populations), and the variation of that risk by sex, age, etc. Generally, this information will pertain most directly to doses and dose rates higher than those of immediate interest. For radiation-related risk, there is a substantial body of epidemiological information, the most comprehensive of which is based on follow-up of the survivors of the atomic bombings of Hiroshima and Nagasaki, Japan.

ii) How to transfer risk estimates for the informative population to the population of interest, which may differ from the first population in specified ways such as baseline cancer rate, smoking prevalence, patterns of reproductive history, other possible dose-response modifiers. Also, random and biased errors in dose reconstruction for the first

population, which should not affect risk estimates for members of the first population, may bias the application of dose-specific risk estimates to the second population. A similar problem exists for biased ascertainment of cancer cases, e.g., because of inaccuracies of death certificates.

iii) How to extrapolate risk from high to low doses and from high to low dose rates, including dose and dose-rate effectiveness factor (DDREF) and departures from the LNT hypothesis such as hormesis and low-dose threshold.

***b)* The likelihood that a particular individual will develop cancer as a result of his or her exposure**. Note that this likelihood is not verifiable at the individual level; the individual either will or will not develop cancer, and the estimate of the individual's probability, or excess probability, of developing cancer is verifiable only if we assume that information on a population also pertains to the individual.

Thus, ***b)*** reduces to ***a)***, and is addressed as follows:

i) Identify the individual as a member of some population with the exposure history and other characteristics of the individual insofar as the relevance of these characteristics to risk is known or estimated.

ii) Estimate the exposure-related increase in cancer rate for that population.

iii) Treat the individual as a randomly sampled person from the population, i.e., a possible cancer event is treated as a Bernoulli random variable with probability $p = r'$ as given in *a)* above. Note that r' is itself an uncertain quantity.

The several kinds of required information discussed under ***a)*** are qualitatively different. Many of them are subjective in nature, requiring expert judgment.

6.2 Sources of uncertainty

6.2.1 Statistical estimate of excess risk per Gy.

The epidemiological information from a radiation-exposed population is summarized by a statistical estimate, of excess absolute or excess relative risk (EAR or ERR, respectively), the uncertainty of which can be expressed by confidence limits or, more comprehensively, by a probability distribution derived from the statistical likelihood contour of the estimate. This probability distribution defines likelihood-based statistical confidence limits at all confidence levels, and may depend upon sex, exposure age, attained age, and other identifiable risk modifiers. Figure 6.1 represents an example of a likelihood-based statistical uncertainty distribution for excess relative risk of cancer at

ages 50 or older following a 1 Gy, whole-body, acute exposure at age 40. The estimate is based on a linear-model dose-response analysis of LSS tumor registry cancer incidence data (Thompson 1994) for males, reanalyzed in the context of adjudication of compensation claims for possibly radiation-related cancer (NCI/CDC 2003). In that analysis, it was found that most variation of ERR by exposure age was confined to ages under 30, and that most variation by attained age occurred at ages under 50. A model was used based on log-linear splines in exposure age and attained age such that there was no variation in ERR per Gy by exposure age after 30 and by attained age after 50. The resultant statistical uncertainty distribution for ERR per Gy at older exposure ages and attained ages is approximately lognormal with 5th and 95th percentiles (90% confidence limits) 0.18 and 0.43.

This statistical uncertainty distribution is the basis for the numerical demonstration presented below. However, summary results are also given, later in this chapter, for calculations based on the fitted estimate for a female population, with a lognormal statistical uncertainty distribution and 90% confidence limits 0.45 and 0.72, and for a population evenly divided by sex, for which the confidence limits are 0.33 and 0.53.

Estimates of excess absolute risk (EAR) for age-specific risk, or for lifetime risk starting from age 50, can be obtained by scaling the ERR distributions by the appropriate age-specific or lifetime baseline cancer rates. However, since in most applications the population of interest is not the LSS population and the exposure of interest is not to an acute dose of 1 Gy, it is computationally convenient to develop the ERR estimate for the population and exposure of interest and then convert to EAR.

6.2.2 Diagnostic misclassification.

Based on autopsy-based analyses by Sposto et al (1992) of misclassification of cancer as noncancer on death certificates, NCRP Report 126 (1997) introduced an uncertain correction factor for combined-site cancer mortality risk estimates, subjectively distributed as normal with 5^{th} and 95^{th} percentiles 1.02 and 1.18, respectively. No correction factor was deemed necessary, however, for cancer morbidity as determined by the RERF Tumor Registry, and none is applied in the present exercise. (Here, (or 90% “probability limits”, here used as a general term to include statistical confidence limits and uncertainty limits for distributions that have a subjective component - see footnote 1 to Chapter 2)

6.2.3 Dose-reconstruction errors.

Application of epidemiological information from one radiation-exposed population to a second population is problematic because errors in dose reconstruction for the first population are unlikely to be repeated in the second; therefore, dose-specific risk estimates should be corrected before being applied to the second population. Also, lifestyle, environmental, and other factors may differentially modify radiation dose response in the two populations.

NCRP Report 126 treated bias correction for dose-reconstruction error in the A-bomb survivor population, involving 5 different factors: random errors in individual dose estimates (following Pierce et al, 1991), uncertainty about the magnitude of the neutron component of dose in Hiroshima, uncertainty about the relative biological effectiveness weight, relative to gamma dose, applied to the neutron component of individual dose, uncertain neutron dose, and uncertain gamma dose. A full rationale is given in the NCRP report (NCRP 1977) to which the reader is referred for details. With the implementation of a new A-bomb survivor dose reconstruction system, designated DS02 (Preston et al. 2004), the details will change. For present purposes, it is enough to note that dose reconstruction is a source of bias and uncertain error, which can contribute to the uncertainties of risk estimates and should be taken into account. For illustration, we use the subjective uncertainty distribution of the combined correction factor described in Figure 3.6 of NCRP Report 126 and redrawn for Figure 6.2 of the present report, which was calculated as approximately normal with mean 0.84 and 90% uncertainty limits 0.69 to 1.0. The resulting corrected uncertainty distribution for ERR at 1 Gy is approximately lognormal with mean 0.26 and 90% limits 0.15-0.46 (Figure 6.3).

6.2.4 Transfer between populations

Also uncertain is the relationship between radiation-related excess risk and baseline cancer rates in the two populations. This is an important consideration if population baseline rates differ substantially. For example, current age-specific incidence rates for female breast cancer are substantially higher in the United States than in Japan, according to tumor registry data from Hiroshima and the U.S. SEER registry (Parkin, 1997) (Figure 6.4). In the figure, breast cancer risk among female A-bomb survivors exposed to a breast tissue dose of 1 Gy at age 15 is represented as a constant multiple of age-specific baseline risk beginning at age 25. The two dashed curves tracking the US

age-specific baseline rates represent two of many different ways of transferring the A-bomb survivor estimate to a US population. The lower of the two dashed curves was calculated as the sum of the US baseline rate plus the radiation-related excess (absolute) rate in the A-bomb survivors (additive transfer). The higher curve was calculated as the product of the US baseline rate times the estimated radiation-related relative risk among the A-bomb survivors (multiplicative transfer). If the baseline rate curves were the same, the additive and multiplicative transfer methods would give the same solution. Because the baseline rates are so different, the lifetable-averaged (over age) estimates of excess risk differ by three-fold.

In the case of breast cancer, there is epidemiological evidence that the additive transfer model is more realistic than the multiplicative model (Preston, 2002; Little and Boice, 1999; Land, 1980b), but there is not enough evidence to rule out alternatives. For stomach cancer there are some data favoring multiplicative transfer (Carr, 2002; Boice, 1988; Inskip, 1990). For most other site-specific cancers, there is little or no relevant information on transfer between populations. NCRP Report 126 considered only total cancer mortality, which is about 40% and 80% higher in the US than in Japan for males and females, respectively (Pisani, 1999). In Report 126, subjective uncertainty about population transfer was expressed as an uncertain multiplicative correction factor, distributed as lognormal with 5th and 95th percentiles at 0.70 and 1.65, respectively, to be applied to the multiplicative transfer model estimate (NCRP, 1997).

For site-specific cancers a more detailed approach is needed because standardized rates may differ between the two countries by as much as 10- to 15-fold in either direction (e.g., for liver, stomach, prostate), although for most sites rates are more comparable. The approach used for the updated NIH radioepidemiological tables report (NCI/CDC, 2003) for most cancer sites was to weight equally all possible linear combinations of the multiplicative (M) and additive (A) model estimates, $p \times M + (1-p) \times A$, by assuming p to be a random variable distributed approximately uniformly over the unit interval. This subjective approach was motivated by (1) the consideration that differences in baseline rates might reflect differential exposure to both cancer initiators (consistent with additive transfer) and cancer promoters (consistent with multiplicative transfer) and (2) an almost complete lack of relevant epidemiological information for most cancer sites. The general EPA approach for site-specific cancer risk was similar, but on a logarithmic scale: the logarithm of the excess risk was assumed to be a linear mixture between the logarithms of the multiplicative and additive transfer model estimates (EPA, 1999), where the uncertain

mixture parameter *p* was assumed to be uniformly distributed over the unit interval. The EPA approach tends to yield somewhat lower risk estimates than the NCI/CDC approach. For the few sites where information on population transfer was available, the NCI/CDC approach was to favor one simple transfer model over the other, e.g., for breast cancer, 0.5 probability was placed on additive transfer and 0.5 on the uniform model; for stomach cancer, probability 0.33 was placed on multiplicative transfer and probability 0.67 on the uniform model.

For all cancers except skin, as a group, the sex-age-standardized ratio of American to Japanese rates was assumed to be 1.3 (Parkin, 1997). Multiplicative transfer of LSS-based excess relative risk would involve applying the same ERR to U.S. baseline rates, whereas for additive transfer the LSS-based ERR would be divided by 1.3 to obtain the same absolute excess in the two countries. The resulting uncertainty distribution for ERR at 1 Gy in a U.S. population, after application of the NCI/CDC approach, is approximately lognormal with 90% limits 0.13-0.41, and mean 0.25 (Figure 6.5).

