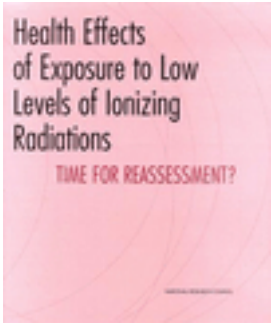


**Health Effects of Exposure to Low Levels of Ionizing Radiations: Time for Reassessment?**

Committee on Health Effects of Exposure to Low Levels of Ionizing Radiations (Beir VII), National Research Council

ISBN: 0-309-58052-8, 86 pages, 8.5 x 11, (1998)

# Health Effects of Exposure to Low Levels of Ionizing Radiations

## TIME FOR REASSESSMENT?

**Committee on Health Effects of Exposure to Low Levels of Ionizing Radiations (BEIR VII)**
**Board on Radiation Effects Research**
**Commission on Life Sciences**
**National Research Council**

NATIONAL ACADEMY PRESS
Washington, DC 1998

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competencies and with regard for appropriate balance.

This report was prepared under EPA Assist ID No. X825133-01-0 between the National Academy of Sciences and the US Environmental Protection Agency.

ISBN: 0-309-06176-8

A limited number of copies of this report are available from
National Research Council
Board on Radiation Effects Research
Room 342
2101 Constitution Avenue, NW
Washington, DC 20418
(202) 334-2232


Printed in the United States of America

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# Preface

Humans have always lived in the presence of low levels of ionizing radiation arising from cosmic rays and emissions from radioisotopes in the air, in water, and on the land. Relatively small populations receive a range, usually small amounts, of occupational exposures, or are exposed to larger doses from diagnostic or therapeutic medical procedures. Other groups have received exposures from radioactive fallout from bomb tests, or from radiation accidents such as Chernobyl. New information has become available in recent years on large exposures of workers in nuclear facilities in the former Soviet Union and on populations affected by their hazardous wastes. In the latter case, precise doses are often difficult to establish, but the data are of particular relevance to radiation protection because they relate to long term low dose-rate exposures. However, the observations on the atomic-bomb survivors in Hiroshima and Nagasaki continue to be the main source of information.

The health effects of acute or chronic ionizing-radiation exposure, such as cancer, are superimposed on the effects that arise from endogenous chemical reactions or exogenous exposures to carcinogens in the environment. Radiation is different from other carcinogens in that in principle it is possible, even if often difficult, to estimate radiation doses whereas, although the mechanisms of action of many chemical carcinogens have been elucidated, environmental- or occupational-exposure doses are poorly known if at all.

The ability to measure radiation doses implies that it is possible to quantify the hazard so as to estimate the mortality arising from low doses of radiation. Low-dose radiation effects cannot be estimated by direct observation, because of the large numbers of background cancers arising from other causes, which usually are not known. For high acute exposures, as in Japan, or for some medical procedures, sufficiently precise data can be obtained to permit extrapolation to lower acute exposures or to lower chronic exposures, assuming a knowledge of the relation between effect and dose and the effect of dose rate. However, extrapolation to low exposures is attended by large uncertainties because the shape of dose-response curves is not well known, especially at the lower doses, and because of uncertainty in background levels (zero added dose). Often, in the absence of reliable data, it is assumed that extrapolation to low doses should be linear and without a threshold, a straight line connecting high dose with zero radiation dose (and zero excess cancers). That point of view is controversial. Some investigators believe that there is a threshold dose—a dose below which radiation has no deleterious effect. Others cite data indicating that the shape of a dose-response curve close to the zero dose has a slope much greater than that of the straight-line that represents interpolation between zero dose and high doses.

The uncertainties in the magnitude of low-dose effects led to a request from the US Environmental Protection Agency (EPA) to the National Research Council that a committee be formed to consider recent data derived from molecular, cellular, animal, and human epidemiologic studies and to evaluate whether it would be feasible to improve the estimated risks to humans posed by exposures to low levels of ionizing radiation. As a result, the Research Council's Commission on Life Sciences authorized the Board on Radiation Effects Research (BRER) to form the Committee on Health Effects of Exposure to Low Levels of Ionizing Radiations (referred to as BEIR VII because it is the seventh committee

in a series that began with the Committee on Biological Effects of Ionizing Radiations). The Committee was to carry out a preliminary scoping (phase-1 study) to review and evaluate the scientific literature pertinent to the biologic and health effects of low-level ionizing radiation and make a concerted effort to learn about the status of all relevant research in progress. The committee was charged to determine, on the basis of those data, whether sufficient information had become available since the 1990 BEIR V report to warrant a comprehensive reassessment of health risks in a phase-2 study by an enlarged BEIR VII committee.

The phase-1 committee, organized in January 1997, had 8 members with expertise in molecular, cellular and animal radiation biology and in human epidemiology and radiation dosimetry. The committee met first in March to summarize what it knew about relevant advances since the 1990 BEIR V report and to organize 2 workshops. The workshops were to encompass invited speakers and position papers at publicized meetings on epidemiology and on the impact of new biologic knowledge on risk assessment. A 2-day committee meeting in June included a half-day workshop devoted to epidemiology and a 2-1/2 day committee meeting in July included 2 days devoted to the impact of new biologic knowledge on risk assessment. A 2-day meeting in August, overlapping a BRER meeting, was devoted to summarizing the committee's conclusions derived from the members' own reading, discussion, and the workshops and to begin the writing of the committee's report.

This BEIR VII phase-1 report includes an executive summary recommending a full, BEER VII phase-2 study, and describes the general structure of the phase-2 study. Chapters on epidemiology, cellular and molecular considerations, animal studies, and mechanistic cancer modeling provide the background information for a phase-2 study and the committee's rationale for endorsing such a study.

Richard B. Setlow, Chairman

# Contents

**HEALTH EFFECTS OF EXPOSURE TO LOW LEVELS OF IONIZING RADIATIONS:**
***Time for Reassessment?***

# EXECUTIVE SUMMARY

The US Environmental Protection Agency Office of Radiation and Indoor Air asked the National Research Council to evaluate whether sufficient new data exist to warrant a reassessment of health risks reported in *Health Effects of Exposure to Low Levels of Ionizing Radiations (BEIR V)* in 1990. To respond to this request, the National Research Council assembled the Committee on Health Risks of Exposure to Low Levels of Ionizing Radiations. The work of the committee was conducted in what was called the BEIR VII phase-1 study. To assist the committee during its deliberations, various scientists were consulted for advice, and a workshop on the impact of biology on risk assessment was held in collaboration with the Department of Energy Office of Health and Environmental Research. The intent of the workshop was to address the implications of new understanding of the biologic basis of radiation injury and carcinogenesis for risk assessment.

The following is a synopsis of the conclusions of the BEIR VII phase-1 study:

**Information that has become available since the 1990 publication of *Health Effects of Exposure to Low Levels of Ionizing Radiations (BEIR V)* makes this an opportune time to proceed with BEIR VII phase-2, a comprehensive reanalysis of health risks associated with low levels of ionizing radiations. Such a study should begin as soon as possible and is expected to take about 36 months to complete**.

*The committee based that judgment on the following considerations:*

- Substantial new epidemiologic evidence has accumulated since the 1990 BEIR V report was published. The present committee's phase-1 report cites 39 new epidemiologic studies that fall into this category (see Table 1). Additional studies that have a direct bearing on the subject should become available in the next 3 years, the estimated period required to complete the phase-2 study.
- Some of the new epidemiologic data are on subjects on which information previously had been sparse, such as cancer mortality in those exposed to whole-body irradiation in childhood.
- Studies of carcinogenesis completed since publication of the last BEIR report have focused on mechanisms and the cellular and molecular events that are involved in the neoplastic process. The understanding of molecular events involved in carcinogenesis has increased significantly. Mechanisms that might be involved in radiation carcinogenesis have been identified. Further knowledge of these mechanisms that should become available in the next 3 years might affect estimation of the radiation-response curve at low doses.
- Over the next few years, investigators will be applying two closely linked approaches using animal models of carcinogenesis. These will likely contribute to a better understanding of mechanisms of radiation-induced cancer. In the first of these two approaches, genetically engineered mice with alterations in specific genes will be used to determine the influence of these genes on the susceptibility of the mice to radiation-induced cancer. In the second approach, studies will be conducted of the inherent differences in susceptibility to radiation-reduced cancer among different mouse strain, the objective being to identify the genes involved in controlling susceptibility. Researchers responsible for this new generation of animal studies are taking advantage of the current rapid developments in molecular genetics. Progress on both approaches should be substantial over the next few years. Significant results of relevance to risk estimation are expected to be available for the proposed BEIR VII phase-2 study.
- Evidence regarding specific biologic events that can affect the shape of the dose-response curve at low doses is accumulating. Information on such phenomena as DNA repair, signal transduction, chromosomal instability, "bystander" effects, and adaptation, although preliminary, might eventually affect risk analyses of low-dose and low-dose-rate exposures.

*The Committee Recommends That the Individuals Responsible for the Proposed Phase-2 Study*

- Include a comprehensive review of all relevant epidemiologic data related to low-LET (low linear energy transfer), i.e. sparsely ionizing, radiation.

