

HEALTH EFFECTS OF LOW DOSES OF RADIATION: PERSPECTIVES ON INTEGRATING RADIATION BIOLOGY AND EPIDEMIOLOGY



National Council on Radiation Protection and Measurements



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October 27, 2015

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Preface

To gain a greater understanding of the biological interactions and health effects of low doses of ionizing radiation, the National Council on Radiation Protection and Measurements (NCRP) had increased its activities on this subject in past years. The NCRP 2008 Annual Meeting was entitled *Low Dose and Low Dose-Rate Radiation Effects and Models* (NCRP, 2009a). In December 2008 NCRP held a workshop that involved 30 participants with expertise in the effects of low doses of radiation. After the workshop summary was completed,¹ a decision was made to convene a panel of experts to produce an NCRP commentary related to critical issues and research needs for gaining a better understanding of effects of low doses of radiation. The advisory panel met in August 2010, and NCRP began the preparation of a commentary in 2012. This Commentary provides general perspectives on the integration of results of basic science studies, including biomarkers and bioindicators of cancer and other diseases, with epidemiologic studies on health effects of low doses of radiation. Such integration may contribute to reducing uncertainty on the types and levels of effects that occur after exposure to low doses of ionizing radiation (*i.e.*, the presence or absence of adverse outcomes, or the possibility of a benefit) and under what conditions the various effects may occur. The Committee members have expertise and experience in the following areas related to low doses of ionizing radiation: epidemiology, radiation biology, radiation oncology, biostatistics, health physics, and dosimetry.

This Commentary was prepared by Scientific Committee 1-21 on Multiplatform National Program for Providing Guidance on Integrating Basic Science and Epidemiological Studies on Low-Dose Radiation Biological and Health Effects. Serving on the Committee were:

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Executive Summary

For over 100 y, epidemiologic studies have assessed the health effects of ionizing radiation exposure from multiple sources: occupational, accidental, environmental, military and medical. These efforts have been successful in evaluating radiation-related adverse effects (particularly cancer) at or above ~100 mGy (absorbed dose). Since there are greater uncertainties inherent in epidemiologic studies of exposed individuals at lower doses, it is generally agreed that the effects observed at 100 mGy and above are more reliable than those observed at <100 mGy. Despite a history of over 70 y, the field of radiation risk estimation has not been able effectively to make direct use of radiation biology data from laboratory animal, cellular and molecular studies. Rather, such data have been used in a largely supportive role for the epidemiology-based risk estimates. The reasons for this are that there has been a reliance on the available human epidemiology as this was perceived to be the most relevant despite the identified uncertainties, and much of the available radiation biology data were not collected with their use in risk assessment in mind and so they are difficult to fully utilize in such an application.

The current state of knowledge on the effects of low-dose and low dose-rate radiation² remains uncertain with respect to understanding the shape of the dose-response relationship and the level of risk at low doses, despite the fact that significant information on the biochemical effects of low doses of radiation has been obtained over the past two decades. This uncertainty can impact actions taken with regard to radiation in areas such as radiation protection guidance, medical practice, compensation programs, environmental contamination issues, and communication to members of the public. Thus, there is a need to identify novel and innovative approaches to reduce these uncertainties.

The focus of this Commentary is on identifying further means to integrate results of basic science studies in radiation biology with epidemiologic studies on human health effects of low doses of radiation. In this regard, it is proposed that more extensive use be made of informative biomarkers of exposure and early cellular effects and bioindicators of adverse health outcomes (cancer and noncancer diseases). A *biomarker* is a biological phenotype [e.g., chromosome alteration, deoxyribonucleic acid (DNA) adduct, gene expression change, specific metabolite] that can be used to indicate a response at the cell or tissue level to an exposure. A *bioindicator* is a cellular alteration that is on a critical pathway to the disease endpoint itself (i.e., necessary, but not by itself sufficient for the endpoint), such as a specific mutation in a target cell that is associated with tumor formation. Towards this end, the Commentary reviews general approaches to risk assessment (Section 2), existing epidemiologic studies that have incorporated biological endpoints (Section 3), and areas of radiation biology with potential relevance to low-dose risk (Section 4).

Given major advances in our understanding of the etiology of diseases, host susceptibility, and the cellular processes affected by radiation, coupled with the rapid development of new technologies, there is a new opportunity to integrate information from multiple disciplines in risk assessment. For example, understanding of low-dose specific mechanisms based on radiation biology data should be incorporated where possible into the process of extrapolating

²For the purpose of this Commentary, for low linear-energy transfer (LET) radiation, a low absorbed dose is <100 mGy delivered acutely, and a low absorbed-dose rate is <5 mGy h⁻¹ for any accumulated dose (Section 1.1).

from epidemiologic data at higher doses to predict responses at low doses and low dose rates. The key to successfully doing this is to identify and select appropriate endpoints that inform the process of extrapolation of risk from the higher doses to low doses. Such biological endpoints utilized for extrapolation purposes need to be predictive of the apical endpoint for which risk is being estimated (*i.e.*, a bioindicator predictive specifically of radiation-related cancer or noncancer outcome). While some approaches have been suggested, an essential problem is the lack of bioindicators (key events) that are specific for radiogenic disease. It is not yet feasible to assess the robustness of key events in the radiation risk assessment process at low dose and dose rates because to date too little reliable information has been developed. However, the development of powerful new technologies that include genomic and proteomic analysis at the whole genome level and gene expression analysis coupled with computational approaches offers considerable encouragement to the use of key events in a risk assessment framework (Tollefsen *et al.*, 2014).

Biologically based dose-response (BBDR) models have been suggested as a way forward for low-dose risk estimation. However, developing such models requires a fuller understanding of the relevant biology than we currently have, as well as the collection of sufficient data for parameterization of the models. Awareness of such modeling needs should be integrated with experimental design, to ensure the collection of relevant data.

Radiation-induced genomic instability is one example of a specific biological response that may have relevance to modeling of low-dose radiation risk. It is important to determine if specific types of genomic instability are capable of transforming a cell from a normal to a cancer cell, or furthering the progression of a premalignant cell towards a more aggressive cancer phenotype. Chromosome instability has been shown to persist for as long as a year, but there is little information concerning how cells pass the unstable phenotype through succeeding somatic cell generations.

Another gap in our understanding of radiation risk is the role of individual variation in genetic susceptibility and interaction with radiation. Clinical studies following radiation therapy procedures over the years have identified genetic disorders and a few high penetrance alleles that convey increased risk of radiation-related cancer and other endpoints. Such alleles are often demonstrated in studies of second cancers, but the magnitude of effect is unknown and likely to vary. As each person is exposed to a different array of lifestyle and environmental factors in addition to any low-dose radiation exposure, the potential impact of interactions between these factors, genetic factors, and radiation exposure must also be considered.

The nascent field of systems biology, in which global information from multiple levels (*e.g.*, transcriptomics, proteomics and metabolomics) is integrated to develop a clearer picture of a process, for example, response to radiation, may also prove informative for risk prediction. A systems level understanding of radiation response could lead to a more comprehensive understanding of the mechanisms involved, and the interactions between mechanisms, thus supporting more robust biologically based models of risk.

The following proposals are made for closing the gaps towards an integrated approach of basic science studies in radiation biology with epidemiologic studies on health effects of low doses of radiation:

- focus on key events (*i.e.*, bioindicators) and modifying factors in adverse outcomes of ionizing radiation exposure, as outlined in the parallelogram approach presented in Section 1 [Figure 1.1 (for mechanistic studies) and Figure 1.3 (specific for bioindicators)], rather than simple biomarkers of exposure;
- develop BBDR models to provide a path forward in low-dose radiation risk assessment;
- explore the feasibility of registries of patients with exposure to medical-imaging procedures (particularly those associated with higher patient doses) and linked with



biological specimens and outcome data, in order to provide for standardized research planning and data collection for prospective risk assessment;

- maintain and develop the specialized facilities required for studies conducted at low doses and low dose rates; and
- promote and expand interdisciplinary training and integrated cross-professional research programs devoted to understanding and quantifying radiation health effects at low doses in order to meet the growing needs of radiation science for the next generation of radiation researchers, and for communication with policymakers and members of the public.