6.2.5 DDREF

In general, epidemiological estimates of overall and site-specific cancer risk related to radiation exposure are statistically consistent with a linear dose response (leukemia, with a linear-quadratic dose response, is an exception). For the same reasons that data restricted to low doses tend to be uninformative about radiation-related excess risk, this apparent linearity does not rule out, on statistical grounds, the possibility of increased, decreased, or even absent excess risk per unit dose at very low doses. For various reasons discussed in Chapters 2 and 3, linear-model estimated excess risk is often divided by a dose and dose-rate effectiveness factor (DDREF) at low doses and low dose rates. The ICRP (1991) recommended a DDREF of 2 for radiation protection purposes, and the United Nations Scientific Committee on Effects of Ionizing Radiation (UNSCEAR, 1993) recommended that the chosen DDREF be applied to chronic exposures at dose rates less than 6 mGy per hour averaged over the first few hours, and to acute exposures at total doses less than 0.2 Gy. This recommendation was adopted by the EPA (1999). Continuous, subjective uncertainty distributions for DDREF were used in uncertainty analyses carried out for NCRP Report 126 (NCRP, 1997), the EPA (1999), and by an expert committee advising the Colorado Department of Public Health and Environment (Grogan, 2000) (Figure 6.6). The Grogan uncertainty distribution differs from the NCRP distribution mainly in allowing a small probability that risk per unit dose

might increase at very low doses. Thus, the NCRP and EPA distributions allowed for the possibility of DDREF values between 1 and 5, while the Grogan et al distribution included DDREF values as low as 0.2. The uncertainty analysis for the revised NIH radioepidemiological tables report postulated a discrete subjective uncertainty distribution for DDREF, with non-zero probabilities assigned to 0.5, 0.7, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 (Figure 6.7).

Application of a DDREF factor greater than 1 reduces estimated risk, and an uncertain DDREF introduces additional uncertainty in estimated risk. Applying the different DDREF assumptions summarized in Figures 6.6 and 6.7 to the adjusted uncertainty distribution for risk in Figure 6.5 resulted in roughly lognormal uncertainty distributions for ERR per Gy at low doses and dose rates, with mean values substantially less than the mean value 0.25 for ERR at 1 Gy for acute exposures corresponding to the uncertainty distribution in Figure 6.5, and upper 95% uncertainty limits somewhat less than the value 0.41, also from Figure 6.5. By model, means and upper limits were 0.12 and 0.20, respectively, for the EPA DDREF, 0.11 and 0.23 for the NCRP model, mean 0.12 and upper limit 0.28 for the Grogan et al model, and mean 0.17 and upper limit 0.36 for the NCI/CDC model (Figure 6.8).

6.2.6 Variation by sex

The above results apply to males. Carrying out the same calculations based on the statistical uncertainty presented in Section 6.2.1 for a female population yields an ultimate uncertainty distribution, using the NCI/CDC DDREF model, with mean 0.355 and 95th percentile 0.69. For a population divided equally by age and sex, the mean is 0.26 and the upper limit is 0.50.

6.2.7 Expression of excess risk in absolute terms

For U.S. males, the lifetime baseline cancer risk, tabulated by National Cancer Institute's SEER program (http://seer.cancer.gov/faststats/html/dev_all.html), from age 50 given cancer-free survival to age 40, is 45.3%. Thus, the estimated lifetime excess cancer risk per Gy associated with a low-dose, low-LET, whole-body radiation exposure is roughly lognormal with mean 0.17 H 45.3% =7.7% and 95th percentile 0.36 H 45.3% = 16.3%; the 5th percentile is 0.066 H 45.3% = 1.1%. For females, the corresponding baseline risk is 35.5%, and the uncertainty distribution for lifetime EAR per Gy has mean

0.355 H 35.5% = 12.6% and 90% uncertainty bounds (0.146, 0.69) H 35.5% = (5.2%, 24.5%). For a population evenly divided by sex, the baseline risk from age 50 given survival to age 40 is 40.4%, and lifetime EAR per Gy is roughly lognormal with mean 10.5% and 90% bounds (3.8%, 20.1%).

6.2.8 Gradualism in DDREF and threshold effects.

A rule that a DDREF should apply at acute doses below (say) 0.2 Gy and not at 0.2 Gy and above, or at dose rates less than 6 mGy per hour but not at dose rates marginally higher than that value, is contrary to experience with stochastic phenomena, and would be difficult for practical applications, e.g., in adjudicating compensation claims for radiation-related cancer. Accordingly, in the recent revision of the NIH radioepidemiological tables report (NCI/CDC 2003), DDREF was gradually phased in, from 1 to its (uncertain) full value, over an interval of decreasing dose of acute exposure. Similarly, a threshold dose, below which there is presumed to be no radiation-related risk, is generally not thought of as a value associated with the abrupt disappearance of risk, but a (possibly uncertain) value greater than zero Gy at which the gradual disappearance of excess risk with decreasing dose becomes complete. Thus, a threshold or possible threshold would, like the DDREF, be phased in gradually with decreasing dose.

For simplicity of presentation, phasing in DDREF and/or a threshold is ignored in the following discussion.

6.3 Allowing for the uncertain possibility of a threshold.

The threshold concept has practical importance only if the threshold dose is high enough to justify ignoring, for radiation protection purposes, a substantial range of exposures that would otherwise be of concern. A reasonable way to include the threshold concept in an uncertainty analysis is to multiply the uncertain dose-specific excess relative risk, adjusted for the DDREF and other factors discussed in the preceding paragraphs, by a threshold factor distributed as a Bernoulli random variable taking value zero with probability $p(D)$ and value one with probability $1 - p(D)$, where $0 \# p(D) \# 1$ and p is a possibly uncertain, decreasing function of radiation dose D. Some examples will illustrate the impact of uncertainties regarding whether a threshold exists or the dose level of that threshold.

Example 1--Threshold and dose level certain. Known threshold at 10 mGy: $p(D) = 1$ for $D \# 10$ mGy and $p(D) = 0$ for $D > 10$ mGy (for simplicity, the threshold is not phased in as a function of D). Thus, the uncertainty distribution for excess risk assigns probability 1 to the value zero, below 10 mGy, and is the same as that without a threshold (e.g., the NCI/CDC distribution in Figure 6.8) above 10 mGy. The mean and 95% upper probability limit on ERR per Gy are unchanged above 10 mGy, but they are both zero below that dose level. This example represents the common conception held by those who believe there is a threshold, albeit the putative threshold dose level may differ from 10 mGy.

Example 2--Threshold uncertain but threshold dose level certain. A threshold may exist at 10 mGy; this possibility is assigned subjective probability **p**, where **p** is a known value such as 5%, 20%, 50%, or 80%. The uncertainty distribution of ERR/Gy risk below 10 mGy assigns probability **p** to zero and, for all other possible values of ERR/Gy, 1-**p** times the probability that would be assigned if there were no threshold. For doses below 10 mGy, the mean of the uncertainty distribution is 1-**p** times the mean of the uncertainty distribution for ERR/Gy if there were no threshold (i.e., if **p** were zero). The 95% upper uncertainty limit is given by limit = $F^{-1}((.95-\mathbf{p})/(1-\mathbf{p}))$ for $\mathbf{p} < 0.95$, and limit = 0 for $\mathbf{p} \$0.95$, where F^{-1} is the inverse cumulative distribution function of the uncertainty distribution in the absence of a threshold (Land, 2002). Plots of the mean and

upper 95% limit, as functions of **p,** are shown in Figure 6.9 for the approximate lognormal uncertainty distribution for ERR/Gy according to the NCI/CDC model as represented in Figure 6.8.

This example shows that, when the probability of a dose threshold is uncertain, the central estimate of the ERR/Gy for low doses decreases linearly with an increasing probability that there is a threshold -- but the 95% upper limit remains quite high until the probability of a threshold reaches 80-90%, after which it falls sharply. This indicates that unless there is consensus agreement that a threshold is very likely, the potential for an appreciable low-dose risk cannot be ruled out.

Example 3--Threshold certain but its dose level uncertain. A threshold is known to exist somewhere between 5 and 25 mGy, but otherwise is completely uncertain: $p(D;D_0) = 1$ for $D \# D_0$, and $= 0$ for $D > D_0$, where D_0 is an uncertain (random) quantity uniformly distributed between 5 and 25 mGy. Estimated ERR/Gy is zero below 5 mGy, but the probability assigned to non-zero values by the uncertainty distribution for risk at dose D increases linearly from zero at $D = 5$ mGy to one (or to the value assigned in the absence of a threshold) at $D = 25$ mGy. The uncertainty distribution for ERR/Gy assigns probability 1 to zero for D below 5 mGy, 100% to the non-threshold distribution for doses above 25 mGy, and probability $(25-D)/20$ to zero and probability $(D-5)/20$ to the non-threshold uncertainty distribution, for $5<D<25$. The mean and upper 95% uncertainty limit at dose D are as given in Example 2, and shown in Figure 6.9, for $\mathbf{p} = (25-D)/20$.

The third example illustrates an important point: even when one is certain there is a dose threshold but is still uncertain as to the dose level at which it occurs, the low-dose ERR/Gy behaves very similarly to the result for Example 2 (which had a fixed threshold dose but uncertainty as to whether there was a threshold). Specifically, there still is some probability that the low-dose ERR/Gy is appreciable.

Example 3a—There is a threshold for each individual in a population, but the dose level varies by individual. Thus, for a randomly chosen individual from the population, there is a threshold, but its dose level is uncertain. Mathematically, this example is essentially the same as example 3.

Example 4--Threshold probability very uncertain but its dose level, conditional on existence of a threshold, is certain. A threshold may exist at 10 mGy, with uncertain

probability. Enough is known (or there is a consensus among experts, which may be a compromise) to characterize the subjective uncertainty distribution of *p(D)*, for $D < 10$ mGy; for example, as

i) uniform between 0 and 1: U(0, 1),

ii) triangular between 0 and 1 with peak at 0: Tr(0, 0, 1),

iii) Tr(0, 0.25 ,1) (peak at $p = 0.25$),

iv) Tr(0, 0.5 ,1),

v) Tr(0, 0.75 ,1),

vi) Tr(0, 1, 1).

In example 4, the proportion of the uncertainty distribution for ERR/Gy assigned to zero is randomly distributed over the unit interval, and the mean and upper 95% limit of the resulting distribution depends on the assumed distribution of *p*. Figure 6.10 shows Monte Carlo estimates of the resulting uncertainty distributions for ERR/Gy for the six cases, again using the NCI/CDC non-threshold distribution from Figure 6.8, and the corresponding means and upper 95% uncertainty limits.

The probability distributions in Figure 6.10 show, not unexpectedly, that if the consensus uncertainty distribution of *p* gives a high weight to the likelihood of a threshold (e.g., subjective distribution *vi*), then the distribution of the low-dose ERR/Gy is weighted toward small values, whereas the opposite is true when the probability of a threshold is less likely (e.g., subjective distributions *ii* or *iii*). Nevertheless, even for distribution *vi* the mean expected low-dose ERR/Gy of 5.7% is about a third as great as under the LNT hypothesis (ERR/Gy = 17%), and it is between 40% and 70% as the LNT value for distributions *i – v*.