- Define and establish principles on which quantitative analyses can be based, including requirements for epidemiologic data and cohort characteristics. In this respect, the committee should consider biologic factors (such as the dose and dose-rate effectiveness factor, relative biologic effectiveness, genomic instability, and adaptive responses) and appropriate methods to develop etiologic models (favoring simple as opposed to complex models), estimate population detriment, and attribute causation in specific cases.
- Assess the current status and relevance to risk models of biologic data and models of carcinogenesis. This should include a critical assessment of *all* data that might affect the shape of the dose-response curve at low doses, in particular, evidence of thresholds or the lack thereof in dose-response relationships and the influence of adaptive responses and radiation hormesis.
- Consider potential target cells and problems that might exist in determining dose to the target cell.
- Consider any recent evidence regarding genetic effects not related to cancer. Any such data, even if obtained from high radiation exposures or at high dose rates, should be considered.

*With Respect to Modeling, the Committee Recommends That the Individuals Responsible for the Proposed Phase-2 Rtudy*

- Develop appropriate risk models for major cancer types and other outcomes, including benign disease and genetic effects. Specifically, the responsible committee should develop models appropriate for use in future development of probability-of-causation tables and should consider the fitting of purely empirical models to original data from studies or combined studies, the fitting of purely empirical models with recta-analytic techniques, and the fitting of semiempirical biology-based models to epidemiologic data.
- Provide examples of specific risk calculations based on the risk models and explain the appropriate use of the models.
- Describe and define the limitations and uncertainties of the risk models and their results. The committee conducting the proposed phase-2 study should be directed to develop best-estimate models for purposes of risk assessments as opposed to developing conservative models for purposes of radiation protection.
- Discuss the role and effect of modifying factors, including host (such as individual susceptibility and variability, age, and sex), environment (high background radiation exposure), and lifestyle (such as alcohol and cigarette consumption).

- Identify critical gaps in knowledge that should be filled by future research.

To accomplish the charge suggested here, the membership of the committee responsible for the proposed BEIR VII phase-2 study will require expertise in epidemiology, biostatistics, radiation physics and dosimetry, molecular biology, risk assessment and communication, cancer modeling, animal and cellular radiation biology, somatic cell genetics, cell-cycle regulation and apoptosis, and the causation and repair of DNA damage induced by ionizing radiation. The committee recommends that the experts chosen have adequate resources and access to data for the computing, statistical analyses, and modeling required to complete the study. A major goal of the BEIR VII phase-2 study will be to better quantify and characterize the uncertainties associated with risk estimates and to produce the most realistic estimates of uncertainties.

# 1

# INTRODUCTION

**I**onizing radiation arises from natural and human-produced sources and can affect essentially all the organs and tissues of the body. Well-demonstrated late effects include induction of cancer, developmental abnormalities, and cataracts. In recent years, increasing concern has centered on the risks of these effects at the low doses and low dose rates experienced by radiation workers and the general public. That concern is influenced, in part, by renewed speculation regarding the postulated nonlinearity of the dose-response relationship at environmental levels of exposure. Assumptions as to the shape of the dose-response curve at environmental levels by regulatory agencies have profound economic and health implications.

A large amount of additional epidemiologic data have become available since the BEIR V report (NRC 1990). New statistical methods are available to increase the analytic power of interpretation of those data. Biologic data are emerging on phenomena that could affect the shape of the dose-response curve at low doses. Low-level radiation exposure might induce genomic instability and thus result in damage to cells many cell generations after exposure. Additional evidence suggests that the clusters of damage produced in the DNA at very low doses of radiation are refractory to DNA repair. Conversely, adaptive or hormetic responses to low levels of ionizing radiation might render cells refractory to later exposures.

This report was prepared by the Committee on Health Risks of Exposure to Low Levels of Ionizing Radiations (BEIR VII), in the Board on Radiation Effects Research of the National Research Council's Commission on Life Sciences to summarize evidence that has accumulated on health risks posed by exposure to low levels of ionizing radiation since the BEIR V report and to determine whether the new information justifies a comprehensive study, which would be called BEIR VII phase-2.

# 2

# EPIDEMIOLOGIC INVESTIGATIONS

This chapter considers epidemiologic evidence that has accumulated since the BEIR V report (NRC 1990). The first section identifies epidemiologic studies of low-LET ionizing radiation that have appeared since the BEIR V report and summarizes their results. The second section discusses recent developments in methodology, and the third the relevance of new data to the possible utility and function of a BEIR VII phase-2 committee. The final section discusses the radioepidemiologic tables.

## NEW EPIDEMIOLOGIC RESULTS

Table 1 summarizes some of the more important epidemiologic data that have been published since 1990, when the BEIR V report appeared, or that were not available to the BEIR V committee. The table includes studies that the current committee expects to produce new and useful epidemiologic data during the term of a BEIR VII phase-2 committee. Table 1 is intended not to be exhaustive, but rather to be a guide to the new epidemiologic data that have become available since 1990, and are expected to become available in the next few years.

Table 1. Summary of epidemiologic studies of low LET ionizing radiation and cancer since 19901

| STUDY | REFERENCE | TYPE OF STUDY | SERIES | SEX | NO. IN STUDY | FOLLOW-UP PERIOD | CANCER SITES REPORTED |
|---|---|---|---|---|---|---|---|
| Ankylosing spondylitis patients | Weiss and others, 1994<br>Weiss and others, 1995 | Cohort<br>Cohort | Mortality<br>Mortality | Male and Female<br>Male and Female | 15,577<br>14,767 | 1935-1992<br>1935-1992 | All cancer and multiple cancer sites<br>Leukemia |
| Atomic-bomb survivors | Preston and others, 1994<br>Thompson and others, 1994<br>Ron and others, 1995a | Cohort<br>Cohort<br>Cohort | Incidence<br>Incidence<br>Incidence | Male and Female<br>Male and Female<br>Male and Female | 93,696<br>79,972<br>80,311 | 1950-1987<br>1958-1987<br>1958-1989 | Leukemia, lymphoma, multiple myeloma<br>Multiple cancer sites (solid tumors)<br>Benign tumors of stomach, colon, and rectum |
| Atomic-bomb survivors | Pierce and others, 1996 | Cohort | Mortality | Male and Female | 86,572 | 1950-1990 | Non leukemias, leukemia, and multiple cancer sites |
| Atomic-bomb survivors | Land and others, 1994a<br>Land and others, 1994b | Case control | | Female | Cases: 196<br>Controls: 566 | 1955-1981 | Breast cancer |
| Atomic-bomb survivors (in utero cohorts)<br>Canadian fluoroscopy | Delongchamp and others, 1997<br>Howe, 1995<br>Howe and McLaughlin, 1996 | Cohort<br>Cohort<br>Cohort | Mortality<br>Mortality<br>Mortality | Male and Female<br>Male and Female<br>Female | 17,601<br>64,172<br>31,917 | 1950-1992<br>1950-1987<br>1950-1987 | Non leukemias, leukemia, and multiple cancer sites<br>Lung cancer<br>Breast cancer |
| Cervical cancer patients | Kleinerman and others, 1995 | Cohort | Incidence | Female | 86,193 | 1935-1990 | Multiple cancer sites |
| Contralateral breast (Denmark) | Storm and others, 1992 | Case control in cohort | | Female | Cohort: 56,540<br>Cases: 691<br>Controls: 691 | 1943-1986 | Breast cancer |

| STUDY | REFERENCE | TYPE OF STUDY | SERIES | SEX | NO. IN STUDY | FOLLOW-UP PERIOD | CANCER SITES REPORTED |
|---|---|---|---|---|---|---|---|
| Contralateral breast (US) | Boice and others, 1992 | Case control in cohort | | Female | Cohort: 4,109 Cases: 655 Controls: 1,189 | 1935-1987 | Breast cancer |
| Fallout from Nevada Test Site | Kerber and others, 1993 Simon and others, 1995 | Cohort Case control | Incidence | Male and Female Male and Female | 2,473 Cases: 1,177 Controls: 5,330 | 1965-1986 1952-1981 | Thyroid cancer and other thyroid disease Leukemia |
| Massachusetts fluoroscopy | Davis and others, 1989 Boice and others, 1991 | Cohort Cohort | Mortality Incidence | Male and Female Female | 13,385 4,940 | 1929-1986 1925-1986 | Multiple cancer sites Breast cancer |
| Multiple diagnostic xrays of scoliosis patients | Hoffman and others, 1989 | Cohort | Incidence | Female | 1,030 | 1935-1986 | Breast cancer |
| Nuclear industry workers (combined analysis) | Cardis and others 1994 Cardis and others, 1995 | Cohort Cohort | Mortality Mortality | Male and Female Male and Female | 95,673 95,673 | 1943-1988 1943-1988 | Multiple cancer sites Solid tumors and leukemia |
| Nuclear workers at Mayak Production Association | Koshurnikova and Shilnikova, 1996 | Cohort | Mortality | Male and Female | 18,879 | 1948-1993 | Lung cancer and leukemia |
| Pelvic radiotherapy for benign gynecologic disease | Inskip and others, 1993 | Cohort | Mortality | Female | 12,955 | 1929-1985 | Multiple hematopoietic cancers |
| Pooled analysis of external radiation and thyroid cancer | Ron and others, 1995b | Cohort Case control | Incidence | Male and Female | 120,000 | 1926-1990 | Thyroid cancer |
| Radiation treatment for benign head and neck conditions (benign thyroid tumors) | Wong and others, 1996 | Cohort | Incidence | Male and Female | 544 | 1939-1991 | Benign thyroid nodules |
| Radiation treatment for | Schneider and others, 1993 | Cohort | Incidence | Male and | 4,296 | 1939-1990 | Thyroid cancer and |