1. Introduction

We live in an era of increased exposure to ionizing radiation, from the proliferation of its use in diagnostic and interventional medical procedures to its nonmedical use for security screening. With the increase in electrical generation by nuclear power, there is a continuing concern surrounding the public exposures that may result from accidents, as dramatically illustrated by the 2011 Fukushima-Daiichi disaster. In such an environment, meaningful risk assessment for low-dose and low dose-rate ionizing radiation exposures is more critical than ever for the improvement of radiation protection, medical practice, environmental protection and remediation, compensation, and communication to members of the public (Locke, 2009; 2011).

Much of the population exposure to increased levels of radiation consists of sparsely ionizing types, such as gamma rays and x rays. Hence, elucidating the health effects of these radiations is of highest concern to members of the public. A fundamental issue in deriving risks at low levels of dose and dose rate is the shape of the dose response for endpoints relevant to human health outcomes and possible modifications by dose rate. This Commentary outlines a framework to integrate data from biological studies with epidemiologic data to illuminate this issue. This framework includes a parallelogram approach for the biological mechanistic studies (Section 1.1) and made more specific by the use of bioindicators of response (Section 1.2). The bioindicators proposed are developed from an adverse outcome pathway and key events approach (Preston, 2015). An auxiliary contributor is the field of microdosimetry and track structure analysis which can give insights into radiation interaction and energy transport. That topic is not covered further in this Commentary, but it supplements the biological and epidemiologic studies by providing a fuller understanding of energy deposition by ionizing radiation in small volumes (*e.g.*, various components of cells).

While epidemiologic studies remain the principal source of information for determining risk and setting exposure limits, statistical considerations (*e.g.*, population size, statistical power) limit the usefulness of such studies in the low-dose range. In recent years, however, considerable progress has been made in low-dose radiation biology at the cellular and molecular levels. Ideally, such work should contribute to our understanding of risk and should help shape radiation protection guidance. In order to effectively integrate the results of radiobiology studies into epidemiology and risk analysis, it is necessary to make multiple comparisons, from cell and molecular studies to *in vivo* studies, and from animal to human on both the cell and molecular level, and on the *in vivo* level.

Conceptually, this approach has been used frequently for predicting outcomes in humans from data collected from cellular and laboratory animal systems. It is generally referred to as a parallelogram approach and has been used frequently in toxicological research, leading to enhanced risk assessments. A generalized scheme for the use of this so-called parallelogram approach is illustrated in Figure 1.1. In Figure 1.1, “experimental” is used for data that are obtained directly from experiments and “calculated” when extrapolation is conducted using ratios (*e.g.*, *in vitro* to *in vivo* or laboratory animal to human) that are derived from the available experimental data. There are many examples of this use over the past 40 y but the following are generally representative: Brewen *et al.* (1973), Edwards and Preston (2008), Kienhuis *et al.* (2009), Preston (2005), and Sobels (1989).

Studies with biological models that more closely approximate tissue architecture are of particular use as a bridge between simple cellular studies *in vitro* and organismal studies

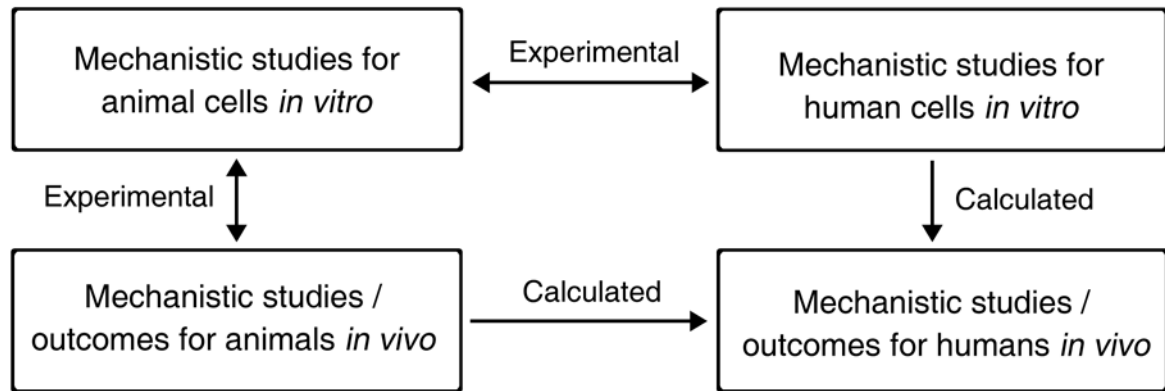


Fig. 1.1. A parallelogram approach for utilizing laboratory animal and *in vitro* cell data to estimate human cancer and noncancer risks for ionizing radiation.

(Bissell *et al.*, 1997). Application of extrapolation to the assessment of human risk has been reviewed in the National Council on Radiation Protection and Measurements (NCRP) Report No. 150 (NCRP, 2005), which concluded that an understanding of the molecular mechanisms of radiation carcinogenesis in different animal species was needed for the most robust extrapolation. Although species-specific differences in critical biological processes need to be kept in mind, a focus on establishing which mechanisms are conserved between models will be useful when making intercomparisons to inform risk in humans.

The major goal of this Commentary is to develop a framework to support the useful incorporation of biological endpoints into epidemiologic studies in order to enhance the understanding of the risks of low-dose and low dose-rate ionizing radiation exposures.

Towards this end, this Commentary reviews general approaches to risk assessment, areas of radiation biology with potential relevance to low-dose risk, and existing epidemiologic studies that have incorporated biological endpoints. The Commentary suggests that focusing on key events and modifying factors for adverse outcomes pathways from ionizing radiation exposure, rather than simple biomarkers of exposure, in concert with the development of BBDR models will provide the best path forward in low-dose radiation risk assessment. These approaches and others are identified in Section 5 and may better enable the integration of basic biology and epidemiologic studies for the advancement of low-dose ionizing radiation risk assessment and radiation protection. In this Commentary, risk assessment for exposure to low-dose radiation includes observing the presence or absence of an adverse outcome, or observing a benefit.

1.1 Low Doses and Low Dose Rates

Studies have been conducted over a broad range of radiation doses and dose rates referred to as low, and what constitutes a low dose and a low dose rate is often due to the context in which dose and dose rate are being considered (*e.g.*, radiobiological research, epidemiologic studies, medical exposure, environmental exposure). For the purposes of this Commentary, the context is levels from radiation sources commonly encountered by individuals in the population (*e.g.*, in the controlled occupational setting, from controlled environmental sources; from ubiquitous natural background sources; from diagnostic and interventional medical procedures). This excludes situations such as radiation therapy in medicine and severe accidental or intentional incidents. Criteria for low dose and low dose rate that include the levels experienced from the common sources are useful for general discussion. It is recognized that

the doses and dose rates from the common sources vary over orders of magnitude, and differences in radiation response over such a large range are yet to be fully understood in terms of mechanisms of radiation action, given our current state of knowledge. The intent here is to identify criteria that include these doses and dose rates, and that are linked to the current understanding of when the effectiveness of dose and dose rate is likely to be less than at higher doses and dose rates.