Example 5 – Dose-dependent uncertain probability of a threshold. Suppose that the uncertainty distribution for a threshold at 10 mGy corresponds to Example 4, distribution *ii*, i.e., Tr(0,0,1), and that the uncertainty distributions for thresholds at 1 mGy and at 0.1 mGy correspond to Example 4 distributions *iv* (Tr(0, 0.5, 1)) and *vi* (Tr(0, 1, 1)), respectively. Then the subjective means and upper uncertainty limits for ERR/Gy would be 11.5% and 27%, respectively, at 10 mGy, 8.6% and 21% at 1 mGy, and 5.7% and 17% at 0.1 mGy. The corresponding values of ERR would be 0.115% and 0.27% at 10 mGy, 0.0086% and 0.021% at 1 mGy, and 0.00057% and 0.0017% at 0.1 mGy. The mean and 95% upper limit for ERR at 0.1 mGy can be compared with the mean 0.0017% and upper limit 0.0036% according to the LNT hypothesis.

Of the five examples above, Example 5 probably best reflects our present state of knowledge about low dose risk, namely, that we are uncertain about the likelihood of a dose threshold, and that in addition, if there should be a dose threshold, we are uncertain about at what dose level it would be. However, as a counter to an agnostic viewpoint, it should be noted that the mechanistic and experimental data discussed in this monograph tend to give weight to a nonthreshold model, as do the solid tumor data in the Japanese atomic bomb study. (In addition to apparent linearity of dose response down to doses below 100 mGy, an analysis by Pierce and Preston (2000) found that a threshold above 60 mGy would be statistically inconsistent with LSS dose-response data for all solid cancers combined.)

6.4 Conclusions

Information on radiation-related cancer risk is needed (1) as guidance for radiation protection efforts, (2) as a basis for informed consent by persons who may be asked to accept a certain level of exposure in the interests of medical research, economic progress, or some other social good, (3) for adjudication of claims and disputes concerning cases of disease possibly related to past radiation exposure, and (4) for risk-benefit analyses of public policy initiatives related to radiation. As mentioned previously in this report, these issues are essentially political, in the sense that different people have different interests and points of view which must be taken into consideration when policies are developed. Moreover, implementation of such policies inevitably involves accommodation and consensus, and it is important that the policies are seen to be derived fairly and openly, on the basis of facts and assumptions that are wholly accessible to those affected by implementation.

Information useful for these purposes includes central estimates of dose-specific risk, but also, lower and (especially) upper probability bounds on risk. Probability bounds can reflect both statistical uncertainty, estimated by fitting a mathematical model to observational data, and subjective uncertainty that may take into account model assumptions that are necessary to calculate estimates but are themselves uncertain. Probability bounds provide a level of transparency substantially beyond that provided by a point estimate, such as the expected (mean) value of the uncertainty distribution for estimated excess risk. A lower probability bound (e.g., a 95% lower confidence limit or

uncertainty limit) greater than zero is evidence that there really is an excess risk; however, the carcinogenicity of ionizing radiation exposure is already well established. A lower bound corresponding to a risk that is intolerably high would, of course, be evidence in support of diversion of financial resources for exposure reduction, even from the viewpoint of those who would bear the expense.

From the viewpoint of those who would bear the risk, if any, associated with exposure, and of those responsible for their protection, the questions of interest concern (1) the extent to which risks associated with a given level of exposure are low enough to be tolerated in view of competing risk and loss of benefits associated with avoidance of that exposure, and (2) whether we can conclude that there is no risk at all associated with a given exposure. An upper probability bound, if less than some "tolerable" level of risk, can be used to help justify a favorable risk-benefit assessment for a particular exposure, and can provide a margin of safety in decisions regarding risk protection or informed consent related to possible hazards of radiation exposure. An upper probability bound of zero or less would be evidence in favor of a threshold or, more likely, a beneficial effect of low-dose radiation.

The implications of a possible, but uncertain, low-dose threshold for radiation protection are summarized by the dependence of the mean value and the upper 95% probability limit on the presumed threshold probability value (Figure 6.9) or on the uncertainty distribution for that probability (Figure 6.10). The mean value of estimated ERR/Gy is proportional to 1-**p** for known threshold probability **p** and proportional to 1-E(p) for an uncertain threshold probability p with expected value E(p). Thus, the effect on the mean value is the same as that of an assumed constant DDREF equal to 1/**p** or 1/E(p). The effect on the upper 95% probability limit is less drastic, unless the assumed probability of a threshold is high. As shown in Figure 6.9, the upper limit decreases with increasing **p**, but the not nearly as steeply as for the mean until **p** approaches the probability level of the upper limit, e.g., about 0.85 in the case of a 95% limit. Obviously, the lower 95% limit (the 5^{th} percentile of the distribution) is zero for $\mathbf{p} \geq 0.05$.

As mentioned earlier in this chapter, an established, universal or near-universal, low-dose threshold for radiation-related cancer risk would obviate concern about risks from exposures at doses lower than the threshold value. Our present information, summarized in NCRP Report 136 (2001) and the present report, offers little support for the existence of a universal low-dose threshold, but it cannot be ruled out as an uncertain

possibility. However, the implications of the uncertain possibility of a threshold are qualitatively not much different from those of an uncertain DDREF: central values and upper uncertainty limits are reduced somewhat, but they do not become zero. Moreover, the argument that radiation protection standards should be relaxed “because it is possible that there may not be *any* risk at low doses” is unlikely to be persuasive to persons who are concerned about the possibility that risk associated with very low doses may be unacceptably high, and it may undermine the more realistic argument that the risk, which is understood rather well compared to that associated with other common carcinogens, is almost certainly less than some stated value which may be considered tolerable, for various reasons such as economic benefits or consideration of risks associated with alternative strategies involving less exposure.

6.6 References

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7. CONCLUSIONS

1. Epidemiological studies of cancer risk following radiation exposure provide the primary basis for estimation of radiation-related risk in human populations. These studies demonstrate the existence of dose response and its modification by other factors, and show some variation by cancer site and by histological subtypes within sites. At low and very low radiation doses, statistical and other variation in baseline risk tends to be the dominant source of error in both epidemiological and experimental carcinogenesis studies, and estimates of radiation-related risk tend to be highly uncertain both because of a weak signal-to-noise ratio and because it is difficult to recognize or to control for subtle confounding factors. Thus, extrapolation of risk estimates based on observations at moderate to high doses continues to be the primary basis for estimation of radiation-related risk at low doses and dose rates.

2. There is no direct evidence, from either epidemiological or experimental carcinogenesis studies, that radiation exposure at doses on the order of 1 mGy or less is carcinogenic, nor would any be expected because of the considerations outlined in Conclusion 1. There is, however, epidemiological evidence, unlikely on the wholel to be an artifact of random variation, linking increased cancer risk to exposures at doses on the order of 10 mGy. This evidence includes several case-control studies of leukemia and solid cancers among different populations of children exposed in utero to x-ray pelvimetry, cohort studies of breast cancer among women given multiple fluoroscopy examinations during treatment for tuberculosis or scoliosis, with average breast doses on the order of 10 mGy per examination, and the observation that risk of mortality and morbidity among atomic bomb survivors from all solid cancers combined is linear in radiation dose down to about 100 mGy.

3. Overall, relevant animal tumor data from experimental carcinogenesis studies tend to support a dose response that, at low doses, is linear with no threshold. This inference does not conflict with experimental evidence for reductions in excess risk per unit dose at low doses or with fractionation and/or protraction of dose. Recent cytogenetic and molecular studies provide direct support for the view that

the critical radiation-associated events in the tumorigenic process are predominantly early events involving DNA losses targeting specific genomic regions harboring critical genes. The predominant importance of DNA DSB induction and post-irradiation error-prone NHEJ repair for the induction of aberrations, and the apparently critical role for radiation-induced aberrations in the pathogenesis of cancer in these experimental models, argue against the proposition of a low dose threshold in the dose response.

4. There is evidence from both epidemiological and experimental studies that specific tissues and cancer sites may for various reasons vary from the general rule articulated in Conclusions 1-3, in the sense that radiation carcinogenesis is markedly and disproportionately less likely to occur at low doses than at high, and may even suggest a threshold. Examples are the small intestine, bone, and skin. However, these appear to be the exception rather than the general rule; experimental studies of radiation-related life shortening, which represent the integrated dose responses for a variety of tumor types, suggest linear dose responses over a wide range of doses.

5. Ionizing radiation is able to produce a unique type of damage in which multiple lesions are encountered within close spatial proximity. Even a single track through a cell is likely to induce these unique clustered damages. This type of damage may not be generated frequently endogenously or by other exogenous agents, and thus, there may not have been a strong selective pressure driving efficient repair. Although cells have a vast array of damage response mechanism that facilitate the repair of DNA damage and the removal of damaged cells, these mechanisms are not fool proof. Moreover, clustered radiation–induced lesions pose a particular problem and current emerging evidence suggests that closely spaced lesions can compromise the repair machinery. On this basis, there is not any strong evidence for a radiation dose below which all radiation-induced damage can be repaired with fidelity.

6. Although many of the cells containing such radiation-induced damage may be eliminated by damage response pathways involving cell cycle checkpoint control and apoptotic pathways, it is clear from analysis of cytogenetics and mutagenesis

that damaged or altered cells are capable of escaping these pathways and propagating. This further argues against the likely possibility of a threshold for radiation-induced cellular effects.

7. The processing and misrepair of radiation-induced DSBs, particularly complex forms, are responsible for chromosome/gene alterations that manifest as chromosome aberrations and mutations. Current understanding of mechanisms and quantitative data on dose and time-dose relationships support a linear dose response at low doses with no compelling evidence for the existence of a threshold dose below which there would be no effect.

8. When considered as a whole, the emerging results with regard to radiation-related adaptive response, genomic instability, and bystander effects suggest that the risk of low level exposure to ionizing radiation is uncertain, and a simple extrapolation from high dose effects may not be wholly justified in all instances. However, a better understanding of the mechanisms for these phenomena, the extent to which they are active *in vivo*, and how they are interrelated is needed before they can be evaluated as factors to be included in the estimation of potential risk to the human population of exposure to low levels of ionizing radiation.