| STUDY | REFERENCE | TYPE OF STUDY | SERIES | SEX | NO. IN STUDY | FOLLOW-UP PERIOD | CANCER SITES REPORTED |
|---|---|---|---|---|---|---|---|
| benign head and neck conditions (thyroid cancer and thyroid nodules) | | | | Female | | | nodules |
| Radiation treatment for breast cancer | Curtis and others, 1992 | Case control in cohort | | Female | Cohort: 82,700 Cases: 90 Controls: 264 | 1973-1985 | Leukemia |
| Radiation treatment for peptic ulcer | Griem and others, 1994 | Cohort | Mortality | Male and Female | 3,609 | 1937-1985 | Multiple cancer sites |
| Radiotherapy for Hodgkin disease (breast cancer) | Hancock and others, 1993 | Cohort | Incidence and Mortality | Female | 885 | 1961-1990 | Breast cancer |
| Radiotherapy for Hodgkin Disease (gastrointestinal cancer) | Birdwell and others, 1997 | Cohort | Incidence and Mortality | Male and Female | 2,441 | 1961-1993 | Multiple cancer sites (gastrointestinal only) |
| Radiotherapy for metropathia hemorrhagic anemia | Darby and others, 1994 | Cohort | Mortality | Female | 2,067 | 1940-1991 | Multiple cancer sites |
| Radiotherapy for pituitary adenoma | Brada and others, 1992 | Cohort | Incidence | Male and Female | 334 | 1962-1986 | Multiple cancer sites (solid tumors only) |
| Radiotherapy for skin, hemangioma in childhood | Furst and others, 1990 | Case control in cohort | | Male and Female | Cohort: 14,647 Cases: 94 Controls: 359 | 1920-1986 | Multiple cancer sites (solid tumors) |
| Radiotherapy for thymus enlargement | Shore, 1990 | Cohort | Incidence | Male and Female | 7,450 | 1953-1989 | Skin cancer |
| Radiotherapy for uterine bleeding | Inskip and others, 1990 | Cohort | Mortality | Female | 4,153 | 1925-1984 | Multiple cancer sites |
| Tinea capitis (Israel) | Ron and others, 1989 Ron and others, 1991 | Cohort Cohort | Incidence Incidence | Male and Female Male and Female | 10,834 27,060 | 1950-1986 1950-1980 | Thyroid cancer and other thyroid disease Melanoma, other skin cancer and benign skin tumors |

| STUDY | REFERENCE | TYPE OF STUDY | SERIES | SEX | NO. IN STUDY | FOLLOW-UP PERIOD | CANCER SITES REPORTED |
|---|---|---|---|---|---|---|---|
| Women treated for infertility | Ron and others, 1994 | Cohort | Mortality | Female | 816 | 1925-1991 | Multiple cancer sites |

| STUDY | REFERENCE | DESCRIPTION |
|---|---|---|
| In utero exposure | Doll and Wakeford 1997 | A review of case-control and cohort studies of childhood cancers. |

[1] Table 1 is a summary of the more important epidemiologic data that have been published since the 1990 publication of the BEIR V report or that are expected to provide new and useful data during the 3-year term of the proposed BEIR VII phase-2 study. Although not exhaustive, the list should serve as a guide to some of the pertinent new and upcoming epidemiologic data on the subject.

The following list presents categories where additional data have become available since the BEIR V report.

1. Nonleukemia cancer mortality. In a recent mortality update from the Japanese atomic-bomb survivor Life Span Study cohort, Pierce and others (1996) modeled mortality to the end of 1990. This extension of the existing data added 10,500 persons to the cohort with DS86 doses and 1,227 nonleukemia cancers to the mortality data. The increase in the number of cancer deaths for analysis was particularly noticeable among the members of the cohort who were under the age of 20 years at the time of the atomic bombings; in this category the number of deaths increased from 545 to 889 in the most recent 5-yr period of follow-up. The primary focus of this analysis was on modeling the risk of the nonleukemia cancers as a single entity, in that the authors concluded that the apparent variation in site-specific cancer risks could often not be distinguished from random variation. With that approach, the preferred risk model was a linear excess-relative-risk model; the excess relative risk (ERR) per sievert was lower for men (0.375) than for women (0.774) and was reduced with age at exposure by the same exponential factor for men and women.
2. Mortality in the British series of patients treated with x-rays for ankylosing spondylitis. This data has been updated (Weiss and others 1994, 1995).
3. Mortality among radiation workers. A combined analysis of risk estimates can be compared with those obtained at higher doses from other series (Cardis and others 1994, 1995).

4. Site-specific analyses:

- Leukemia: includes a downwind study (Preston and others 1994; Weiss and others 1994; Simon and others 1995).
- Breast cancer: possibility that sensitive groups might show up in form of high excess relative risk for early-onset cancer, new evidence on risk of exposure to radiation for various reproductive histories, and new data on transfer of risk between populations with different baseline risks (Land and others 1994a,b; Tokunaga and others 1994; UNSCEAR 1994; Land 1995a).
- Lung: Cancer evidence relating to dose and dose rate effectiveness factor (DDREF) (Howe 1995).
- Gastrointestinal cancers: longer follow-up periods by studies such as Birdwell and others (1997).
- Lymphatic and hematopoietic cancers other than leukemia (Thompson and others 1994).
- Lung, salivary gland, skin, and central nervous system cancers: evidence of specificity of radiation-related risk in terms of histologic subtype (Land and others 1993, 1996; Land 1995b; and data from the Radiation Effects Research Foundation).
- Thyroid cancer (including that caused by Iodine-131): combined analysis of childhood exposure to x rays and gamma rays (Kerber and others 1993; Ron and others 1995b); given the recent National Cancer Institute report (National Cancer Institute 1997) estimating thyroid doses to the US population from Iodine-131 in fallout from the Nevada Test Site, BEIR VII phase-2 will be expected to address the issue of thyroid cancers induced by Iodine-131.
- Other cancers (including atomic-bomb incidence series not addressed above).
- Noncancer outcomes.

5. New data on radiation-related risk in patients known to be genetically susceptible to cancer:

- Retinoblastoma patients (Tucker and others 1987; Eng and others 1993; Hawkins and others 1996; Wong and others 1997): evidence that ERR for bone sarcoma (Tucker and others 1987) and bone sarcoma and soft-tissue sarcoma (Wong and others 1997) increases with increasing therapeutic radiation dose to the tumor site with dose-specific relative risks comparable with those in survivors of other childhood cancers treated with radiation. Given evidence that baseline rates of bone and soft-tissue sarcoma are orders of magnitude higher among survivors of heritable retinoblastoma, this suggests that the *excess rate* (or absolute risk) of radiation-related cancer is also orders of magnitude higher among heritable retinoblastoma patients, and that, therefore, these patients constitute a genetic subpopulation highly susceptible to radiation-related bone and soft-tissue sarcoma.

- Swift and others (1991) hypothesis regarding the protein mutated in ataxia telangiectasia (ATM) and breast cancer and increased susceptibility to radiation-related breast cancer.
- International Commission on Radiological Protection study group on genetic susceptibility to radiation-related cancer (Cox and others in press).

## ADVANCES IN METHODOLOGY

In addition to new data, there have been advancements reported for analytical methods including:

- Adjustment for bias due to random errors in dosimetry (Pierce and others 1990; Gilbert 1998).
- Systematic presentation of sources of uncertainty in various components of risk estimates and their combined influence (NCRP 1997).

## CONSIDERATIONS FOR A BEIR VII PHASE-2 COMMITTEE

From the epidemiologic point of view, the prime motivation for a BEIR VII phase-2 study is the substantial increase in the mount of epidemiologic data that have been published since the BEIR V report. That applies particularly to some subjects on which data have previously been sparse, for example, cancer mortality in those exposed as children to whole-body irradiation. The new data permit the development of richer risk models and alternatives to models presented in the BEIR V report. Furthermore, there have been methodologic developments, such as the incorporation of dose measurement errors in fitting risk models.

The primary purpose of a BEIR VII phase-2 study would be to present a balanced overview of the new epidemiologic evidence and in particular to synthesize results from all the relevant studies, giving appropriate weight to the value of each study.

The committee could develop a generalized strategy for risk modeling and illustrate it with specific examples. Ideally, the strategy would be applied to all relevant exposure circumstances and outcomes; if this task were too onerous, the committee could at least develop a generalized approach that could be applied by others to other relevant situations.

In a general strategy for modeling, models should provide a good fit to the empirical epidemiologic data, be biologically plausible, be readily understood by the scientific community in general (which argues in favor of simple, rather than complex, models), and take into account all the relevant epidemiologic and biologic data.

Obviously some specific issues would have to be considered in such models, including the dose and dose rate effectiveness factor (DDREF) and the shape of the dose-response curve, the temporal distribution of risk after exposure, and the interaction of radiation with other risk factors and with other possible modifying factors, such as sex, age at exposure, attained age, and population differences.