In assessing human health effects (in particular cancer), the International Commission on Radiological Protection (ICRP, 1991) applied a dose and dose-rate effectiveness factor (DDREF) of two to obtain risk estimates for absorbed doses <200 mGy and from higher doses when the dose rate is <100 mGy h^{-1} . The U.K. National Radiological Protection Board (NRPB) concluded that for the purposes of applying a dose-rate effectiveness factor in radiation protection, dose rates of <0.1 mGy min^{-1} (when averaged over ~ 1 h) and acute doses of <100 mGy may be regarded as low (Muirhead *et al.*, 1993). This dose rate is equivalent to 6 mGy h^{-1} . Wakeford and Tawn (2010) more recently agreed with the NRPB guidance and suggested that a low dose is <100 mGy delivered acutely and a low dose rate is <5 mGy h^{-1} for any accumulated dose. The rationale for selecting such dose and dose-rate values appears to be driven by the desire to link them to what is believed to be the linear portion of a linear-quadratic dose-response relationship (Wakeford and Tawn, 2010). For the purpose of this Commentary, for low linear-energy transfer (LET) radiation, a low absorbed dose is <100 mGy delivered acutely, and a low absorbed-dose rate is <5 mGy h^{-1} for any accumulated dose.

1.2 Use of Radiation Biology Data to Reduce the Uncertainty in Risk Estimates

There is extensive literature on the health effects of radiation exposures (occupational, accidental, environmental and medical) in human populations. Many of these involve relatively high acute doses, although statistical associations at lower doses (around a few tens of milligray) and lower dose rates have been reported. Given the greater uncertainties inherent in epidemiologic studies of exposed individuals at low doses than at higher doses, it is generally agreed that effects observed at ~ 100 mGy and above are more reliable than those observed at <100 mGy. In this regard, despite a history of over 70 y, the field of radiation risk estimation has not effectively made direct use of radiation biology data from laboratory animal, cellular and molecular studies. Rather such data have been used in a largely supportive role for the epidemiology-based risk estimates. The reasons for this are that there has been a reliance on the available human epidemiology as this was perceived to be the most relevant despite the identified uncertainties (*e.g.*, observational studies with uncontrolled confounding factors) and much of the available radiation biology data were not collected with their use in risk assessment in mind and so they are difficult to fully utilize in such an application.

Risk estimates for radiation-related cancer and noncancer effects at low doses (chronic and acute) have relied almost exclusively on epidemiologic data. Exceptions are for the estimation of DDREF and relative biological effectiveness of high-LET radiation that have relied quite significantly on data from biological studies, although also supported by some but not all epidemiologic data. Given the major advances in understanding the etiology of chronic diseases (especially cancer, although with an increasing emphasis on certain noncancer diseases) and of the fundamental cellular processes affected uniquely by low-dose and low dose-rate radiation (both targeted and nontargeted), it is prudent to consider how this information can be incorporated into the risk assessment process. In particular, it is critical that the use of data and mechanistic insight from biological studies be incorporated into the process for extrapolating from epidemiologic data at medium or high doses to predict responses at low doses and low dose rates (Section 1.1). Given these goals, two issues to be addressed herein are:

- how can informative endpoints be selected that can enhance the process of extrapolation of “risk” from high or medium to low doses; and
- how can data from laboratory animal, cellular and molecular studies best be integrated with epidemiologic studies to better predict risk and account for unique processes occurring after low-dose but not high-dose exposures?

Assistance with the task of estimating the health effects of radiation exposures at low doses and low dose rates can be found in the approach being developed for estimating risks from exposures to chemicals (*e.g.*, Boobis *et al.*, 2006; EPA, 2005; Julien *et al.*, 2009; Preston, 2015; Seed *et al.*, 2005; Thomas *et al.*, 2012; Vinken, 2013). The use of mechanistic and other biological data is necessary for the risk assessment process for chemicals because of the lack of epidemiologic data for almost all chemicals (environmental or occupational exposure). The process is built upon a framework of adverse outcome pathways and key events for the development of adverse health effects following exposure to a chemical or chemical mixture. An adverse outcome pathway for both cancer and noncancer endpoints is defined as a sequence of key events and processes starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in formation of an adverse outcome (EPA, 2005). It should be noted that this adverse outcome pathway and key event approach has not been used for an entire risk assessment process for any chemical. A prototype example has recently been developed by Adeleye *et al.* (2015). For radiation, this adverse outcome pathway and key events approach will be enhanced because, in fact, epidemiologic data are available that allow for integration with the biology.

For ionizing radiation, for example, cancer is considered to be produced by a DNA-reactive mode of action. A *key event* is defined as an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element (EPA, 2005). Preston (2015) has recently presented a proposed adverse outcome pathway and associated key events for low doses and low dose rates of ionizing radiation. It is more difficult to utilize such a general approach for noncancer effects given the wide range of mechanisms whereby adverse outcomes (*e.g.*, neurological, reproductive, cardiovascular) can be produced. However, an initial approach is described by Seed *et al.* (2005) for a range of chemical classes and noncancer endpoints.

Preston and Williams (2005) developed a set of key events by which a DNA-reactive chemical can produce a metastatic cancer. A modified version of this scheme can be considered as being applicable to ionizing radiation. As a starting point for the development of an adverse outcome pathway for radiation-related cancer, an initial key event is considered to be a DNA double-strand break (DSB), if in turn it can be converted into a mutation. The set of key events in Figure 1.2 is a progression from the initial key event to a malignant tumor (*i.e.*, an adverse outcome pathway) (Preston, 2015). It is possible to expand this approach to include radiation effects on preneoplastic lesions that may have developed prior to exposure. Another approach considers radiation effects on any of the critical processes that are considered hallmarks of cancer development (Boss *et al.*, 2014; Hanahan and Weinberg, 2011).

For the purpose of maximizing the use of biological data in the dose-response assessment for adverse health outcomes, it is informative to distinguish between biomarkers and bioindicators. A *biomarker* is a biological phenotype (*e.g.*, chromosome alteration, DNA adduct, gene expression change, specific metabolite) that can be used to indicate a response to an exposure at the cell or tissue level. In this regard, it is generally a measure of the potential for development of an adverse outcome such as cancer (*e.g.*, a predictor of exposure level). A *bioindicator* is defined as a cellular alteration that is on a critical pathway to the disease endpoint itself (*i.e.*, necessary, but not by itself sufficient for the endpoint), such as a specific mutation in a target cell that is associated with tumor formation. Thus, a bioindicator can be perceived as