9. Probability limits on risk provide additional information relevant to radiation protection. In particular, a high lower limit attests to the reality of danger associated with a given exposure, and a low upper limit provides assurance as to the relative safety, and presumably the acceptability, of the exposure when seen in the context of other hazards of daily life. The information reviewed in this report, from epidemiology and from experimental studies of animal, cellular, and molecular models, is consistent with proportionality between radiation-related cancer risk at low doses and at low dose rates, including the dose delivered by a single photon. It is also consistent, given uncertainties about the roles played by repair and apoptosis at very low doses, with the existence of a dose threshold at a dose level so low that radiation-related risk under the linear, no-threshold (LNT) hypothesis would be statistically indistinguishable from random variation in baseline risk. However, the uncertain possibility of a threshold does not drastically reduce either central estimates or upper probability limits for low-dose risk

compared to those obtained using the LNT hypothesis, unless that possibility is assumed to be very likely. To the contrary, the evidence reviewed in this report suggests that a universal low-dose threshold above (say) 1 mGy of low-LET radiation is an unlikely possibility. Thus, the LNT hypothesis remains the most prudent risk model for guidance of radiation protection.

TABLES

Table 2.1 Some sources and amounts of ionizing radiation exposure (unless noted, from Mettler and Upton (1995))

Exposure	Effective dose, in mSv	
Natural background (world population)	Normal background areas	High background areas
Cosmic rays	0.38 / year	2.0 / year
Terrestrial (rays	0.46 / year	4.3 / year
Radionuclides in tissue	0.25 / year	0.6 / year
Inhaled radionuclides	2.5 / year	
Medical diagnostic (U.S. population)	Per exam	
Skull	0.22	
Cervical spine	0.20	
Chest	0.08	
Cholioangiogram	1.89	
Lumbar Spine	1.27	
Upper gastrointestinal series	2.44	
Abdomen (KUB)	0.56	
Barium enema	4.06	
Intravenous pyelogram	1.58	
Pelvis	0.44	
Hip	0.83	
Extremities	0.01	
CT scan, head or body	1.11	
Pediatric CT scan, abdomen[1]	25 (stomach dose)	
Single screening mammogram[1]	3 (breast dose)	
Astronaut, 3-day space shuttle mission[2]	2 – 3	
Astronaut, 60-day space station mission[2]	50	
Average cumulative occupational dose in monitored radiation workers[3]	Cumulative reported badge dose 20	
Average neutron-weighted colon dose for LSS population with doses between 0.005 and 4 Gy [4]	Colon dose 200	

[1] Brenner et al, 2003; [2] NCRP Report 138, 2001; [3] Gilbert, 2001; [4] Preston, 2003; computed using data set downloaded from RERF web site (Radiation Effects Research Foundation, 2003)

Table 2.2. Parameter estimates corresponding to the general dose-response model,

$$ERR(D) = \alpha D \times (1 + \beta D) \times \exp(-\gamma D),$$

where D is neutron-weighted (weight = 10), reconstructed radiation dose to the colon from the atomic bombings and ERR(D) is the dose-related excess relative risk of solid cancer morbidity, 1958-87, among members of the Radiation Effects Research Foundation's Life Span Study cohort of survivors of the bombings.

Parameter	Estimate	90% CI	p-value
α	0.52	0.16, 0.83	.02
β	0.94	0*, 6.8	.28
γ	0.84	0*, 0.68	.07
α	0.71	0.56, 0.87	<.001
β	0[§]	----	----
γ	0.11	0*, 0.24	.07
α	0.57	0.48, 0.68	<.001
β	0*	----	----
γ	0[§]	----	----
Analysis restricted to survivors with estimated doses 2 Sv and less			
α	0.40	0*, 0.85	.24
β	0.92	0*, 3.0	>.5
γ	0.53	0*, 1.3	>.5
α	0.61	0.35, 0.76	<.001
β	0.045	0*, 0.68	>.5
γ	0[§]	----	
α	0.64	0.54, 0.74	<.001
β	0[§]	----	
γ	0*, 0[§]	----	

* Estimate constrained to be $0. [§]Estimate set = 0.

Table 2.3 Modification of radiation-related risk by individual and lifestyle factors, and by other exposures.

Organ site / cancer	Population	Factor	Main factor effect on risk	Interaction with radiation exposure	References
Female breast	LSS cohort	Young age at 1st full-term pregnancy	Decreased	Multiplicative[1]	Land et al, 1994
"	"	Multiple births	Decreased	Multiplicative[1]	"
"	"	Lengthy lactation history	Decreased	Multiplicative[1]	"
"	NY mastitis series	Assoc with 1st delivery	Increased	Not tested	Shore, 1980
"	Massachusetts TB fluoroscopy series	Exposed yr of 1st delivery	Increased (NS)	Not tested	Boice & Stone, 1978
Lung& bronchus	LSS cohort	Smoking history	Increased	Additive[2]	Pierce, 2003
"	U.S. Uranium miners	"	"	NS closer to mult. than to additive	Lubin & Steindorf
Basal cell skin ca.	LSS cohort	Sun-exposed cf. covered areas of skin		Additive[2]	Ron, 1998
"	NY tinea capitis series	White cf. Black patients	Higher in whites	Multiplicative[1]	Shore, 2002
Liver	LSS cohort	Hepatitis C infection	Increased	Strongly synergistic	Sharp, 2002
Female breast	LSS cf. Euro/American pops	Population rates	Japan rate 4-fold < US	Additive[2]	Preston, 2002
Stomach	LSS cf. US peptic ulcer patients	Population rates	Japan rate 12-fold > US rate	NS, closer to multiplicative than to additive	Carr, 2002

[1] Additive interaction model rejected (statistically inconsistent with data); [2] Multiplicative interaction model rejected

Table 2.4. Statistical power calculations for a hypothetical study in which baseline cancer risk is known to be 10%, and the unknown radiation-related excess risk is 10% at 1 Gy and proportional to dose between 0 and 1 Gy.

Radiation dose	Excess risk	Total risk	Standard deviation of the estimated excess risk under the null and alternative hypotheses		Population size N needed for 80% power to detect the excess risk at the 5% significance level
1 Gy	10%	20%	$0.316 / N^{1/2}$	$0.447 / N^{1/2}$	124
100 mGy	1%	11%	$0.316 / N^{1/2}$	$0.332 / N^{1/2}$	6,800
10 mGy	0.1%	10.1%	$0.316 / N^{1/2}$	$0.318 / N^{1/2}$	624,000
1 mGy	0.01%	10.01%	$0.316 / N^{1/2}$	$0.316 / N^{1/2}$	61.9 million

Table 2.5. Distribution of subjects, solid cancers, and estimated radiation-associated, excess solid cancers among 79,901 exposed members of the Life Span Study cohort of Hiroshima-Nagasaki atomic bomb survivors (Pearce and Preston, 2000).

Estimated colon dose	Number of subjects	Number of solid cancers	Estimated number of radiation-associated excess cancers*
Exposed beyond 3000 m	23,493	3,230	0
<5 mGy, exposed within 3000 m	10,159	1,301	1
5-100 mGy	30,524	4,119	77
100-200 mGy	4,775	739	60
200-500 mGy	5,862	982	164
0.5-1 Gy	3,048	582	177
1-2 Gy	1,570	376	165
>2 Gy	470	126	80

* Fitted values, linear dose response

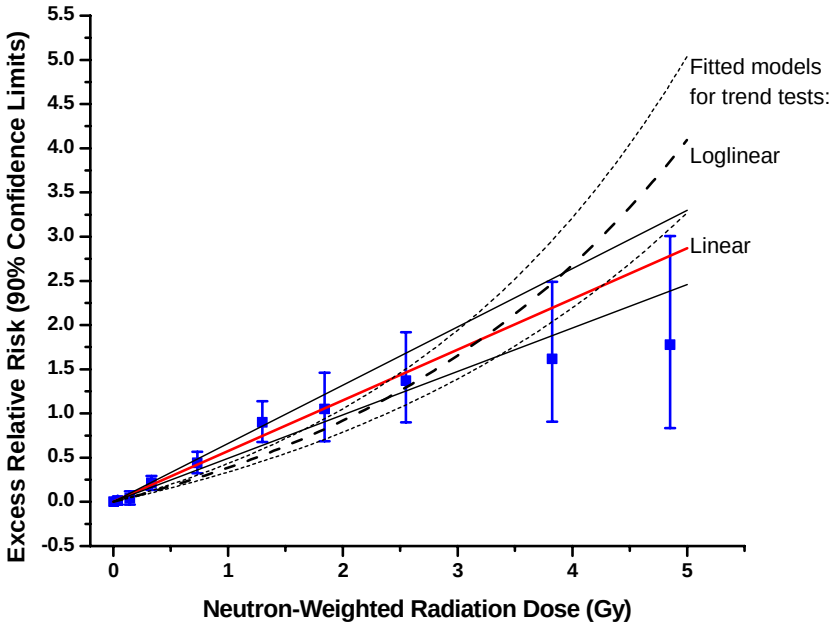


Figure 2.1. Dose-specific excess relative risk of solid cancer among atomic bomb survivors, 1958-87, by interval of neutron-weighted, estimated radiation dose to the colon. Fitted dose-response functions correspond to statistical tests of increasing trend according to the linear ($RR = 1 + \alpha D$) and log-linear ($RR = \exp(\beta D)$) dose-response models. The baseline risk is adjusted for city of exposure (Hiroshima or Nagasaki), sex, and 5-year intervals of exposure age and age at observation for risk, using a saturated model.

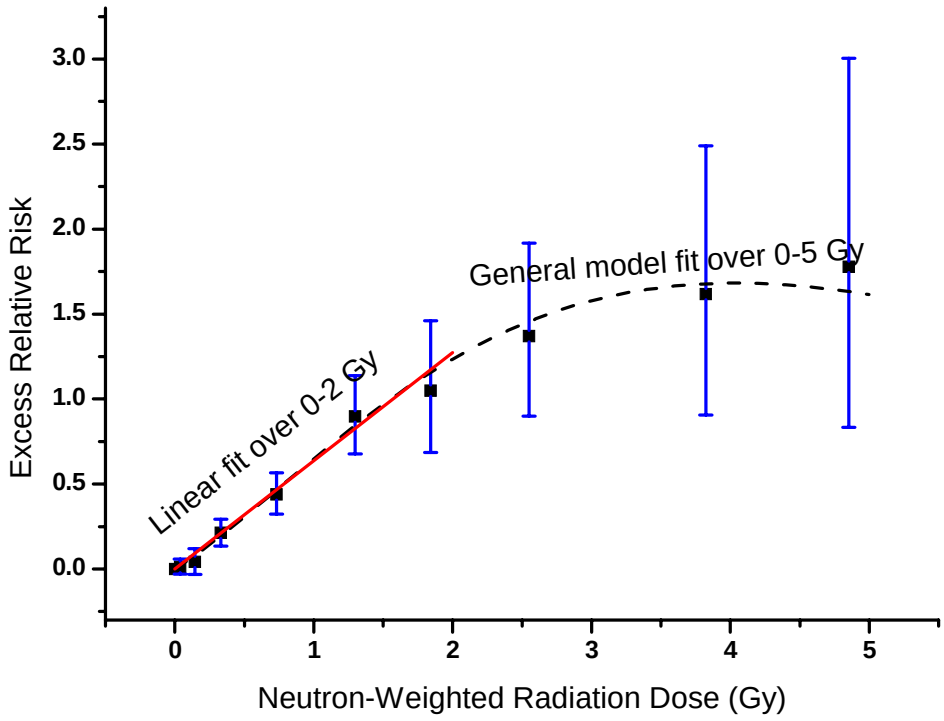


Figure 2.2. General dose-response model, $ERR(D) = \alpha D \times (1 + \beta D) \times \exp(-\gamma D - \delta D^2)$, fit to the dose-response data of Figure 1, and linear dose-response model, $ERR(D) = \alpha D$, fit to the data subset restricted to radiation doses between zero and 2 Sv. Details of the parameter estimates are given in Table 2.2.