Approaches to the modeling process could include:

- Fitting of purely empirical models to original data from studies or combined studies.
- Fitting of purely empirical models with meta-analysis; this is relatively underdeveloped and might be particularly useful when there are a number of studies of a particular outcome such as esophageal cancer.
- Fitting semiempirical biologically based models to epidemiologic data to improve understanding of the biologic basis of some of the empirical effects observed.
- Fitting (and testing) of simple models now being used in radiation protection, such as linear nonthreshold models in which the estimated relative risk at 1 sievert might depend upon age at exposure but remains invarient over time after exposure (with a minimal latent period) or an otherwise similar quadratic (linear-quadratic) model with an appropriate DDREF and particular attention given to the principal contending alternatives. Such alternatives include hormesis, threshold models, the Kellerer-Barclay model, and supralinear models.

Committee members will be selected who will be able to access original data from completed or ongoing studies, or who will be able to directly contact the original investigators.

## RADIOEPIDEMIOLOGIC TABLES

The NIH radioepidemiologic tables, mandated by Congress, were developed to meet a perceived need for an objective way to present and evaluate compensation claims for adverse health outcomes, such as cancer, that might be related to radiation exposure. The concept is simple: given a documented history of exposure to radiation $d_1, \ldots, d_k$ at ages $a_1, \ldots, a_k$ and a cancer diagnosis at age A, compute the ERR of a cancer at that age. The "probability of causation" (NIH 1985), or "assigned share" (NRC 1984), computed as ERR/(1 + ERR), is an informed quantitative estimate of the proportion of similar cancers at that age, in a large population of similar people with similar exposure histories that, would not have occurred in the absence of exposure, that is, the proportion of such cancers attributable to radiation. The ERR might depend on exposure history and age at diagnosis, but also on sex, time from each exposure until diagnosis, history of exposure to other carcinogens (such as tobacco), and other risk modifiers (such as reproductive history). Thus, all relevant factors known to influence radiation-related risk can be incorporated, as can various sources of uncertainty.

Estimation of radiation-related risk for radioepidemiologic tables is a useful check for the modeler and it relates to actual applications of the model such as claims for compensation in individual contested cases. Each possible estimate is to be treated as the current scientific consensus judgment in a particular case. For example, a model that produces a sharp change in estimated probability of causation, depending on whether exposure occurred at age 39 or 40 yr, would lack credibility even though it might fit the data better than a model with a smooth exponential decline in ERR with increasing age at exposure. It can be argued that a model that agrees well with scientific observations while avoiding anomalies that would seem unreasonable and capricious as a basis of real-life decisions would suit the interests of the sponsors of BEIR VII.

There is a legal requirement for periodic revision of the 1985 radioepidemiologic tables, which now are out of date in view of changes in understanding of radiation-related risk over the last decade or so. Any such revision presumably will depend heavily on the algorithms developed in the most recent BEIR reports, modified as necessary to meet the requirements for plausibly equitable decisions in individual cases. It would be appropriate for the BEIR VII phase-2 committee to produce its risk estimates in a format that would enable scientists revising the radioepidemiologic tables to incorporate the latest BEIR estimates.

# 3

# CELLULAR AND MOLECULAR CONSIDERATIONS

## DNA DAMAGE AND ITS REPAIR

The genetic material of cells is DNA, which is distributed among the chromosomes of eukaryotic cells and is bound to structural and other proteins. Because the two strands of DNA are complementary—a purine base (adenine or guanine) on one strand pairing with a pyrimidine base (thymine or cytosine) on the other strand—the information contained in the sequences of bases is redundant. In a haploid human cell, there are about $3 \times 10^9$ base pairs of DNA, which includes about 100,000 genes whose sequences specify all the structures and reactions that make up the cell and the entire human being, including the control of DNA replication and cell division. If there are DNA sequence changes in germ cells that affect offspring, the changes that result are hereditary changes or mutations. Changes in the normal sequences of bases in somatic-cell DNA as a result of endogenous reactions or exogenous agents might alter the normally well controlled cellular processes and result in loss of homeostatic regulatory mechanisms, loss of inhibition or stimulation of cell growth and division, or cell death. The uncontrolled and metastatic growth of tumor cells, derived from previously normal cells, is associated with changes in DNA sequence in somatic cells.

DNA in humans is a large molecule subjected to hydrolytic attack and to endogenous oxidative and other damage at 37ºC. For example, it has been estimated that 2-10 × $10^3$ DNA purines (of a total of about 3 × $10^9$) turn over in each human cell each day (Lindahl 1993). Over a 70-yr lifetime, depurination could affect 10% of a person's DNA. Furthermore, DNA alterations caused by the deamination (removal of an amine group) of cytosine and 5-methyl-cytosine (and to a lesser extent adenine and guanine) lead to coding changes that must be rectified.

In addition to the damage that results from its normal chemical bond breakage and reunion errors, DNA is assaulted by reactive oxygen species generated by "leakage" from mitochondria, flavin-catalyzed reactions, and many other sources, including phagocytosis and inflammation (Beckman and Ames 1997). The superoxide radical ($O_2$-), formed by one-electron reduction of molecular oxygen, is generated in all aerobic cells. Chemical or enzymatic dismutation of $O_2$- produces hydrogen peroxide, $H_2O_2$. The toxicity of these species has been attributed to the highly reactive hydroxyl radical ($OH^{\cdot}$), which can be formed by reactions of $O_2$- and $H_2O_2$. Floyd (1995) has estimated that about 1% of the oxygen consumed by human cells is diverted to oxidizing cellular protein and that 0.001% of the oxygen molecules damage DNA and RNA; these numbers undoubtedly increase under conditions of oxidative stress, such as during chronic inflammation. Although protein and small molecules, such as glutathione, serve as scavengers for reactive oxygen and thus protect the nucleic acids, there is a considerable amount of oxidative DNA-base damage per cell per day (Saul and Ames 1986). However, the steady-state level of DNA damage is low, so most of the spontaneous and metabolically-generated damage is apparently repaired efficiently and correctly. Poor repair would allow the accumulation of excessive DNA damage that could interfere with DNA replication and transcription and ultimately threaten survival. Thus, although DNA in cells is frequently damaged, the damage is counteracted by DNA-repair processes.

Added to the sources of spontaneous damage and metabolically produced oxidative DNA damage is natural background radiation. The principal sources of external exposure from natural sources are cosmic radiation and naturally occurring radionuclides in the earth/soil. The primary sources of internal exposure are radionuclides, such as potassium-40, deposited within tissue. Collectively, these two sources deliver effective (whole body) close rates to members of the US public that range from 1 to 2 mSv per year. One sievert represents an amount of absorbed energy equivalent to 1 J/kg, adjusted to take into account the quality factor of the radiation. Artificial radiation sources, such as x-rays used in medical diagnosis and radiopharmaceuticals used in nuclear medicine, add an additional effective dose rate to the average member of the US public of about 0.50 mSv per year—0.40 mSv from medical x-rays, and about 0.14 mSv from nuclear medicine (NCRP 1989). The total effective dose rate from these two artificial sources is thus about half that from the natural background sources cited above. In addition, naturally occurring indoor radon and its

airborne radioactive decay products, whose concentrations vary widely from one geographic location to another, add an estimated effective dose rate of 2 mSv per year to the average member of the US public (NCRP 1987). Ionizing radiation produces $OH^{\cdot}$ and other radicals ($e_{aq}^-$ and H atoms) by interacting with cellular water and exerts the bulk of its biologic effects in cells through these free radicals, in particular $OH^{\cdot}$. Ionizing radiation produces several classes of damage to DNA, including single-strand breaks (SSBs) and double-strand breaks (DSBs) in the DNA chains, DNA-DNA covalent cross-links, DNA-protein covalent cross-links, and a large variety of oxidative changes in the nucleotide bases (Hutchinson 1985; Ward 1988). The identified oxidative base products of ionizing radiation are chemically identical with those produced by other oxidizing agents, such as $H_2O_2$ in the presence of iron or copper ions, and those resulting from the normal metabolic production of free radicals that are byproducts of the transport of electrons to oxygen in mitochondria (Dizdaroglu and others 1987, 1991a,b; Gajewski and others 1990; Nackerdien and others 1991; Dizdaroglu 1992; Beckman and Ames 1997). Ionizing radiation damages DNA both through direct deposition of energy in the DNA (which is considered to include the first layer of tightly bound water) and indirectly through the generation of $OH^{\cdot}$ radicals in the water within the immediate vicinity of the DNA. Early experiments demonstrated that about 70% of the DNA damage can be prevented by the addition of $OH^{\cdot}$ scavengers (Roots and Okada 1972). Because $OH^{\cdot}$ is so highly reactive, it has been estimated that only the radicals formed within about 3 nm of the DNA can react with it (Ward 1994).

It has been argued in both the scientific and lay press that the quantity of spontaneous and metabolically generated DNA damage is many orders of magnitude larger than that resulting from low, protracted doses of radiation from environmental sources implying that the contribution from low doses of ionizing radiation is trivial (Billen 1990; Beckman and Ames 1997)—in other words, that the DNA damage produced by background radiation and the even higher doses to which some workers are exposed does not add appreciably to the extensive spontaneous and metabolic damage and can be ignored.