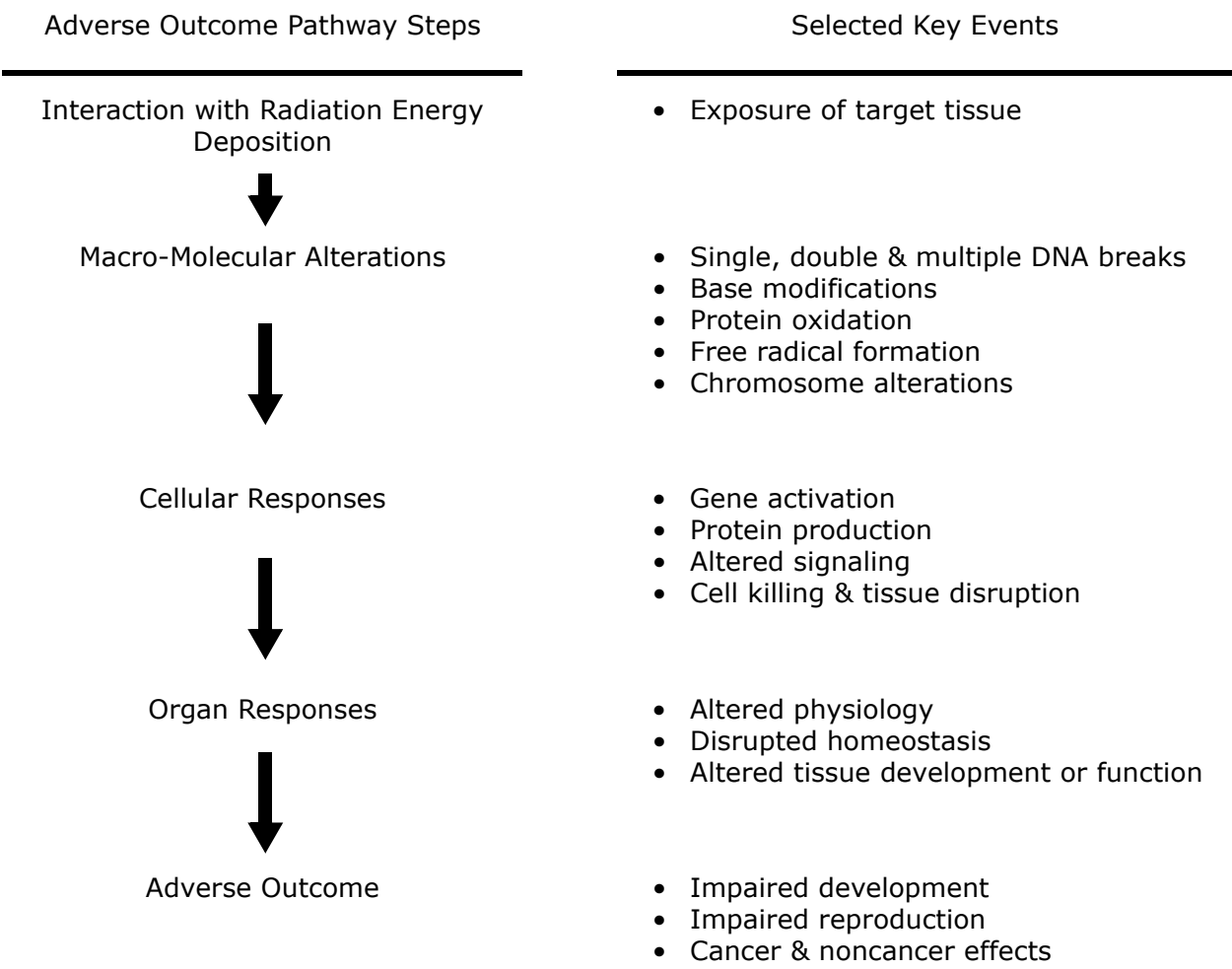


Fig. 1.2. Schematic representation of an adverse outcome pathway leading to cancer or noncancer diseases, showing each step along the proposed pathway and a selection of the possible associated key events for ionizing radiation exposures (Preston, 2015).

informing on the shape of a dose-response curve for the disease outcome or on cancer frequency itself, and therefore, is equivalent to a key event. As such a bioindicator is not a potential confounder, and should not be adjusted for in the analysis. Nor is it usually an effect modifier. In a logical way, the more proximate a key event (bioindicator) is to the apical endpoint (e.g., cancer), the more predictive of this outcome it will be.

Thus, for the purposes of risk assessment, it is most pertinent to design research studies to assess bioindicators of response rather than simply biomarkers of an exposure. In addition, it is essential to describe the predicted human relevance of key events (bioindicators) developed in nonhuman assay systems (Boobis *et al.*, 2006). Figure 1.1 can be modified (Figure 1.3) to show how bioindicators can be used as the data input for a typical parallelogram approach (Sobel, 1993). In this approach, bioindicators determined in laboratory-animal studies *in vivo*, and in human and laboratory-animal cellular studies *in vitro* or *in vivo*, are used for extrapolation to the box in the bottom right hand corner (bioindicators in humans *in vivo*) (Figure 1.3) for integration with human data on bioindicators or from epidemiology. The types of informative bioindicators for cancer risk assessment include: cancer-specific (target tissue) chromosome alterations and gene mutations, target tissue cell killing and regenerative cell

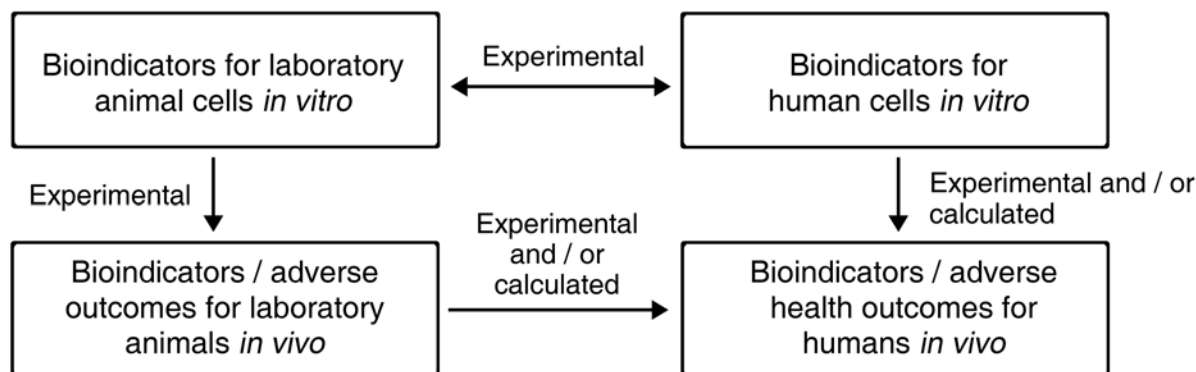


Fig. 1.3. Modified Figure 1.1, showing how bioindicators can be used as the data input for a parallel approach (Sobels, 1993).

proliferation, and target tissue inflammatory responses. Genovese *et al.* (2014) has provided data that suggest that “A subset of the genes that are mutated in patients with myeloid cancers is frequently mutated in apparently healthy persons; these mutations may represent characteristic early events in the development of hematologic cancers.” This type of DNA sequencing approach can be extended to investigate other cancer types.

Key events provide the critical input for dose-response modeling; so-called associated events or modulating factors can influence the nature of the overall response for both cancer and noncancer outcomes. Consideration of such events and factors is particularly important for quantitative assessments of dose response.

A modulating factor can be considered as a biological feature, including control mechanisms or host factors that can modulate the dose-response relationship of one or more key events, thus altering the probability or magnitude of the outcome. As such, some modulating factors might be considered as “inevitable” (fixed) because all humans are subject to their influence; examples are sex, age and race. Other modulating factors, for example, are more aptly described as “optional” (controllable): these include smoking, diet and occupation. Yet other modulators are acquired (*e.g.*, disease state). It is relatively straightforward to incorporate the inevitable modulators into a risk assessment process but much more problematic to adequately account for the optional or acquired ones without considering individual risk values. Laboratory animal and cellular and molecular data might be developed that can account for the magnitude of the influence of modulating factors on risk but currently this is very rarely done and frequently it is not possible to do.

An associative event is a biological process that is not itself a key event but is a reliable biomarker for a key event(s). Thus, an associative event can be a biomarker for exposure or closely linked to the mechanism of disease formation (*e.g.*, nonspecific chromosome alterations and mutations, nonspecific gene expression changes). Recent research has identified two novel classes of events, epigenetic changes and nontargeted events [*e.g.*, bystander effects (Section 4.6.2) and genomic instability (Section 4.5)] that are sometimes considered as associative events. However, at present it is not clear whether epigenetic changes are associative, but based on the evidence to date it appears unlikely that nontargeted events are associative, since not all radiation exposures are associated with instability in human subjects (Tawn *et al.*, 2000; 2011; 2015; Whitehouse and Tawn, 2001). It should be noted that nontargeted effects might provide an explanation for the development of degenerative conditions that are associated with the development of chronic inflammation.