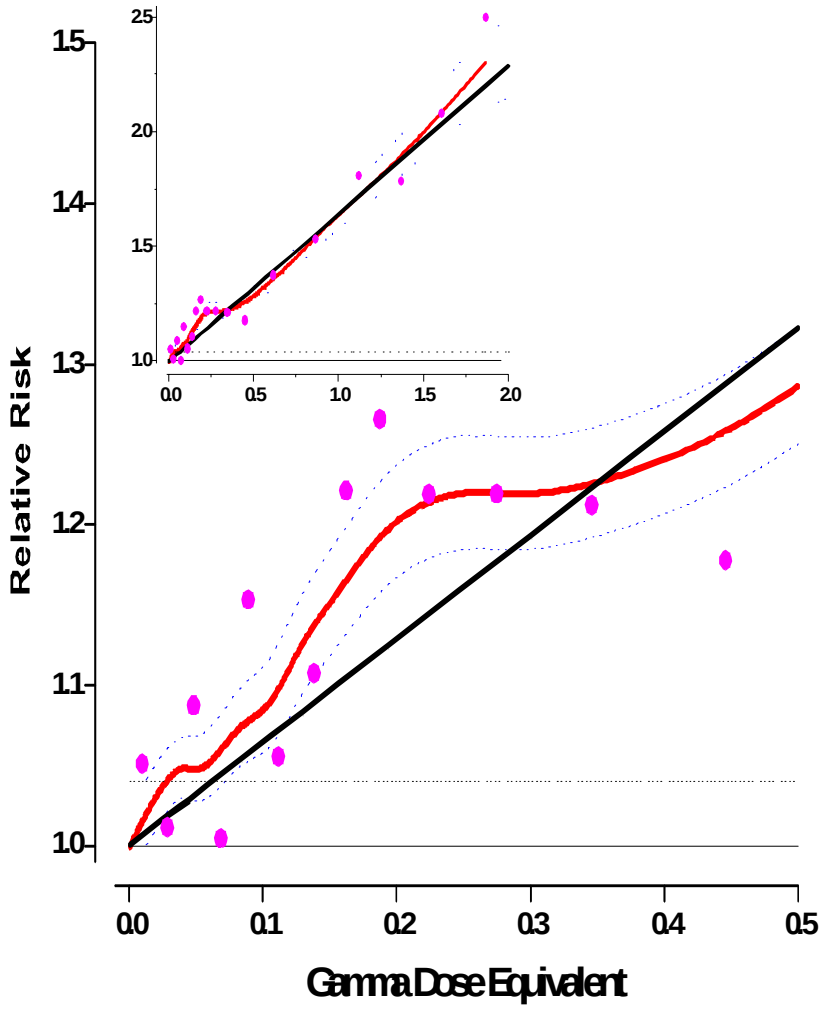


Figure 2.3. Estimated low-dose relative risks. Dose-specific cancer rates over the 1958-94 Follow-up period relative to those for an otherwise similar exposed person, averaged over the follow-up and over sex, and for age 30 at exposure. The dashed curves represent 1 standard error limits for the smoothed curve. The straight line is the estimated linear dose response for 0-2 Sv (see inset). The unity baseline corresponds to zero-dose survivors exposed within 3 km of the bombs. The horizontal dotted line represents the alternative baseline if survivors exposed beyond 3 km had been included. (Pierce and Preston, Radiation Res. 2000, 154:178-186.)

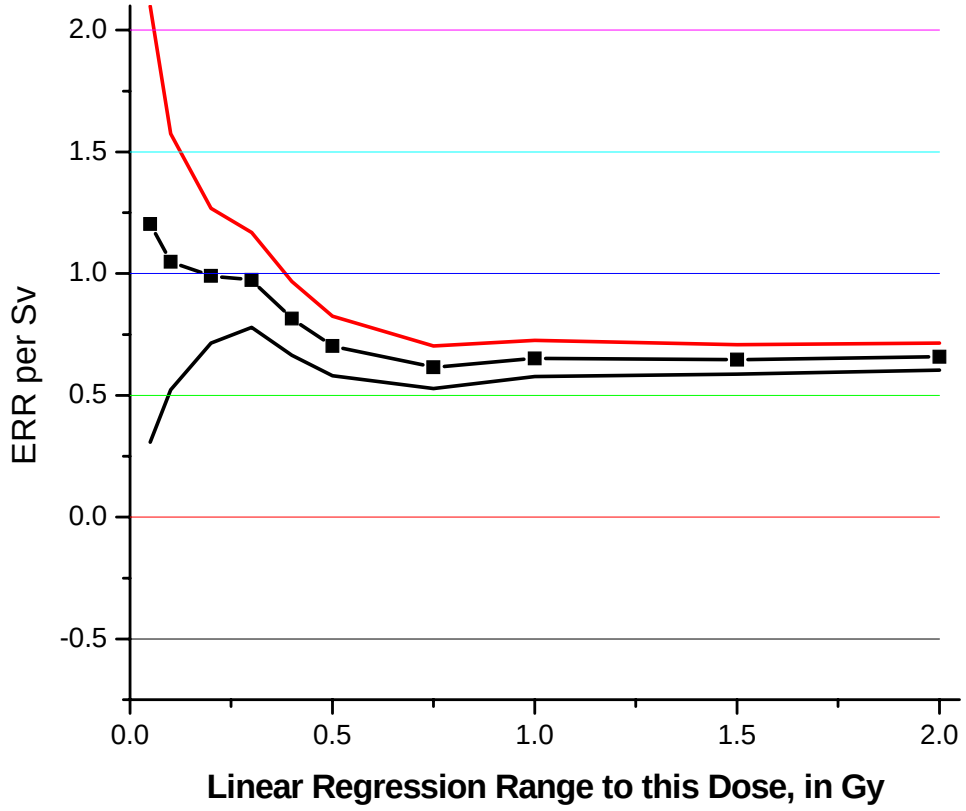


Figure 2.4. Linear regression estimates of the ERR per Gy (points and connecting line, with error bounds of ± one SE) for solid cancer incidence, based on Poisson regression over dose intervals of differing ranges from zero to the horizontal coordinate of the plotted point. The analysis is limited to proximal survivors exposed at distances under 3 km.

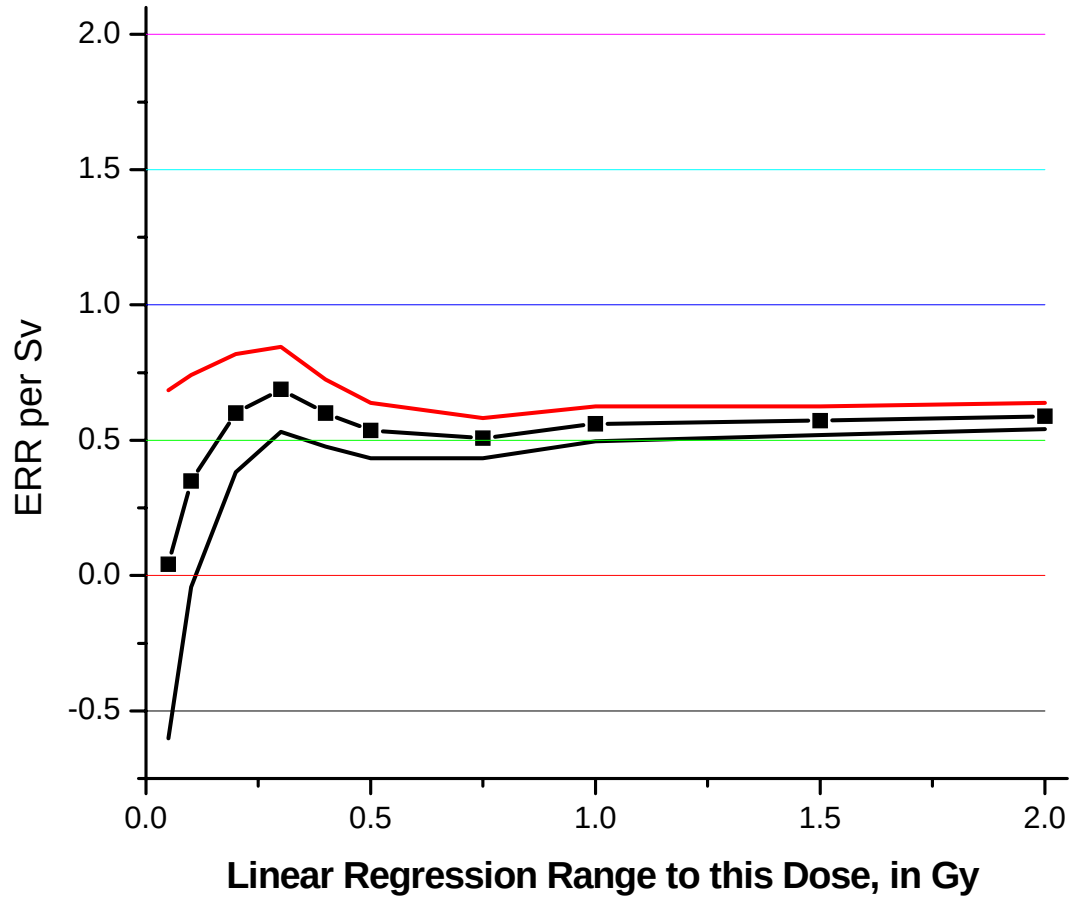


Figure 2.5. Linear regression estimates of the ERR per Gy (points and connecting line, with error bounds of ± one SE) for solid cancer incidence, based on Poisson regression over dose intervals of differing ranges from zero to the horizontal coordinate of the plotted point. The analysis is based on all exposed survivors with estimated radiation doses less than 2 Gy.