A counterargument is based on unique aspects of ionizing radiation damage to DNA. Accumulated evidence shows that the products of ionizing radiation differ from chemically generated oxidation products in the microdistribution of the damage rather than in the chemistry of the individual lesions (Ward 1981, 1988, 1994). A portion of the energy of ionizing radiation, primarily that from secondary electrons, is deposited in large-enough packets to produce clusters of $OH^{\cdot}$ radicals. Clusters of ionization were first observed in a cloud chamber (Wilson 1923), then extended to liquid water (Samuel and Magee 1953), and later shown to result from properties of the radiation-track structure (Goodhead 1989, 1994; Pimblott and Mozumder 1991). Because $OH^{\cdot}$ has a very short range owing to its high reactivity, it can produce a cluster of damage within a

few base pairs along the DNA if the cluster is generated within 3 nm of the DNA. Ward and others (1985) have referred to such lesions as multiply damaged sites (MDSs). The probability of clustered damage increases with dose and linear energy transfer (LET) but is independent of dose rate because it results from the passage of a single-particle track (Prise and others 1994; Holley and Chatterjee 1996; Rydberg 1996). A DSB resulting from a single energy deposition is the most obvious example of a MDS, but other combinations of strand breaks, cross-links, and base or sugar products can also occur (Ward 1994). Furthermore, both direct interactions of radiation with DNA and reactions of $OH^{\cdot}$ contribute to the complexity of MDSs (Nikjoo and others 1997).

A second property of ionizing radiation that might distinguish it from chemical radicals is the extensive production of peroxyl radicals due to initial damage to molecules other than DNA (Floyd 1995; Milligan and others 1996). Peroxyl radicals produce oxidized bases but not DNA strand breaks and might account for the greater-than-expected yield of base damage, as opposed to strand breaks, observed in irradiated cells (Nackerdien and others 1992), as well as the production of double base lesions by single radicals that have been observed in irradiated oligonucleotides (Box and others 1995).

Ward has calculated that 5 μM $H_2O_2$ can produce 15 Gy-equivalents of SSBs in mammalian-cell DNA in 30 min through $OH^{\cdot}$ generation catalyzed by iron ions bound to DNA; on the basis of these SSB yields, 1000 Gy-equivalents are required to kill cells (Ward and others 1985). Similarly, on the basis of the mount of Oxidative base damage excreted by rats each clay (4,600 molecules of thymine glycol, an amount equivalent to that produced by 4.7 Gy of ionizing radiation per day), or the measured mount of 8-oxoguanine generated daily in each rat liver cell (80,000 molecules or 40 Gy-equivalents), base damage cannot be of consequence in the killing of cells by ionizing radiation (Ward 1988; Beckman and Ames 1997). In fact, at the $D_{37}$ dose for cell-killing, it has been calculated that each cell will have sustained 2.5 million SSBs for $H_2O_2$ and 400,000 pyrimidine dimers for UVC radiation. In contrast, the $D_{37}$ dose for low-LET ionizing radiation produces only 1,000 SSBs but 40 DSBs, a type of damage that is not characteristic of lethal doses of $H_2O_2$ or UVC radiation. Such data suggest DSBs are the critical lethal phenomena. DSBs and other MDSs are peculiar to ionizing radiation and a few radiomimetic agents, such as bleomycin and neocarzinostatin.

The mount of energy deposited that can yield MDS increases with LET, and MDSs are generally thought to explain the increased biologic effectiveness of high-LET radiation and the poor repairability of the induced DNA damage. At the least, clustering will create complex DSBs within up to 10 bp or so (Ward and others 1985; Holley and Chatterjee 1996). Because of the wrapping of DNA around nucleosomes and the organization of the chromatin fiber, some clusters might include DSBs at two or more sites that are several kilobase pairs apart or even removed from each other by the distance of a chromosomal loop of about 100 kbp (Lobrich and others 1996; Rydberg 1996). A relationship between the protein composition of the nuclear matrices of cells deficient in

the repair of DSBs and chromosomal-loop dynamics is consistent with the idea that chromosome structure affects both DNA and cellular radiosensitivity (Roti Roti and others 1993; Malyapa and others 1994, 1996). Chromatin proteins and condensation can also directly affect access of $OH^{\cdot}$ to DNA and thereby protect DNA from damage (Ljungman 1991; Ljungman and others 1991; Warters and Lyons 1992; Elia and Bradley 1992; Chiu and others 1992; Xue and others 1994). A more open structure can make active chromatin domains more sensitive than the bulk condensed chromatin to radiation damage (Chiu and others 1982; Bunch and others 1992). The nuclear matrix and its associated DNA can also suffer excess damage, both DSBs and DNA-protein covalent cross-links, became of its more open structure relative to the bulk chromatin and because of the binding of metal ions capable of catalyzing the formation of additional $OH^{\cdot}$ (Chiu and others 1986, 1993, 1995; Balasubramaniam and Oleinick 1995).

For cells to survive without mutations, DNA damage must be faithfully repaired. Whereas spontaneous damage is readily repaired in repair-competent cells, the DSBs and clustered lesions produced by even low-LET radiation are likely to be repaired with difficulty or incorrectly, if at all (Ward 1988, 1994). However, conventional assays for monitoring the yield and repair of DSBs would not detect the majority of MDSs (ones that contain one or no initial strand breaks) and would treat complex DSBs as simple ones. One approach to study the repair of clustered damage is to synthesize oligonucleotides that contain defined sets of damage and to monitor the ability of specific repair enzymes to act on those sequences, as opposed to sequences that contain simple types of damage. One study has demonstrated impairment of repair if two base damages lie within 5 bp of each other (Chaudhry and Weinfeld 1995). Given the very large number of possible combinations of lesions within MDSs and the several types of enzyme systems that might attempt repair, considerably more work is needed on this subject. If two or more DSBs occur within a single chromosomal loop, the fragment between the two breaks is theoretically no longer bound to the nuclear matrix and might be more difficult to repair.

Within the limits of detection of standard assays of DNA damage, induction of DSBs and other lesions in cellular DNA is generally found to depend linearly on radiation dose (Iliakis and others 1992; Lange and others 1993). Assays for the measurement of removal of base damage or the rejoining of SSBs or DSBs reveal that repair begins in cells as soon as radiation damage occurs. DSB rejoining proceeds rapidly with apparently biphasic kinetics; the half-time for the first (rapid) repair phase has been estimated at about 10-20 min and that for the second phase about 0.5-2.0 h (for example see Metzger and Iliakis 1991). The initial rate of damage removal decreases modestly with increasing dose, and the extent of residual unrejoined DSB might increase with dose. The data suggest that the enzyme systems for DSB rejoining are constitutively present in repair-competent cells. However, such measurements are made at supralethal radiation doses and cannot detect the removal of all the lesions; furthermore, some components of repair of the measured DSBs might be inducible.

The steady-state level of DNA damage is low, so extensive spontaneous damage must be rapidly and effectively repaired. Errors in DNA replication, such as the placing of a thymine opposite a guanine, create mismatches that are corrected by "proofreading" activities of the DNA polymerase complex and removal of the newly incorporated incorrect base before the next base is added. Alternatively, if the mismatch is not at the growing end of a DNA chain, mismatch-repair enzymes remove the wrong nucleotide, and the resulting gap in one strand is filled in properly by a DNA polymerase. Defects in the mismatch-repair enzymes have been associated with genetic instability and the human familial syndrome hereditary nonpolyposis colon cancer (Modrich 1994; Fishel and Kolodner 1995; Marta and Boland 1995). Most oxidative base damage and SSBs, including those derived from ionizing radiation and from metabolic sources, are efficiently repaired by the base-excision repair pathway, which initiates the removal of damaged bases via the generation, by one of several specific N-glycosylases, of an apurinic-apyrimidinic (AP) site, which is then a substrate for associated AP endonucleases (Demple and Harrison 1994; Wallace 1994). Some kinds of oxidative base damage are also repaired via the nucleotide-excision repair pathway that is thought to be the primary repair mechanism for UVC-induced pyrimidine dimers and bulky adducts (Sancar and Tang 1993; Sancar 1995). In spite of the importance of those repair systems, none of the human syndromes that are characterized by a sensitivity to ionizing radiation have been attributed to defects in the repair of oxidative damage, with the possible exception of Cockayne syndrome, discussed below.