To fully use such key event (bioindicator) data to inform dose-response assessment for adverse health effects, it is necessary to utilize or develop some form of a BBDR model.

Although several approaches have been described that suggest the viability of this type of modeling (Eidemuller *et al.*, 2011; Kaiser *et al.*, 2014; Little, 2010; Luebeck *et al.*, 2013; Moolgavkar and Knudson, 1981; Moolgavkar and Luebeck, 1992; Moolgavkar and Venzon, 1979; Shuryak *et al.*, 2010), these approaches may be of limited value for reliably predicting cancer risk because they do not incorporate any biological data other than the generalized mutational evidence supporting the multistage model. Some recent approaches have perhaps provided a greater reliance on auxiliary biological data (Heidenreich and Rosemann, 2012; Heidenreich *et al.*, 2013). The further development of truly informative models along these lines has to be a research priority given the importance of reliable estimates of radiation-related health effects at low doses and low dose rates.

The discussion herein describes an approach for integrating data from radiation biology and epidemiology in the dose-response assessment phase of risk assessment for radiation-related cancer and noncancer effects (Morgan and Bair, 2013). It proposes the development and application of mode of action and key events (bioindicators) as providing a link between epidemiology and radiation biology data at the animal, cell and molecular levels. For example, emerging large-scale and high-quality epidemiologic studies that focus on chronic low-dose radiation, such as the Million Person Study of Low-Dose Radiation Health Effects (Boice, 2012; 2014a; Bouville *et al.*, 2015), might conceivably be used in conjunction with the key-event approach to reduce overall uncertainty in low-dose and low dose-rate cancer and non-cancer risk estimates (Preston, 2015).

2. General Approaches to Risk Assessment

2.1 Risk Assessment Process and Associated Uncertainties

Risk estimates for radiation-related cancer mortality and incidence and those for noncancer diseases can play a critical role in the development of dose limits used for radiation protection purposes. A recent example of the use of risk estimates for cancer is provided by the approach for establishing the standards used by the National Aeronautics and Space Administration for limits on days in space for astronauts (Cucinotta *et al.*, 2013; NA/NRC, 2012a). The estimates of radiation risk currently used by international and national bodies such as ICRP, NCRP, the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), the National Academies/National Research Council (NA/NRC), and the U.S. Environmental Protection Agency (EPA) rely heavily upon epidemiologic data on cancer and noncancer diseases in a variety of exposed populations (particularly atomic-bomb survivors and people with estimated or known doses from occupational, medical and environmental exposures).

The general approach to risk assessment is to extrapolate from health effects measured at medium to high doses to predict those expected at low doses. Currently, for cancer the extrapolation by international and national bodies typically utilizes a linear-nonthreshold model (ICRP, 2007; NA/NRC, 2006; NCRP, 2001). The estimates obtained in this way are corrected for a DDREF to allow for calculated reductions in effect from high to low dose and from high to low dose rate. There are challenges to this approach largely because of the reliance on the linear-nonthreshold model and the uncertainty in a chosen value for DDREF. For noncancer diseases, the approach employed by ICRP (2007; 2012) is to calculate a dose for a practical threshold level of disease response (<1 % chance of occurrence) (ICRP, 2012) based on effects at medium to high doses. The appropriateness of this approach has been discussed recently in light of new data on cataracts and cardiovascular disease that addresses the general use of threshold models for noncancer diseases (ICRP, 2012). Clearly, the issue requires further consideration and the development of additional informative data.

Because of the reliance on epidemiologic data, it is important to utilize the most recent and relevant high-quality data for developing risk estimates and to take advantage of enhancements in computational modeling approaches. However, at the same time, it is essential to appreciate that any approach used will inevitably have associated uncertainties that should be considered when applying risk estimates for protection purposes. NCRP Report No. 171 (NCRP, 2012) considers the types and magnitude of the several uncertainties, including those associated with epidemiologic study design and conduct, that are a component of the risk assessment process for cancer, noncancer effects, and heritable effects following radiation exposure. Consideration of this issue is timely because new data have recently become available for cancer incidence, occurrence of effects other than cancer (particularly for cataracts and cardiovascular disease) and heritable effects (ICRP, 2007; 2012; UNSCEAR, 2001). NCRP (2012) builds upon the analyses in other recent reports (NCRP, 2007; 2009b; 2009c) of the sources and magnitude of uncertainties in the estimation of organ doses from exposure to external and internal sources of radiation.

Given the heavy reliance on epidemiologic studies, a significant component of uncertainty associated with these risk estimates will be accounted for by comprehensive assessments of

uncertainties in these epidemiological studies. It is of note that little direct use has been made of the extensive data from ~70 y of radiation biology study in estimating radiation-related health risks (ICRP, 2007; NA/NRC, 2006; NCRP, 2005); the data have generally provided support for the epidemiology-based approach currently used. The most significant uses of laboratory animal and cellular data have been for developing a value for DDREF and values for quality factors and radiation weighting factors used in radiation protection. However, even with the use of these experimental data, uncertainties associated with these factors are large, and contribute significantly to the overall uncertainty in risk estimates (Cucinotta *et al.*, 2013). Sections 2.2 through 2.4 consider the types of uncertainty, their magnitude and potential approaches for reducing these uncertainties.

2.2 Modeling Uncertainties: Dosimetric, Epidemiologic and Biological

Epidemiology is by its nature observational rather than experimental. As discussed in NCRP (2012) and UNSCEAR (2008a), the precision of epidemiologic risk estimates is driven primarily by the:

- range and distribution of doses;
- sample size;
- duration of and ages at observation;
- baseline frequencies of the health endpoint of interest;
- “true” strength of the radiation-disease association;
- various types of dose uncertainties; and
- degree of accuracy of ascertainment (or, conversely, misclassification or under ascertainment) of the disease of interest.

However, the degree of systematic errors (*i.e.*, bias) of the epidemiologic risk estimates depends on a different set of factors, such as:

- systematic personal reporting errors (with respect to dose-related items, disease experience, or risk-factor covariates), in particular errors that may result from:
 - selection or differential ascertainment effects (resulting in selection or ascertainment bias),
 - recall bias, or
 - follow-up bias (resulting from differential loss to follow-up);
- insufficient statistical adjustment for other risk factors;
- dose-related inequalities in disease ascertainment;
- errors in assigning average values for shared dosimetry factors (*e.g.*, an incorrect estimate of the magnitude of a radioactive release);
- failure to correct for individual measurement errors; or
- failure to adjust for the effects of disease-related covariates.

The importance of these sources of uncertainty varies from study to study. Uncertainties and biases resulting from design and methodologic issues in epidemiologic studies have been considered by a number of scientific committees (NCRP, 2012; UNSCEAR, 2008a). Of special importance are biases, that is, any process at any stage in the conduct of the study that tends to produce results or conclusions that differ systematically from the truth (Sackett, 1979); these biases include many of the uncertainty factors listed above.

Statistical methods to deal with the complexities of measurement error corrections are still evolving (Carroll *et al.*, 2006). A new generation of epidemiologic studies that incorporate

biological measures for estimating radiation risk will help sharpen assessments and correct for some uncertainties and biases.