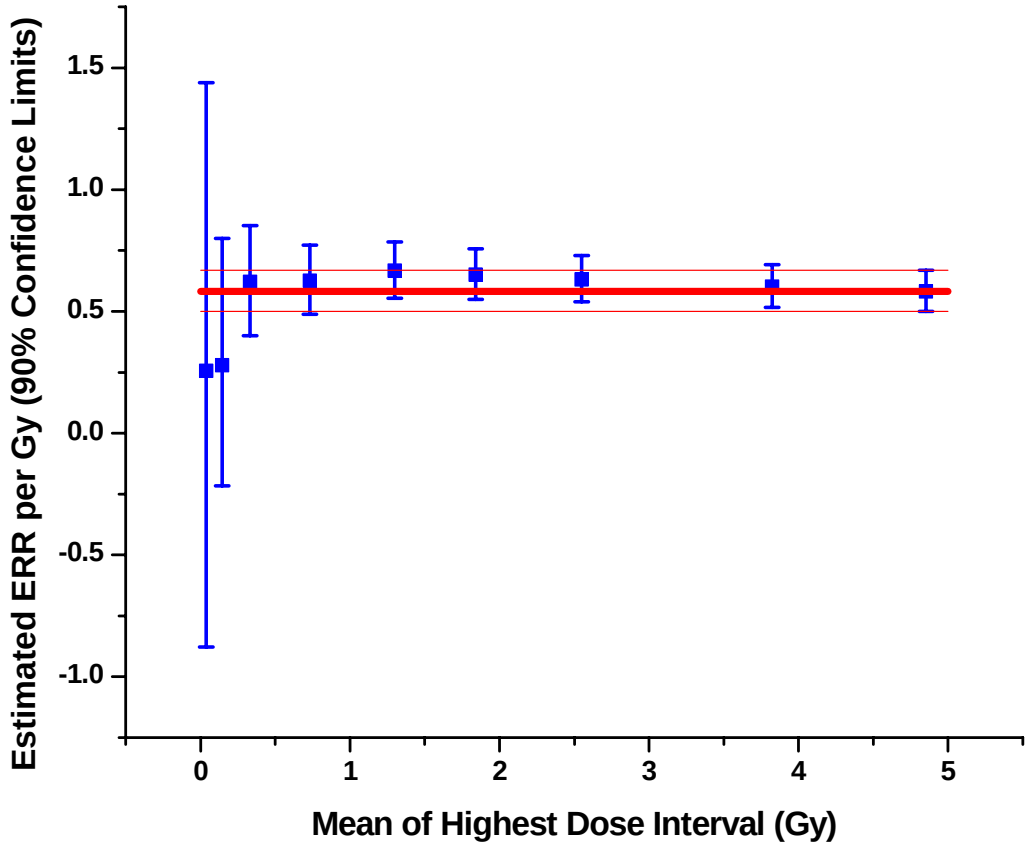
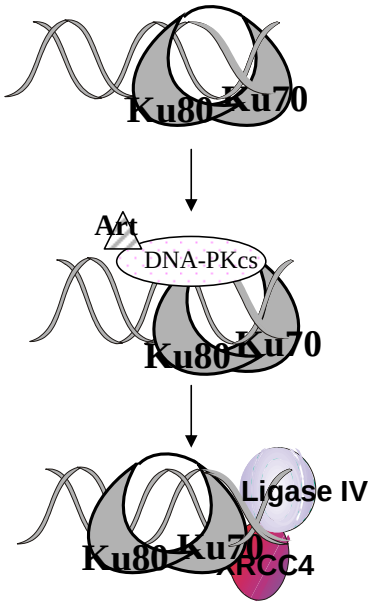


Figure 2.6. All-age linear regression estimates of ERR per Gy for female breast cancer assuming a 12-year minimum latent period, with dose-specific data trimmed from the right. Horizontal placement corresponds to the mean breast tissue dose for the highest neutron-weighted kerma interval included in the regression. Thus, the right-most point corresponds to the full dose range, the next point to the left to doses under 4 Gy, the next to doses under 3 Gy, and so on.

FIGURE 3.1



1) binding of Ku to the double stranded DNA end. The crystal structure of Ku shows that the DNA passes through a cavity in the structure with Ku encircling the DNA

(a single DNA end is shown for simplicity)

2) DNA-PKcs is recruited and the kinase activity activated. Autophosphorylation and phosphorylation of artemis likely ensues, potentially leading to release of DNA-PKcs Artemis nuclease activity may enhance processing of ends.

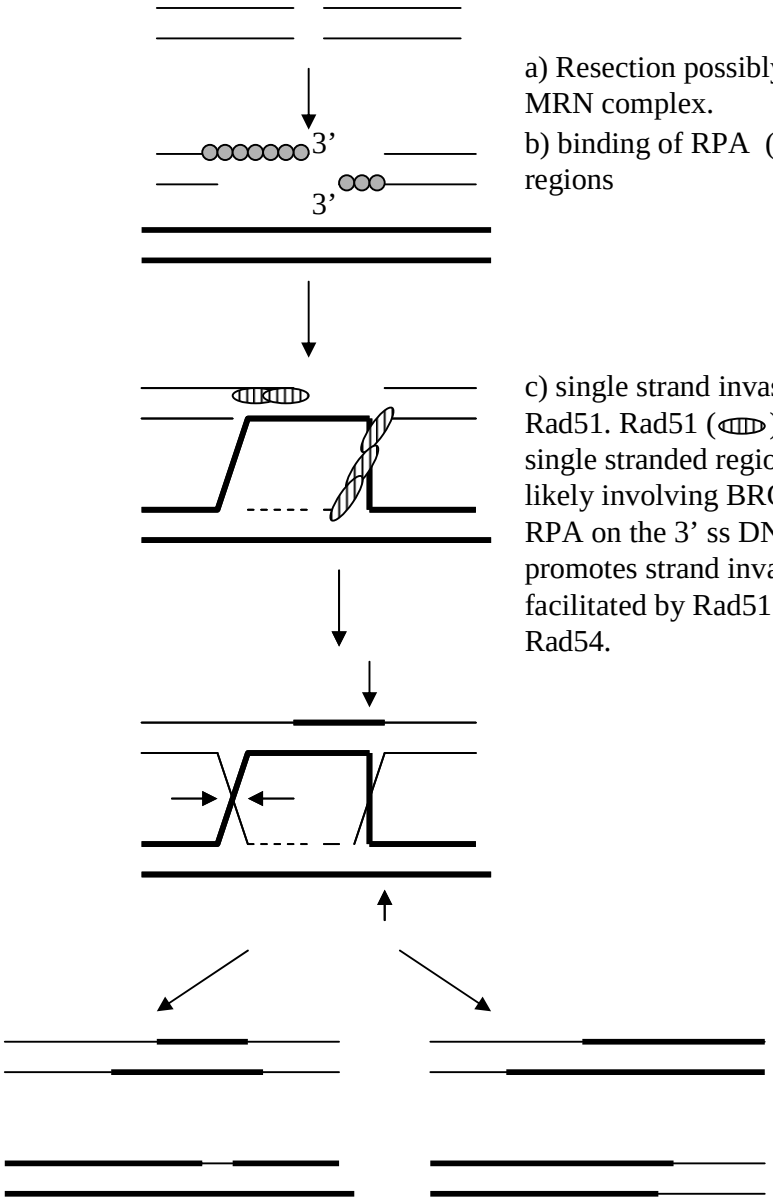
3) Ku enhances the recruitment of DNA ligase IV/XRCC4 complex and Ku translocates inwards to allow ligase IV/XRCC4 access to the DNA ends.

Note that only a single DNA end is shown for simplicity - one function of DNA-PKcs may be to enhance synapsis of the DN A ends.

Fig. 3.1. Model for DNA NHEJ.

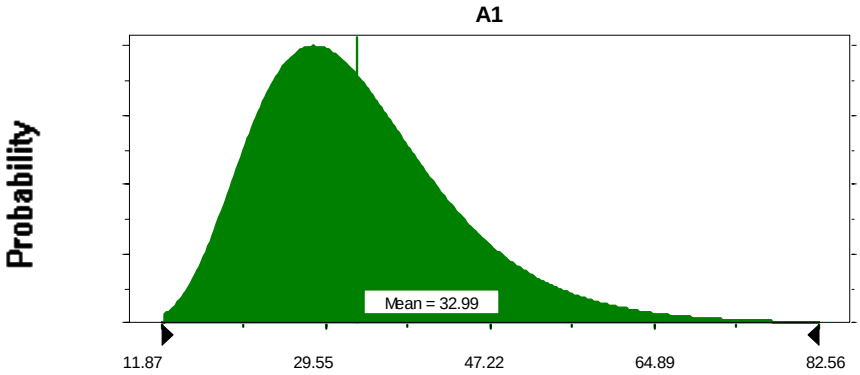
Proposed steps involved in the process

Figure 3.2. Depiction of homologous recombination.



a) Resection possibly carried out by the MRN complex.
b) binding of RPA (●) to 3’ ss regions

c) single strand invasion catalysed by Rad51. Rad51 (▭) is delivered to the single stranded regions via a process likely involving BRCA2. Rad51 replaces RPA on the 3’ ss DNA overhands and promotes strand invasion. This is facilitated by Rad51 paralogues and Rad54.



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10 Figure 6.1. Lognormal distribution with 90% confidence limits 18%-43%, representing
11 statistical uncertainty about percent cancer excess relative risk per Gy.
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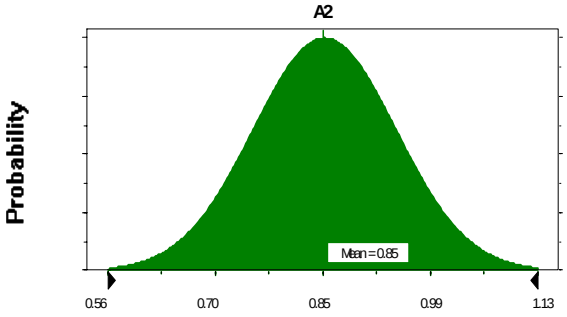

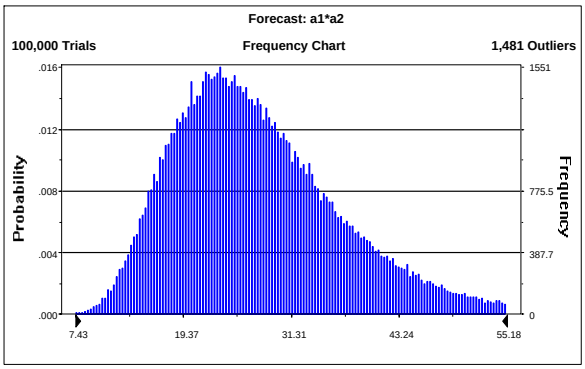

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21
22
23 Figure 6.2. Normal uncertainty
24 distribution for dosimetry bias correction
25 factor, with mean 0.84 and 90%
26 uncertainty limits 0.69-1.00.
27
28

Figure 6.3. Approximately lognormal uncertainty distribution for ERR per Gy corrected for dosimetry bias, with mean 0.26 and uncertainty limits 0.15 – 0.46.