The repair of DSBs in human cells is effected primarily by nonhomologous end joining (NHEJ) and less by homologous recombination and single-strand annealing (Thompson 1996). NHEJ requires the participation of DNA-dependent protein kinase (DNA-PK), the enzyme system that carries out the end-rejoining component of V(D)J recombination in developing immune cells. DNA-PK is composed of a dimer of DNA-end-binding proteins Ku-70 and Ku-86 (the Ku autoantigen), which serve as a nucleus for the binding of the 470-kDa catalytic subunit (DNA-PKcs) (Jeggo and others 1995). Cells deficient in DNA-PK subunits are defective in DSB repair and V(D)J recombination and highly sensitive to ionizing radiation (Biedermann and others 1991; Taccioli and others 1993). DNA-PKcs is a serine-threonine kinase that is a member of the phosphatidyl inositol-3-kinase family (Hartley and others 1995). Another member of this kinase family is ATM, the protein mutated in ataxia telangiectasia (AT), a disease that is also characterized by immune deficiencies and radiosensitivity (Jorgensen and Shiloh 1996). In spite of limited homology between DNA-PKcs and ATM in the kinase domain, the substrates for the two enzymes are different, and cells from AT patients, although highly sensitive to the lethal effects of ionizing radiation and defective in normal radiation-induced cell-cycle progression delays by radiation damage, are not defective in the repair of DSBs (Meyn 1995). Evidence of the existence of other rejoining mechanisms, such as one operating in late S/G2 phase of the cell cycle, has also been obtained (Whitmore and others 1989). The fidelity of the rejoining process is largely accurate to within ±100 nucleotides (Lobrich and others 1995), but the nucleotide-sequence accuracy of the process remains to be determined.

## CONCLUSIONS

Interestingly, the enzymes of DNA repair in normal mammalian cells are constitutively present and do not require induction to repair DNA damage. Thus, if ionizing-radiation damage is produced as a linear function of dose, if a component of that damage is unique (such as MDSs) and not found as a segment of the background DNA damage, if the requisite repair enzymes do not require induction, and if the repair rate is not markedly altered as a function of dose, one could conclude that even the lowest dose of radiation can be biologically significant. In contrast, radiation damage can trigger a plethora of inducible processes, some of which can affect damage-recognition processes, repair, or the cellular responses to initial or unrepaired damage.

## CONDITIONED AND INDUCIBLE RESPONSES TO RADIATION

As elaborated in the previous section, the cell contains a variety of mechanisms for repairing or tolerating damage deposited in DNA by spontaneous or endogenous events, as well as by environmental radiation and chemical agents. The capacity of these repair systems is set at some constitutive level such that a steady-state concentration of DNA lesions remains in the genome of normally growing cells. It is interesting and important that this steady-state concentration is nonzero and that some of the lesions that persist are known to contribute to mutagenesis and other potentially deleterious biologic end points. In principle, the frequency of spontaneous mutations would be lower if the constitutive efficiency of DNA-repair were higher. We do not yet understand how the efficiency of the DNA repair systems is regulated so as to maintain the steady-state concentration of lesions, but we do appreciate that the constitutive repair systems provide only a limited capacity to deal with additional damage that might be inflicted by external threats, such as ionizing radiation. We have also learned in recent years that not all lesions are equally accessible to recognition by repair enzymes. Intragenomic DNA repair is heterogenous; lesions in some domains of the genome are poorly repaired; whereas those in others are repaired with relatively high efficiency (Hanawalt 1991). Thus, some bulky DNA adducts in the highly repetitive DNA sequences found near centromeres are poorly repaired, in comparison with the overall genome, and lesions that block transcription appear to be preferentially repaired. In particular, the lesions in the transcribed DNA strand that arrest the progression of polymerase II are preferentially repaired. Perhaps this so-called transcription-coupled repair mode has evolved because the stalled RNA polymerase otherwise encumbers recognition and repair of the arresting lesion (Hanawalt 1994). The existence of intragenomic DNA-repair heterogeneity means that we need to understand the fine structure of lesion processing in relation to the particular genes or genomic domains responsible for the biologic end point of interest, such as cancer. In addition to the heterogeneity of DNA repair at the level of the gene, there is heterogeneity at the level of the nucleotide. Thus, a given type of lesion might be much more efficiently repaired in one nucleotide-sequence context than in another. In some cases (for example, in the p53 tumor suppresser gene) the sites of slow repair have been shown

to correlate with the sites that are most frequently found to be mutated in tumors (Tornaletti and Pfeifer 1994).

The cell is not passive in its response to environmental genotoxic threats. A wide variety of genes are known to be activated by such agents as ultraviolet light (UV) or ionizing radiation, although only a few of them have been directly implicated in DNA repair in mammalian cells. Genes inducible by x-rays include the p53 tumor-suppresser gene, the proliferating-cell nuclear antigen (PCNA), and the DNA polymerase ß. Polymerase ß is used in the primary pathway of base-excision repair, so it is important for the repair of some of the principal types of base damage produced by radiation. PCNA is the "sliding clamp" that ensures processivity of DNA polymerase δ/ε for both chromosomal replication and repair replication in the process of nucleotide-excision repair. Additional DNA-repair genes will probably be shown to be inducible in mammalian cells. In model bacterial systems, several inducible systems are now well understood at the biochemical level and clearly involve up-regulation of DNA-repair gene expression. Thus, the SOS system, controlled by the recA-lexA regulatory circuit, results in the induction of the *uvrA, uvrB*, and *uvrD* genes (and others), with consequent enhanced efficiency of nucleotide-excision repair. In the adaptive response to alkylation damage, glycosylases specific to DNA lesions and a 6-alkyl-guanine transferase are induced, thereby leading to greatly enhanced tolerance of agents that produce alkylation damage.

We know much less about the induction of specific DNA-repair pathways in mammalian cells, but some reported phenomena are consistent with the existence of such pathways. Thus, the basic phenomenon of "Weigle reactivation" that originally led to the discovery of the SOS response in bacteria, has also been confirmed in mammalian cellular systems. In brief, that phenomenon involves the enhanced survival of UV-damaged virus when the host cells have been preconditioned by exposure to low doses of UV or other DNA-damaging agents. In bacteria, the enhanced survival is now known to be due primarily to the up-regulation of genes involved in the damage-recognition step of nucleotide-excision repair, as noted above. Similarly, the UV-induced and p53-dependent up-regulation of global excision repair in human cells might be due to enhanced expression of DNA-repair genes (Ford and Hanawalt 1995, 1997).

A number of recently reported provocative phenomena in mammalian cell systems deserve careful study to determine their biochemical mechanisms and possible relevance to the low-dose response to radiation and the question of linearity of that response. It has been shown, that in cultured human lymphocytes, low doses of radiation result in the "protection" of the cells from the chromosomal aberrations or mutations that would otherwise result from later exposure to radiation (Olivieri and others 1984; Sanderson and Morley 1986; Wiencke and others 1986; Kelsey and others 1991; Wolff 1992; Shadley 1994) or to some chemical agents that produce DSBs. In human fibroblasts, a low chronic exposure to radiation was found to reduce the frequency of micronuclei derived from later acute radiation exposure; this finding is evidently correlated with an increased

rate of DSB repair. Furthermore, it has been shown in C3H10$T_{1/2}$ cells that the frequency of spontaneous neoplastic transformation can be reduced by a factor of 3 or 4 by a single exposure of the quiescent cells to radiation doses as low as 0.1 cGy. However, it is important to note that the subclone of C3H10$T_{1/2}$ cells used in these experiments exhibited an unusually high level of spontaneous transformation and that the basis for that phenotype is not understood (Azzam and others 1996).

Some recent studies suggest that important biologic effects, including the induction of sister chromatid exchanges and changes in gene expression, can occur in an irradiated population in cells that have received no direct radiation exposure. These so-called bystander effects might be a consequence of communication among cells in the population, although in some cases the results might be explained by indirect effects, such as radiation action on components of the culture medium. Recent evidence has implicated the up-regulation of oxidative metabolism and the production of active oxygen species as mediators of the effects. It is important to appreciate that the intercellular communication that exists in the normal tissue environment of cells in an intact organism (such as a human) is a complication that ultimately limits the utility of model cultured-cell systems in vitro. Another interesting phenomenon is the induction by ionizing radiation of a type of genomic instability whereby important biologic effects occur in the progeny of irradiated cells after many generations of cell replication. The occurrence of this effect has now been confirmed in a number of laboratories for end points that include mutagenesis, cytogenetic changes, and reproductive failure (Kadhim and others 1992; Grosovsky and others 1996; Morgan and others 1996; Little and others 1997). The mechanisms by which this instability is induced and maintained over a long period remain to be elucidated.

The p53-regulated pathways are important and have received much recent attention because mutations in the p53 gene are found in a large percentage of human tumors. p53 is regulated primarily at the level of translation and the stability of the protein, and it is involved in cell-cycle checkpoints, in apoptosis, and in nucleotide-excision repair. In the cancer-prone Li-Fraumeni syndrome, fibroblasts expressing only mutant p53 exhibit little apoptosis and are therefore radiation-resistant. Interestingly, they are deficient in global nucleotide-excision repair but proficient in transcription-coupled repair (TCR) (Ford and Hanawalt 1995). The loss of p53 function can lead to genomic instability by reducing the efficiency of genomic repair whereas cellular resistance is ensured through the operation of TCR and the elimination of apoptosis. Recent reports from several laboratories suggest that an important inducing signal for p53 stabilization and consequent apoptosis is the arrest of transcription at lesions in the DNA. In the case of Cockayne syndrome, characterized by deficiency in TCR, p53 and apoptosis are induced by much lower doses of radiation than in normal cells or in xeroderma pigmentosum complementation group C cells, which are proficient in TCR but deficient specifically in global genomic repair (Ljungman and Zhang 1996). The p53-induction pathway might be of particular relevance to low-dose radiation effects because of the demonstration that some base damage (such as thymine glycol damage) is subject to TCR and that people with Cockayne syndrome are defective in the TCR of this type of damage (Leadon and Cooper

1993). Cells from people with Cockayne syndrome have been shown to be sensitive to radiation and to UV radiation; this leads to the suggestion that the characteristic developmental problems in this hereditary human disease are be caused by cell (particularly neuron) loss due to enhanced apoptosis (Leadon and Cooper 1993; Hanawalt 1994).