2.3 Transfer of Risk Between Populations of Different Countries

One can estimate the risk in the U.S. population or some other target population in a number of different ways. Assuming that the source data on radiation risk is the Japanese atomic-bomb survivor cohort, one can estimate risks by applying the excess relative risk coefficient derived from the Life Span Study (LSS) cohort and using the baseline in the United States to derive the excess risk in the U.S. cohort. This is often called “purely multiplicative projection” of risk from the Japanese to the U.S. population. One can also estimate the risk to the U.S. population by using the excess absolute risk derived from the Japanese cohort and applying this directly to estimate the excess cancer risk in the U.S. population. This is often called “purely additive projection” of risk from the Japanese to the U.S. population. These two risk estimates for any source and target population will have different values. It is often thought that the uncertainty in the transfer of risk to the target population can be defined as the range given by the purely multiplicative and purely additive risk projections (Berrington de Gonzalez *et al.*, 2012; Kocher *et al.*, 2008; Land *et al.*, 2003), and the central estimate may be taken as some weighted average of the two. Another challenge is evaluating how representative the Japanese population exposed acutely in 1945 is to healthy populations today receiving chronic exposures over time. The factors associated with living in a war-torn country with issues of malnutrition, infections and deprivation may be important in generalizing results to other populations.

Despite the relatively extensive data on radiation risk, the question of how to apply risk estimates derived from a population of one country to a population of a different country remains unanswered. It should not be surprising that the relationship between radiation-related and underlying risk in populations of different countries is not consistent for different cancer sites. The available data suggest that there is no simple solution to the problem (Muirhead *et al.*, 1993; UNSCEAR, 1994).

The available information suggests that, depending on circumstances, additive or multiplicative models for transfer of risk between populations of different countries, or indeed the use of a hybrid approach, such as that employed by Little *et al.* (1999a) and Muirhead and Darby (1987), may be appropriate. The principal scientific committees implicitly assume that risk transfer is intermediate between additive and multiplicative (ICRP, 2007; NA/NRC, 2006). The subject is discussed in NCRP (2012), highlighting the complexity of the evidence (*e.g.*, for breast cancer a more nearly additive transfer was suggested, whereas for liver cancer multiplicative risk transfer was more likely to be valid).

When considering the issue of how to transfer site-specific cancer rates from one population or exposed group to another, it is necessary to also consider the influence of factors other than radiation on the cancer rates. Thus, much of environmental, nutritional and occupational cancer epidemiology is concerned with identifying risk factors that might account for some part of the variation of site-specific underlying cancer rates among populations. While there has been much progress, the problem is complex and there is only limited information on the interaction between radiation dose and lifestyle, or constitutional factors, in terms of cancer risk. Thus it is likely that, for the foreseeable future, the most useful information relevant to transferring 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. There has been some work comparing aggregate radiation-associated cancer mortality risks in humans and in various animals (Carnes *et al.*, 2003), suggesting that a simple scaling of radiation-related mortality may be appropriate. However, several key radiation-related cancers



(thyroid, breast) and intermediates (thyroid nodules) are not linked with high case-fatality rates. So mortality may not be the best measure. Without more detailed investigation taking account of the different spectrum of tumors in humans and in these experimental systems, and their distinct etiology, this approach may not be very productive.

Sometimes a meta-analysis, based on published findings from several studies, may be performed. Where feasible, as noted below, it is preferable to combine the original data and analyze them using a common format, in other words to perform a pooled analysis. Pooled analyses have been conducted to assess:

- cancer in various cohorts of radiation workers (Cardis *et al.*, 2007);
- effects of radon daughter exposure in relation to lung-cancer risk in underground miners (UNSCEAR, 2000);
- thyroid cancer risk in various (mainly medically exposed) cohorts (Ron *et al.*, 1995); and
- breast cancer risk in various populations (Howe and McLaughlin, 1996; Little and Boice, 1999; Preston *et al.*, 2002).

Less commonly, analyses combining cohort and case-control data, for example in relation to leukemia risk (Little *et al.*, 1999b), have been conducted.

2.4 Extrapolating Low-Dose Effects from *In Vitro* to *In Vivo*; Animal to Human

Several decades of laboratory research have given us a detailed view of molecular and cellular events that occur in response to acute moderate to high doses of radiation, including activation of DNA damage checkpoints, DNA repair pathways, mutagenesis, genomic instability, cell transformation, and the regulation of cell death pathways (Hall and Giaccia, 2011). The radiation-induced genetic changes observed in cells *in vitro* are similar to those observed in radiation-induced tumors in animal models. As a result, studies of model genetic loci are frequently extrapolated to cancer relevant genetic changes seen in animals and humans. Such genetic events include oncogene activation *via* chromosome translocation or gene amplification and inactivation of tumor suppressor genes *via* point mutations or larger scale changes such as deletions, insertions and inversions.

The complexities of extrapolation between animal models (including rodents and canines) and humans has been reviewed in depth in NCRP Report No. 150 (NCRP, 2005), which concluded that studies of life shortening in animals may provide reliable estimates of radiation risks to humans. The strength of life-shortening data is that it integrates more completely the detrimental effects of radiation in the entire animal, rather than focusing on just one endpoint such as cancer induction. NCRP (2005) further concluded that risk extrapolation from animal models to humans will:

- require robust, well annotated databases that must be widely disseminated to investigators interested in radiation risk estimation;
- include experimental data from animal studies and data from human epidemiologic studies;
- further develop our understanding of molecular mechanisms of cancer and other disease induction; and
- account for effects of age and genetic heterogeneity (or lack thereof) of animal models versus humans.

While the benefits of life-shortening studies are clear, the most recent study that validated risk estimates for humans using life-shortening measures in mice and dogs demonstrated that there was little effect on life span with acute radiation doses <1 Gy (Carnes *et al.*, 2003). Earlier studies of life shortening in mice indicated that acute radiation doses reduced life span by

~28 d Gy⁻¹ (Grahm, 1960). However, with protracted, low dose-rate exposures (<0.1 Gy d⁻¹), life shortening was greatly reduced to ~4 d Gy⁻¹ (Grahm and Sacher, 1968). Therefore, despite the advantages of life shortening as an integrated endpoint of the detrimental effects of radiation, this approach does not appear to have sufficient sensitivity to detect low-dose or low dose-rate effects on animal life span, and even with strong tools to extrapolate risk from animal models to humans (Carnes *et al.*, 2003) this lack of sensitivity poses problems when attempting to use these animal data to extrapolate human risk to low-dose or low dose-rate radiation exposures. There are also certain methodological concerns with life-shortening studies. Even when cancer and other age-specific disease rates are linear functions of radiation dose, life shortening is a nonlinear function of dose. In the manner in which this analysis is sometimes performed, the relevant measure is derived as a nonlinear function of certain regression coefficients derived from the life table (Carnes *et al.*, 2003), and it would appear that the incorrect error structure was assumed.

Extrapolations of *in vitro* cell culture results to animals and to human cancer risks also pose many challenges due to significant environmental and genetic differences among cells in culture versus cells in animals or humans. For example, long-term studies of cells in culture have historically focused on cancer cell lines, which are not only different from the normal cells from which they were derived, but are often genetically unstable. Normal (primary) cells are genetically stable, but their limited replicative capacity (~50 cell divisions) precludes long-term studies of an individual primary cell culture. Cancer cell lines are convenient models because they are immortal, and can therefore be studied long term and shared among laboratories. It is now possible to immortalize primary cells by expressing telomerase reverse transcriptase (in animals and humans), which maintains telomere lengths, but this introduces another departure from normal cells.

Cells in culture are generally studied as monolayers, which is far different from their *in vivo* three-dimensional (3D) organization. The development of 3D artificial tissue models has provided an important experimental bridge between traditional monolayer models and animal models. At least one study demonstrated similar effects of radiation on cell killing, chromosome aberrations, and DSB induction and repair in two-dimensional (2D) monolayers of breast epithelial cells versus 3D tissue models (Lin *et al.*, 2009a), but other studies document differences in damage signaling and other cellular responses to radiation in 2D versus 3D tissue models (Barcellos-Hoff and Costes, 2006). For example, 3D tissue models have been used to investigate transcriptome and secretome changes in response to low- versus high-dose radiation (Mezentsev and Amundson, 2011; Zhang *et al.*, 2014), and there is evidence of reduced high-dose radiation cytotoxicity in long-term 3D cultures versus 2D cultures, reflecting differential apoptotic responses in 2D versus 3D cultures despite similar effects on checkpoint delay (Sowa *et al.*, 2010). Taking this a step further, cell signaling *in vitro* may also differ markedly from the behavior of the same cell type in its normal tissue environment within an animal, where communication among cells of different types may contribute to the response to radiation.