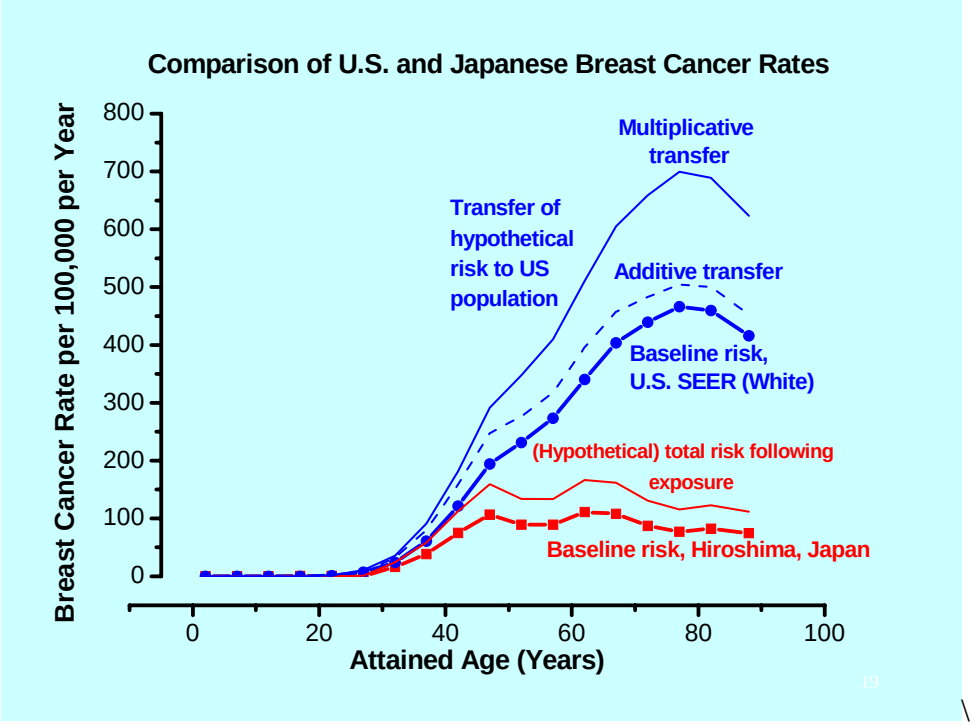

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Figure 6.4. Comparison of age-specific baseline rates for female breast cancer incidence in Japan and the United States (lower and upper polygonal lines with data points, respectively), estimated rate following a hypothetical radiation exposure of a Japanese population at age 15 (lower solid polygonal line without data points), and estimates for a U.S. population obtained by additive (dashed curve) and multiplicative transfer (upper solid curve) of estimated excess risk from the Japanese to the U.S. population.

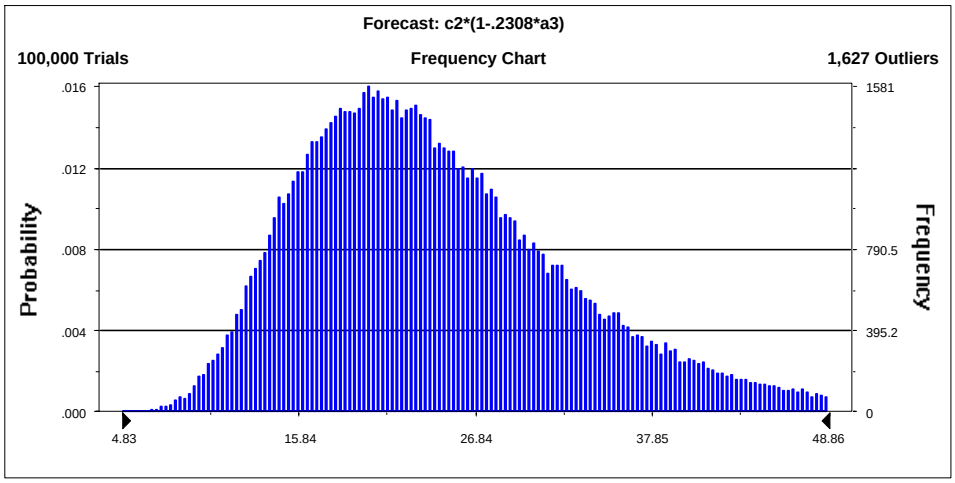


Figure 6.5. Monte Carlo simulation of the uncertainty distribution for cancer ERR (in percent) at 1 Gy, after transfer to a U.S. population: the simulated distribution is approximately lognormal with mean 0.25 and 90% probability limits 0.13-0.41.

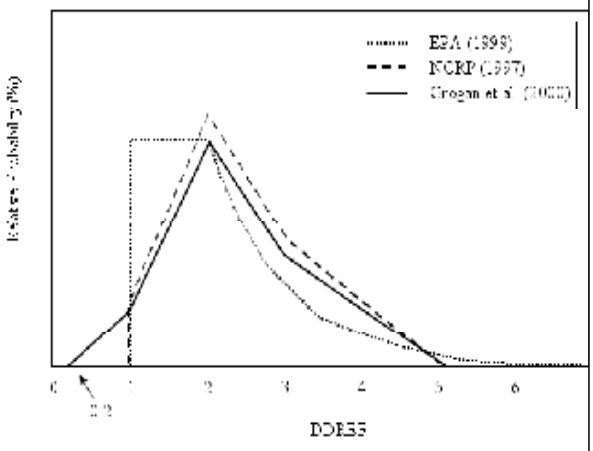


Figure 6.6. Continuous subjective uncertainty distributions for DDREF used in recent analyses.

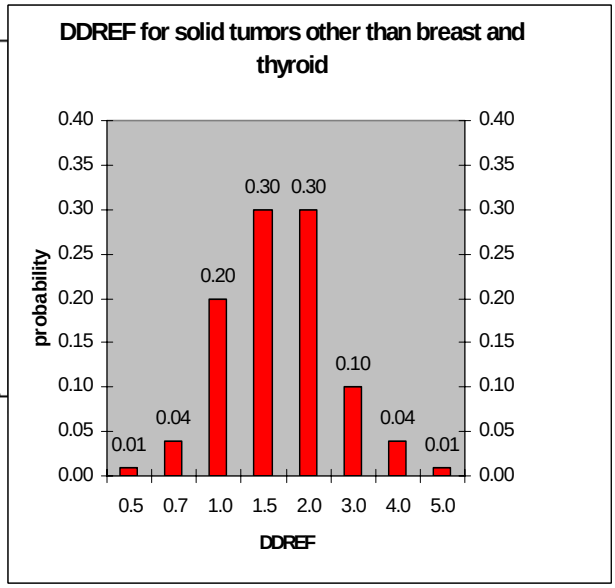
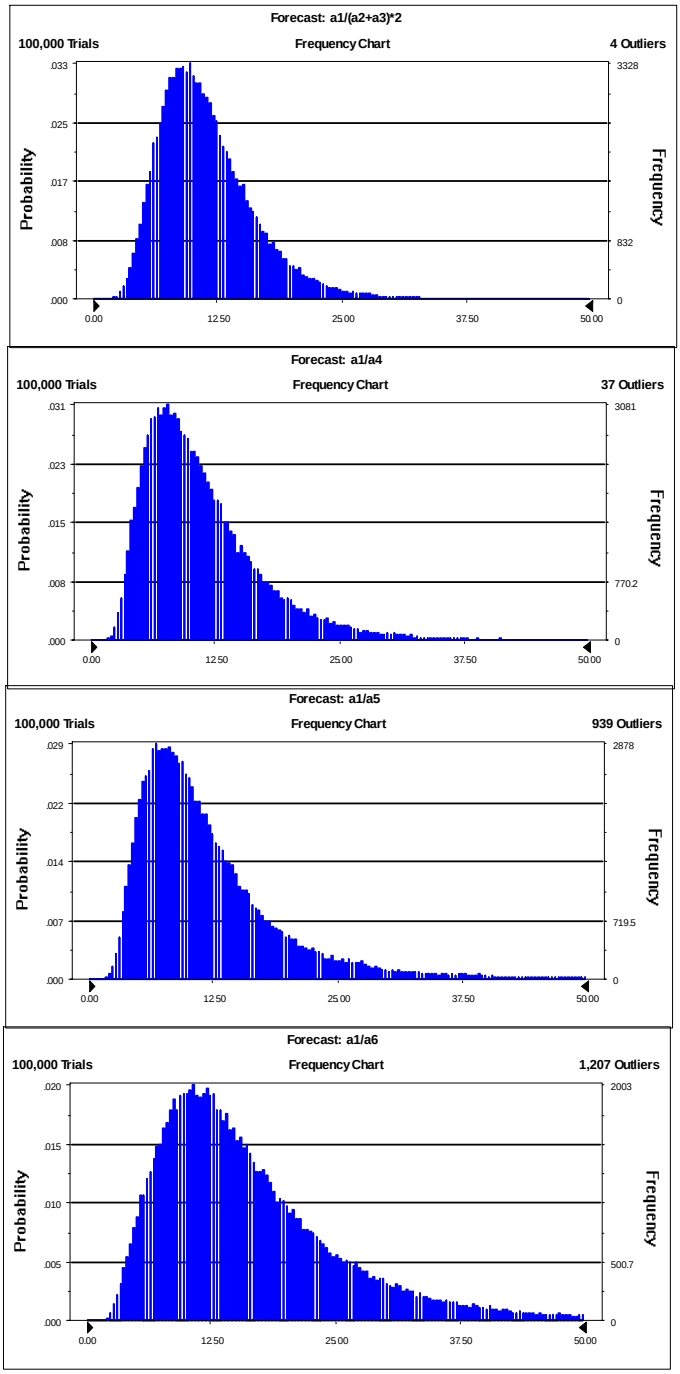


Figure 6.7. Discrete uncertainty Distribution for DDREF used in NCI/CDC (2003) analysis.

Figure 6.8. Influence of DDREF assumptions on uncertainty for ERR/Gy (in percent).