A large number of radiation-induced gene products have been identified by comparing 2D gels after electrophoresis of extracts from irradiated and untreated control cells. Differential screening of cDNA libraries has been used to identify radiation-induced genes. Although most of these remain to be characterized, some of the early radiation-induced genes have been identified, including AP-1 and NF-KB, in addition to p53. In fact, AP-1 and NF-KB sites have been found in many UV-induced and radiation-induced genes, and these factors have also been shown to contribute to the induction of HIV-LTR after UV exposure. Cytokines have been shown to be induced by radiation, including IL-1d, TNF, interferons, IL6, TGFB, and bFGF. An important caution, however, is the finding that pathways of gene induction after radiation exposure might be different in endothelial cells from such pathways in other cells. One must therefore be cautious about generalizing from an inducible response in one type of cell to that in another—or for that matter from cultured cells to cells in a tissue. It is also important to understand the interaction between different repair pathways in that the results of knocking out or up-regulating a particular pathway are often unpredictable. The disruption of mismatch repair has been shown in a number of studies to enhance tolerance to DNA damage, including that produced by reactive oxygen species. Possibly the mismatch-repair system normally interferes with the processing, by nucleotide-excision repair, of some lesions produced by reactive oxygen species.

Comparisons between the widely varied genes induced by UV should be informative. For some proteins, the induction occurs within minutes and can be observed with x-ray doses as low as 10 mGy. The p53 response to x-rays reaches a peak several hours after irradiation but it is transitory and smaller than that after UV exposure (Lu and Lane 1993). Enhanced expression of p53 has also been reported in bystander cells in cultures exposed to alpha rays (Hickman and others 1994). The implication is that substantial communication occurs among the cells in culture and that the biologic effects in cell populations might not be restricted to the responses of the individual damaged cells, as noted earlier. Again, the complication of intercellular communication, when one considers cells in a tissue, is surely important.

The existence of inducible repair systems that improve the efficiency of DNA repair has fueled speculative proposals that low levels of ionizing radiation actually have beneficial, rather than deleterious, effects. These suggestions of hormesis in the radiation response must be considered seriously but critically. Some of the arguments do not take into account the important differences between the DNA-damage spectrum produced by radiation and that produced by endogenous reactive oxygen species—differences that influence the spontaneous mutagenesis level substantially. Thus, an argument for low-dose radiation hormesis goes as follows: If the low-LET background of 1 mGy/yr were

increased to 10 mGy/yr, and that stimulated a 10% increase in DNA-repair efficiency, then mutations due to background radiation would increase from $10^9$/day to $9 \times 10^9$/day (not $10 \times 10^9$/day), and the overall level of background mutations due to endogenous damage would decrease from $10^{13}$/day to $9 \times 10^{12}$/day (for a net decrease of $1 \times 10^{12}$) (Myron Polycove, personal communication).

## CONCLUSIONS

Some epidemiologic data have been cited as consistent with the existence of hormetic mechanisms. However, there have been no carefully controlled studies that negate the conservative view that even very low doses of radiation simply add to the burden of cellular damage and thereby increase the likelihood of deleterious mutagenesis. We need to obtain much more mechanistic information in the general area of inducible responses to DNA-damaging agents such as radiation.

## CHROMOSOMAL ABERRATIONS AND MUTATIONS

Since the acceptance of the unineme structure of chromosomes, it has been generally agreed that the DNA DSB, equivalent to a chromosomal backbone break, is the critical radiation-induced damage that leads to chromosomal aberrations. Experiments with different restriction enzymes that induce specific types of DSBs provide good evidence that both blunt-ended and staggered-ended DSBs can cause chromosomal aberrations (Bryant 1984; Natarajan and Obe 1984; Obe and Winkel 1985; Winegar and Preston 1985). The prevailing concept of the formation of chromosomal aberrations proposes that radiation induces two DSBs that interact with each other to produce aberration configurations—such as dicentrics, reciprocal translocations, and rings—through incorrect rejoining of the broken ends. Two mutually exclusive models are used to describe the formation of aberrations: the "classical" or "breakage-reunion" hypothesis and the "exchange" hypothesis (Lea 1946; Revell 1974; Bender and others 1974; Savage 1989). More recently, it has been proposed that aberrations might be derived from one DNA DSB via nonhomologous, or illegitimate, recombination (Chadwick and Leenhouts 1981; Szostak and others 1983; Goodhead and others 1993), although this hypothesis is not widely held.

Mutations are generally classified as point mutations, which are intragenic and thought of as small changes in a DNA gene sequence, or chromosomal mutations, which are intergenic and thought to result from major alterations in chromosomal structure. Mutations of both types are considered to arise from DNA DSBs, as is supported by evidence from restriction-enzyme cutting experiments (Singh and Bryant 1991), and the second type is clearly associated with large deletions of DNA and possibly chromosomal aberrations. Radiation is generally considered to induce chromosomal mutations rather than point mutations, although point mutations are found after radiation exposure (Vrieling and others 1985; Thacker 1986; Breimer and others 1986; Liber and others 1986; Kraemer and others 1994; Little 1994; Meuth and Bhattacharyya 1994; Simpson and others 1994).

Dose-effect relationships for chromosomal aberrations and mutations induced by sparsely ionizing radiation are invariably interpreted to have linear-quadratic dose kinetics, moving to linear dose kinetics for more densely ionizing radiation, which is more effective per unit of dose than sparsely ionizing radiation (Lloyd and others 1975, 1976; Cox and Masson 1979; Thacker and others 1979; Liber and others 1983; Hei and others 1988; Metting and others 1992; Jostes and others 1994). Dose-rate and dose-fractionation effects are observed for chromosomal aberrations, including translocations (Lloyd and others 1975; Schmid and others 1976) and for mutations (Asquith 1977; Thacker and Stretch 1983; Evans and others 1990; Thacker 1992; Elkind and others 1994). Correlations have been made between chromosomal aberrations and mutations and the cell killing that follows radiation exposure; they suggest that some types of radiation-reduced damage are common to the different biologic end points (Dewey and others 1970, 1971; Thacker and Cox 1975; Cox and others 1977; Bryant 1985).

Recent technologic developments and advances in molecular biology have led to new approaches to the investigation of the mechanisms underlying the induction of both aberrations and mutations. Those developments include the use of prematurely-condensed-chromosome techniques (Hittelman and Rao 1974; Pantelias and Maille 1985; Hittelman and others 1994) to investigate the early response to radiation damage in interphase chromosomes and, more important, the use of fluorescent in sire hybridization (FISH) or "chromosome painting," centromere and telomere staining (Pinkel and others 1986; Gray and others 1991; Natarajan and others 1992; Bauchinger and others 1993; Straume and Lucas 1993; Savage and Simpson 1994; Savage and Tucker 1996), and PCR methods and DNA sequencing (Meuth and Bhattacharyya 1994; Okinaka and others 1994; Singleton and others 1994) to identify the different types of chromosomal aberrations and the DNA-sequence changes in mutations formed after radiation exposure.

Inasmuch as specific types of chromosomal changes are associated with specific cancers and mutations in oncogenes and tumor-suppressor genes are involved in cancer development, it is important to understand the mechanisms underlying the radiation induction of aberrations and mutations. The dependence of the response of these cellular end points on dose, dose rate, and radiation quality needs to be defined because of the relevance of this knowledge to the assessment of low-dose radiation risk.

## Chromosomal Aberrations

Research on radiation-induced chromosomal aberrations has been most fertile and has provided the data leading to the development of theoretical approaches to describe the action of radiation at the cellular level (Lea 1946; Revell 1955). It continues to do so, probably because the aberrations visualized with a microscope represent an early indication of the radiation damage. The increasingly refined methods of staining chromosomes, either by using prematurely condensed chromosomes (PCCs) or by chromosome painting, and the use of specific types of radiation, such as carbon K-characteristic x-rays, continue to provide newer insights into the mechanisms of formation of chromosomal aberrations.

There is no consensus on those mechanisms even though their understanding is of utmost relevance to the assessment of radiation risk at low doses. However, there is a relatively good consensus on the shape of the dose-effect relationship for aberration induction suggesting that it is curvilinear in general and linear at very low doses. Attempts by groups of collaborating cytology laboratories to measure the dose-effect relationship for dicentrics at low doses have shown that linearity can be demonstrated down to 20 mGy but that at lower doses statistical variations mask any effect of radiation; measurements at doses below 20 mGy produced yields of dicentrics that were less than background but not significantly so (Pohl-Rohling and others 1983; Lloyd and others 1988). Those experiments did not reveal any evidence of a supersensitive response at the low doses; the researchers concluded that in view of the very large number of cells scored, it would be "very unlikely that the true response at doses less than 20 mGy will ever be measured directly with these techniques" (Lloyd and others 1992). The dose-effect relationship at doses lower than 20 mGy will have to be inferred from an understanding of the mechanisms of aberration formation.