Animal studies represent a critical translational bridge between *in vitro* studies and understanding low-dose radiation effects in humans. Studies with mice are often performed with inbred populations, which represent a single genotype and are well-suited to learning effects of specific genes in radiation responses, including carcinogenesis. The complementary approach is to employ outbred mice (Grahm, 1958; Weil *et al.*, 1996), which may more accurately reflect effects in outbred human populations with diverse histories of radiation (and other genotoxin) exposures, diet and lifestyles. There is clear evidence of variation in radiation responses in human cells [e.g., cells that are heterozygous for ataxia-telangiectasia mutated (*ATM*), or the retinoblastoma susceptibility gene (Fernet *et al.*, 2004; Kato *et al.*, 2009; Wilson *et al.*, 2010)]. In one study in mice, *Atm* heterozygosity did not enhance radiation

carcinogenesis (Mao *et al.*, 2008), but this study was limited to lymphomagenesis as the 60 week monitoring period was too short to reveal genesis of solid tumors.

To date the full effects of *ATM* heterozygosity on radiation carcinogenesis remain unknown. However, *Atm* heterozygous mice differ from wild type in cellular radiosensitivity (Kato *et al.*, 2006; Spring *et al.*, 2002; Weil *et al.*, 2001), spontaneous carcinogenesis (Spring *et al.*, 2002), and in radiation-induced ductal dysplasia (Weil *et al.*, 2001). Although radiation carcinogenesis studies in outbred mice have been limited, the idea that low-dose ionizing radiation may have significantly different effects in outbred populations is supported by a study of metabolic markers in outbred mice exposed to 100 mGy ionizing radiation (Lee Do *et al.*, 2012), and a study of mammary tissue responses to four weekly doses of 75 mGy (Snijders *et al.*, 2012). Similarly, Okayasu *et al.* (2000) and Yu *et al.* (2001) demonstrated clear differences in (high-dose) radiation carcinogenesis in different strains of mice, tracing the effects to hypomorphic mutations in the DNA-PKcs gene, a key factor in nonhomologous end joining repair of ionizing radiation-induced DSBs. As rodents express DNA-PKcs at ~20-fold lower levels than humans, this highlights yet another point to be considered if attempting to extrapolate experimental results in mice to assess risk in humans.

Extrapolation of risk from animal models to humans depends on conservation of the underlying mechanism. For instance, transgenerational effects have been seen in progeny of whole-body irradiated male mice (Barber *et al.*, 2002; Carls and Schiestl, 1999) but there is little evidence of transgenerational effects in exposed human populations (Dauer *et al.*, 2010; Little *et al.*, 2013), suggesting that at least for this endpoint, there are fundamental mechanistic differences between animal models and humans.

Particularly pertinent to extrapolation of risk from animals to humans with regard to effects of low doses and low dose rates are inverse dose-rate effects, which have been described in several species (mouse, rat, human cells) and several endpoints (cytotoxicity, mutagenesis, tumor induction) (Brenner, 1994; Lubin *et al.*, 1995; Spiess and Mays, 1973; Ullrich, 1984), characterized by greater biological effects of equal doses delivered at low versus high dose rates. While data on inverse dose-rate effects are limited, these effects have been explained in terms of differential checkpoint responses at low versus high dose rates and accumulation of cells in sensitive phases for low dose-rate irradiation. In this model, low dose rates activate checkpoint arrest in late G2 so with time an increasing proportion of the cell population accumulate in this phase. This is not the case for high dose-rate exposures because under these conditions the overall population survival response is almost entirely determined by the radio-resistant late S and early G2 cells (Hall and Giaccia, 2011; Mitchell *et al.*, 1979). The existence of inverse dose-rate effects suggests a DDREF < 1, however, as inverse dose-rate effects are not consistently observed, it would appear that a specific DDREF value cannot be applied in all situations (Nygaard *et al.*, 1992). Inverse dose-rate effects should be further investigated in animal models to refine risk estimates of low-dose and low dose-rate radiation exposures in humans.

Many *in vivo* studies of low dose-rate exposures with total doses <100 mGy have been performed (Nygaard *et al.*, 1992). Of particular note are studies showing that radiation doses <100 mGy cause readily observable damage to spermatogonial cells. Testicular irradiation with incorporated radionuclides has been shown to cause inverse dose-rate effects when the endpoint is induction of sperm abnormalities (Rao *et al.*, 1991), and such exposures can lead to transgenerational effects, including increased cancer among offspring (Hoyes *et al.*, 2001). Timing of conception post-irradiation markedly affected such responses to both low- and high-LET radiation (Lord and Hoyes, 1999). Importantly, testicular irradiation revealed that human sperm are significantly more radiosensitive than mouse sperm (Clifton and Bremner, 1983; de Rooij and Vergouwen, 1991; Meistrich and Samuels, 1985) and human fetal testes are even more radiosensitive than adult testes (Lambrot *et al.*, 2007).



3. Studies Integrating Biology and Epidemiology

There are numerous epidemiologic studies evaluating the presence or absence of radiation-associated cancer (UNSCEAR, 2008a) and certain noncancer effects (UNSCEAR, 2008b). Section 3.1 provides examples of a few studies (primarily for cancer outcomes) that successfully incorporate biological measures, sound epidemiologic methods and radiation measures, which is the main focus of this Commentary. Section 3.2 discusses studies of various noncancer diseases and effects, concentrating in particular on circulatory disease and cataracts. Although currently in preliminary phases, the integrative approach combining multiple measures, including individual dosimetry measures, and the planned analyses of multiple outcomes promise improvements over prior studies designed in singular contexts.

3.1 Epidemiologic Studies Focused on Cancer Outcomes

3.1.1 Chromosome Aberration Studies

A small number of radiation epidemiologic studies have also incorporated measures of chromosome aberrations on a subset of the study population, in particular for support of dose estimation.

Chromosome aberrations in blood lymphocytes are the most thoroughly studied biological sentinel of radiation-induced cellular injury. Unstable asymmetrical aberrations such as rings and dicentrics are used to estimate exposures in the short term, and stable aberrations such as symmetrical translocations are used to estimate exposures many years after they occurred (Ainsbury *et al.*, 2011; Kleinerman *et al.*, 2006; Lucas *et al.*, 1992; Tucker, 2008; Tucker and Luckinbill, 2011). Epidemiologic observations that have incorporated chromosome aberration evaluations include:

- atomic-bomb survivors (Kodama *et al.*, 2001);
- patients treated with radiation for ankylosing spondylitis (Buckton, 1983);
- cervical cancer (Kleinerman *et al.*, 1989);
- benign gynecological disorders (Kleinerman *et al.*, 1994);
- enlarged thymus gland or tonsils (Kleinerman *et al.*, 1990);
- patients receiving diagnostic radiation for tuberculosis monitoring (Kleinerman *et al.*, 1990; Littlefield *et al.*, 1991) or given Thorotrast® (VanHeyden Company, Dresden-Radebeul, Germany) for cerebral angiography (Littlefield *et al.*, 1997);
- workers in radiation occupations (Bhatti *et al.*, 2008; Little *et al.*, 2014a; Tucker *et al.*, 1997a); and
- populations living in areas of high natural background radiation (Wang *et al.*, 1990).