DDREF model (Source)	Uncertainty. for ERR/Gy: Mean	95% limit
EPA (1999)	12%	20%
NCRP (1997)	11%	27%
Grogan et al (2000)	12%	28%
NCI/CDC (2003)	17%	36%



ERR/Gy at low doses

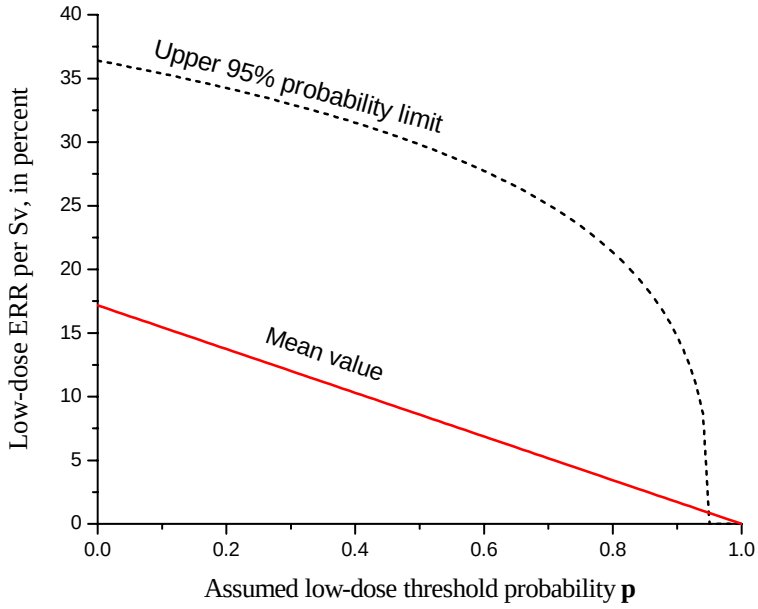
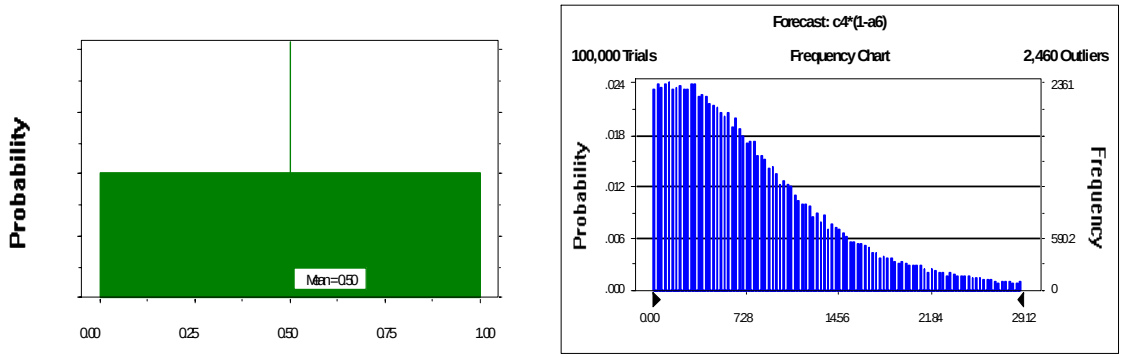


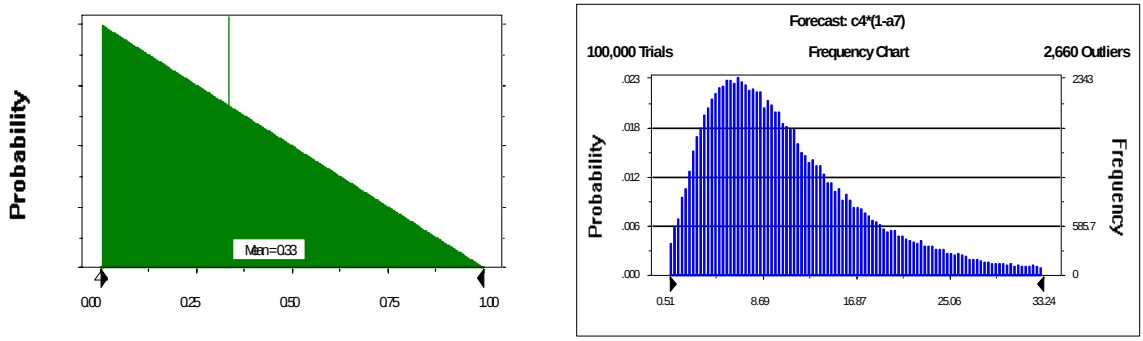
Figure 6.9. Mean and upper 95% probability limit for ERR/Gy as functions of threshold probability **p**, given (in the absence of a threshold) a lognormal uncertainty distribution with mean 0.17 and upper 95% limit 0.36.

Figure 6.10. Effect of uncertain threshold probability on the uncertain distribution for low-dose ERR/Gy.

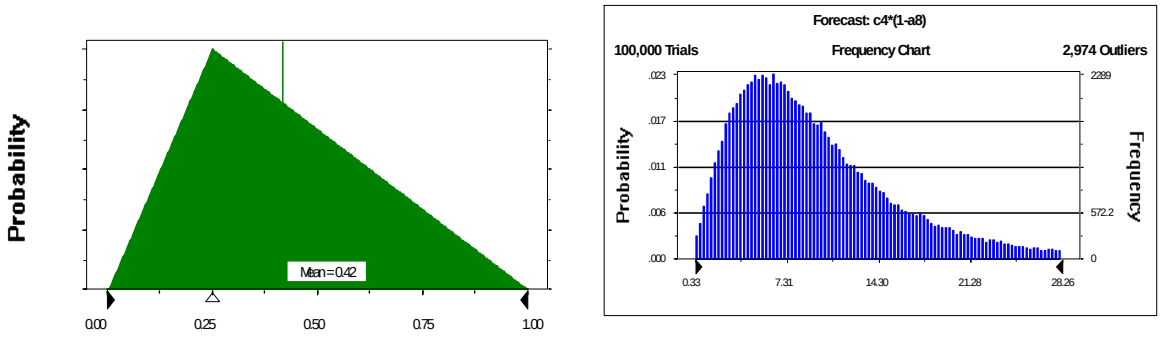
Assumed uncertainty distribution for *p* **Resulting distribution for ERR/Gy**



i) For $p \sim U(0,1)$, the mean ERR/Gy is 8.6% and the 95% upper limit is 23%.



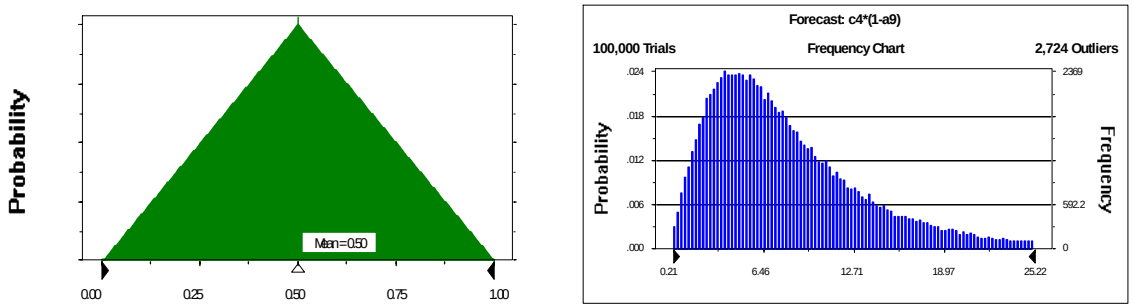
ii) For $p \sim T(0,0,1)$, the mean ERR/Gy is 11.5% and the 95% upper limit is 27%.



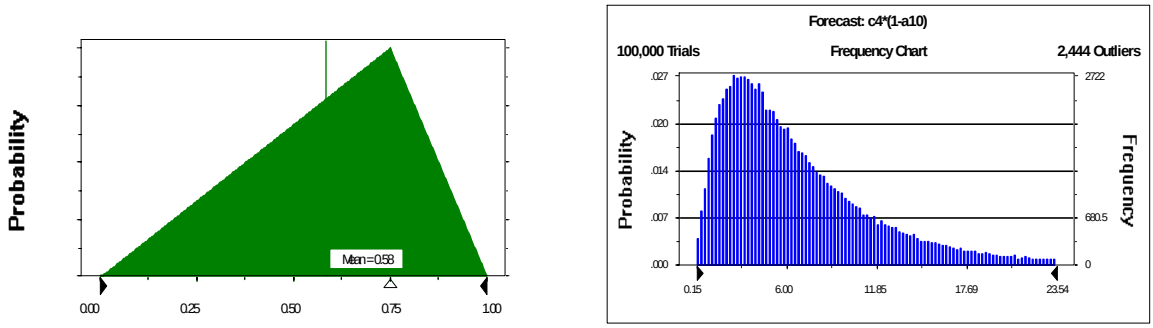
iii) For $p \sim T(0,0.25,1)$, the mean ERR/Gy is 10.4% and the 95% upper limit is 24%.

Figure 6.10 (continued). Effect of uncertain threshold probability on the uncertain distribution for low-dose ERR/Gy.

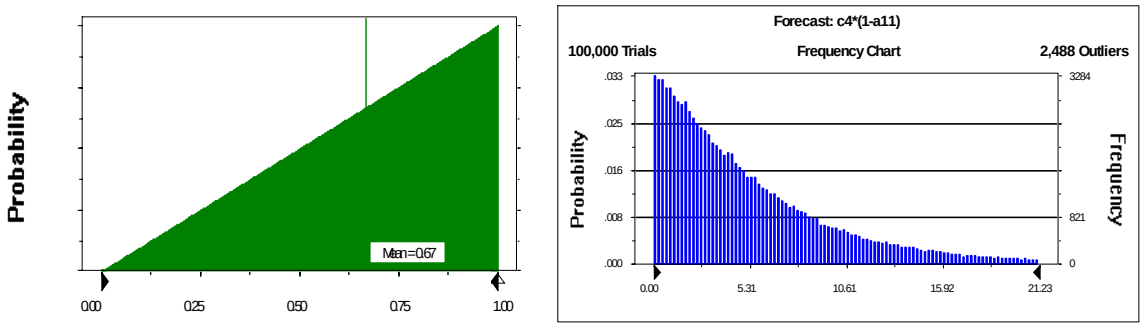
Assumed uncertainty distribution for *p* **Resulting distribution for ERR/Gy**



iv) For $p \sim T(0,0.5,1)$, the mean ERR/Gy is 8.6% and the 95% upper limit is 21%.



v) For $p \sim T(0,0.75,1)$, the mean ERR/Gy is 7.2% and the 95% upper limit is 19%.



vi) For $p \sim T(0,1,1)$, the mean ERR/Gy is 5.7% and the 95% upper limit is 17%.

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