While chromosome aberrations in lymphocytes are indirect indicators of radiation dose and thus correlated with cancer risk, there is evidence that chromosome aberrations may predict human cancer risk independently of such exposure and thus be relevant to the mechanistic and biological understanding of the carcinogenic process (Bonassi *et al.*, 2008; Hagmar *et al.*, 2004; Tucker *et al.*, 1997b). Examples are given below for several epidemiologic investigations where concurrent studies of chromosome aberrations have provided insights into the possible mechanisms of carcinogenesis and other human health detriments.

3.1.2 Atomic-Bomb Survivors

The principal atomic-bomb survivor cohorts consist of the LSS, which provides follow-up for mortality and cancer incidence of ~90,000 survivors with dose estimates, and the Adult Health Study (AHS), which provides biennial medical follow-up for a subcohort of initially ~20,000 LSS members. These two studies are primary sources for investigating the relationship between radiation exposure and cancer and noncancer outcomes. It is from the LSS, for example, that we know that most types of cancer can be associated with radiation exposure, and that increased cancer risk from an acute radiation exposure continues for more than 60 y (through 2009) from initial exposure (Grant *et al.*, 2015a; Hsu *et al.*, 2013; Ozasa *et al.*, 2012; Preston *et al.*, 2007).

There are few known biological effects or lifestyle variables apart from cancer, mortality from various causes, and the standard socio-demographic variables (*e.g.*, age, sex and city) determined in the full LSS cohort. Much more comprehensive biological and lifestyle information was collected in the AHS. The AHS includes a large fraction of LSS members with moderate to high radiation exposures, as well as, for control purposes, survivors who were essentially unexposed. Within this study group a large number of effects and biomarkers have been assessed, many of which have focused on noncancer outcomes. The following presents an overview of those atomic-bomb survivor studies that have incorporated chromosome aberrations as a biological endpoint or a biomarker of exposure. A significant fraction of the AHS members (~4,000 total) have been assessed for stable chromosome aberrations, evaluated in lymphocytes either by traditional Giemsa methods or more recently by fluorescent *in situ* hybridization. Stable chromosome aberrations (primarily translocations) are clearly related to radiation dose but there is a significant inter-individual variability in response to dose (Kodama *et al.*, 2001). In addition, there is an uncertainty in the survivor dose estimates.

Statistically significant excesses of leukemia have been observed among children and adults exposed to the Hiroshima and Nagasaki atomic bombs (Hsu *et al.*, 2013), but not among survivors who were exposed *in utero* (Jablon and Kato, 1970). Studies of chromosome aberrations mirror these epidemiologic observations. There was little evidence of dose response for chromosome aberrations among atomic-bomb survivors exposed *in utero*, whereas dose-response relationships of the expected magnitude were seen for translocation frequencies in lymphocytes of the mothers of those exposed *in utero* (Ohtaki *et al.*, 2004). Murine experimental data (using B6C3F1 mice) confirmed the human observational data, indicating that chromosome aberrations in lymphocytes do not persist after an *in utero* dose of 1 to 2 Gy (x rays) (Nakano *et al.*, 2007). A slightly different, although essentially compatible result was observed in a Sprague-Dawley rat model, where mammary cells from animals irradiated *in utero* with a dose of 2 Gy (gamma rays) appeared to show the normal chromosome aberration dose response, but hemato-lymphoid spleen cells did not (Nakano *et al.*, 2014).

The lack of a dose response at high doses for chromosome aberrations among those exposed *in utero* may provide insights into the differences seen in excess risks among those exposed *in utero* and during early childhood for leukemia. However, it should be noted that, for solid cancers, there is an increased risk following *in utero* exposure, manifest in adulthood, which was considerably lower than for early childhood exposure among those survivors exposed in early childhood (Preston *et al.*, 2008). As one possible explanation for these human and experimental observations, Nakano *et al.* (2007) suggested that the abnormal cells in the fetus were replaced by normal fetal stem cells during the postnatal growth period. Another possibility, suggested by Nakano *et al.* (2014) is that following relatively high doses, the high degree of induced cell sterilization will leave too small a pool of hematopoietic stem cells for any malignancy (*i.e.*, leukemia) to be observed, given the relatively low expected mutation rate (10^{-5} to 10^{-4} per gene per gray). If this phenomenon is true in general, it might be speculated that the

fetus may be less vulnerable to the oncogenic effect of high-dose radiation exposure than the child, but that this may not be the case at lower doses. Another perplexing observation is that while fetal irradiation can induce persistent chromosome aberrations in mammary stem/progenitor cells, this damage apparently does not contribute to an increased risk of mammary cancer (Nakano *et al.*, 2014).

In addition to the atomic-bomb survivors' cohort, other studies (Sections 3.1.3 through 3.1.8) have incorporated chromosome aberration measures to evaluate further whether these markers are predictive of dose and possibly cancer risk.

3.1.3 Pelvic Irradiation Studies

Statistically significant excesses of leukemia have been observed in women treated with pelvic radiation therapy for cervical cancer (Boice *et al.*, 1987; Chaturvedi *et al.*, 2007) and for nonmalignant gynecological bleeding disorders (Sakata *et al.*, 2012). Following radiation treatments for cervical cancer, the leukemia risk increased up to ~4 Gy (mean dose to active bone marrow), and then decreased at higher levels. A similar pattern was seen for radiation-induced chromosomal aberrations suggesting that cell killing might predominate over transformation at very high doses (Kleinerman *et al.*, 1989; Littlefield *et al.*, 1991). Excess leukemia was also reported following lower dose partial-body radiation therapy for benign gynecological disease. However, the level of risk was nearly the same as seen among the cervical cancer patients, about twofold, despite a 10-fold difference in mean dose to active bone marrow (8 versus 0.7 Gy, respectively) (Kleinerman *et al.*, 1994). High-dose cell killing was again postulated as one explanation for this apparent inconsistency. The rate of stable aberrations, which reflects nonlethal damage in surviving stem cells, was also similar among cancer patients and benign gynecological disease patients. Apparently, the lower-dose treatments for benign disorders resulted in much higher chromosomal-aberration yields per unit of dose than seen following radiation therapy for cervical cancer. Assuming that cytogenetically aberrant stem cells that survive radiation therapy contribute to the leukemogenic process, the chromosome aberration data in these two irradiated populations are consistent with the leukemia outcomes in the two populations.

3.1.4 Occupational Studies

Studies of chromosome aberrations among working populations with accurate measures of chronic radiation exposures can provide insights into the ability of human cellular systems to repair radiation damage incurred at a low dose and dose rate. Excess stable translocations, however, cannot be detected at much lower doses than 100 mGy (Tucker and Luckinbill, 2011). Understanding human biological responses to radiation exposure has implications concerning radiation protection guidelines where a DDREF is applied to scale the high dose-rate exposure risks among atomic-bomb survivors to low dose-rate exposure circumstances (ICRP, 2007). Studies of workers have indicated that stable chromosome aberrations can be detected and related to the amount of radiation experienced over time (Evans *et al.*, 1979; Little *et al.*, 2014a; Tucker *et al.*, 1997a).

The ability to detect a biological effect at the chromosome level does not mean that there will be a biological consequence in the population exposed, and it may be informative to contrast chromosome findings in instances where effects have and have not been demonstrated. For Chernobyl cleanup workers from Estonia, a small population (4,810 men) with relatively low doses (mean 100 mGy, maximum 280 mGy), increases in chromosome aberrations (Littlefield *et al.*, 1998) and in leukemia (Rahu *et al.*, 2013) have not been observed. Little *et al.* (2014a) evaluated low-dose occupational exposure and chromosome aberrations in U.S. radiologic technologists and found a consistency with the cytogenetic data among atomic-bomb survivors;