More recent work with fluorescence in situ hybridization (FISH), or chromosome painting, to study the dose-effect relationship for the induction of translocations shows the same curvilinearity as found for dicentrics and a linear response at low doses. Some workers found that the proportion of dicentrics to translocations induced is 1:1 (Nakano and others 1993; Straume and Lucas 1993; Finnon and others 1995; Lucas and others 1995), while others found that there are relatively more translocations, with the proportion varying from 1:2 to 1:1.5 (Natarajan and others 1992; Schmid and others 1992; Bauchinger and others 1993; Tucker and others 1993), although it is expected that the probabilities of formation of symmetrical exchanges (such as reciprocal translocations) and asymmetrical exchanges (such as dicentrics) would be the same. More detailed measurements have revealed, moreover, that some chromosomes seem to be over represented in aberration formtion on the basis of the DNA content of the different chromosomes (Knehr and others 1994, 1996; Slijepcevic and Natarajan, 1994a,b; Finnon and others 1995). Chromosome 4 in humans seems to be particularly over represented. This work suggests that either the induction of chromosomal damage by radiation is nonrandom, or the rejoining of the breaks is nonrandom, but the implications for the mechanism of aberration formation are unclear.

Investigations into the mechanisms of aberration formation have become more sophisticated with the use of the newer PCC and chromosome-painting detection methods and the ultrasoft x-rays. The PCC technique permits the visualisation of interphase chromosomes and the scoring of fragments some 30 min after irradiation. The dose-effect relationship for the fragments is usually found to be linear with dose although the numbers of fragments found are considerably lower than the numbers of DSBs from which they are assumed to be derived. However, the yield of fragments measured in PCC studies has been found to depend on the mitotic cells that are used to induce chromosomal condensation in the irradiated cells (Cheng and others 1993), so the comparison of PCC experiments is compromised, and some care is needed in their interpretation.

Experiments with carbon K-characteristic ultrasoft x-rays, which create very short electron tracks (less than 7 nm), demonstrated an efficient induction of exchange aberrations with a strong linear component in the dose-effect relationship (Virsik and others 1980; Thacker and others 1986). Those results have been interpreted as implying "either that the participating DNA helices must be lying extremely close together at the time of radiation damage, so that one track can effectively damage both helices, or that only one radiation-damaged chromosome is needed to promote an exchange event" (Thacker and others 1986). The results appear, at first sight, to contradict the breakage-reunion concept of aberration formation, although explanations of the ultrasoft x-ray results have been sought in the application of "proximity" concepts to the breakage-reunion theory (Sachs and others 1997) and in the kinetics of formation of aberrations (Brenner 1990; Greinert and others 1995, 1996). However, a chromosome painting study of the induction of complex chromosomal-exchange aberrations, with more than two breaks, by ultrasoft aluminium K x-rays that have a track length of 70 nm has concluded that "for the classical breakage-and-rejoining theory to hold, very large interaction distances are needed . . . unless many sites pre-exist where several different chromosomes come very close together" (Griffin and others 1996). The authors of the study suggest as an alternative that damaged DNA interacts with undamaged DNA to produce an exchange aberration. In a study of the nature of chromatid breaks, using bromodeoxyuridine (BrdU)-differentiated sister chromatids to estimate the proportion of breaks associated with a "color jump" and thus arising from incomplete intra-arm intrachanges rather than from simple breaks, Harvey and Savage (1997) found that the proportion of the color-jump breaks is substantial and almost constant irrespective of radiation dose, radiation quality, BrdU concentration, and cell origin and is similar to the proportion after restriction-enzyme cutting. They concluded that the results are "very difficult to reconcile with the expectations of breakage-and-reunion theory," although they are in line with both the exchange hypothesis and the idea that a single damaged chromosome can interact with an undamaged chromosome to yield an exchange.

The concept of the interaction of a damaged and an undamaged chromosome in exchange formation was not supported by experiments in which irradiated cells were fused with unirradiated cells to determine whether exchange aberrations were formed between the irradiated and unirradiated chromosomes (Cornforth 1990). The low frequency of intergenomic exchanges found suggested that the "majority of radiation-induced exchanges do require damage to both chromosomes." That result casts doubt on the concept of interaction between a damaged and an undamaged chromosome in the formation of aberrations. However, one of the attractive features of the concept is that it is based on a proposal for the recombination repair of DNA DSBs (Resnick 1976), which makes use of known enzymatic processes such as exonuclease degradation, endonuclease nicking, topoisomerase unwinding, and polymerase and ligase sealing—as well as suggesting a role for homology in DSB repair, of which there is increasing evidence in yeast (Resnick and others 1996). The concept also provides a potential link between DNA-repair studies and chromosomal-aberration formation.

There is no clear consensus on the mechanisms Evolved in the formation of chromosomal aberrations. The "classical" theory has been modified by "proximity" factors to take account of the results of the ultrasoft x-ray experiments but remains in doubt as a consequence of the "color-jump" experiments on chromatid breaks, which clearly favor the "exchange" hypothesis or the "recombination" hypothesis. Whichever hypothesis is finally shown to be correct, DNA DSBs and complex damage are currently the implied relevant radiation-induced lesions, and the dose-effect relationship at low doses is assumed by many to be linear.

## Mutations

The type of mutation most often associated with radiation exposure is a large chromosomal deletion that can lead to the loss of the target gene and loss of additional DNA extending on both sides of the gene. The mount of DNA lost in a mutational deletion present in a viable mutant depends on whether the adjacent genes are essential for cell viability; and studies of deletions at different target genes in different cell systems reveal considerable variation in the mount of material lost (Thacker and others 1979; Evans and others 1986; Waldren and others 1986; Thacker 1990). In some cases, the amount of material lost is so large that it can be detected cytologically (Simpson and others 1993, 1994). The target genes most often used in mutation studies are present in the cell in only one functional copy, that is, monosomic, such as the HPRT gene on the X chromosome in male cells whereas most genes are present in the cell in two copies—that is disomic. In the case of disomy, a recessive mutation in one (allele) of the target genes would not be revealed in the phenotype, because of the presence, and activity, of the other copy (allele). Therefore, experimentally, one copy of the target gene normally carries a small inactivating point mutation, and the radiation effect is studied in the other copy. In such studies, it has often been found that the frequency of induced mutants is higher than in the monosomic case (Evans and others 1986; Moore and others 1986; Yandell and others 1986; Bradley and others 1988), most probably because the allele with the point mutation is in a maintained genetic region, so that large deletions in the other target copy can be tolerated. Here, again, the mutant frequency depends on whether essential and active genes are in the neighborhood of the target gene. Differences have been found in the mutant frequencies that result when the target gene is switched from one *TK* allele to the other in lymphoblastoid cells (Amundson and Liber 1991, 1992). Thus, depending on the local genomic situation of the target gene, wide variations in the mutant frequency induced by radiation in different target genes can be expected hacker 1996).

The loss of large mounts of DNA in radiation-reduced mutations makes it difficult to sequence them, but in the few that have been sequenced it has been found that short, direct or inverted repeat sequences are associated with the break points (Miles and others 1990; Nicklas and others 1991; Morris and Thacker 1993; Morris and others 1993). A comparison of the spectra of mutations induced by sparsely and densely ionizing radiation has revealed conflicting data; some results indicate differences between the spectra of different radiation types, and other results indicate very little difference (Thacker 1986;

Gibbs and others 1987; Kronenberg and Little 1989; Lutze and others 1990; Whaley and Little 1990; Aghamohammadi and others 1992; Lutze and others 1992, 1994; Jostes and others 1994; Bao and others 1995; Jin and others 1995; Kronenberg and others 1995; Chaudhry and others 1996). There is some indication that after high-LET radiation, more-complex mutational rearrangements are involved, in addition to the short repeat sequences (Meuth 1990; Simpson and others 1993; Thacker 1996).

The findings of short direct-repeat DNA sequences at sites of large-deletion rejoining, as well as the more complex rearrangements, suggest that a form of illegitimate recombination initiated by a break in DNA is involved in the mutational process. That idea is supported by the results of experiments that reconstructed the process of illegitimate recombination in cell-free conditions by using a DNA substrate with a site-specific DSB and showed that misrejoining is associated with short direct—repeat sequences on either side of the break (North and others 1990; Ganesh and others 1993; Thacker 1994). Research with heterozygotes, in addition to indicating tolerance of large deletions, has also indicated the possibility of mitotic recombination or nondisjunction with a suggestion that recombination is more common than deletion in spontaneous mutants (Fujimori and others 1992; Li and others 1992). There is also an interesting result of a comparison of two cell lines derived from the same original cell but differing in p53 status, DSB rejoining, and recombination ability. The cell line resistant to radiation-reduced killing had a higher radiation-induced mutant frequency, which suggests that it has a higher rate of recombination and can then survive with a concomitant higher rate of mutation (Amundson and others 1993; Xia and others 1994). Delayed apoptosis might well be the reason for this cell's resistance to cell killing (Xia and others 1995; Zhen and others 1995).

In addition to the large deletions induced by radiation, a study of the HPRT and APRT genes has revealed that all types of small mutations occur in response to radiation—such as base-pair substitutions, frameshifts, and small deletions—and that they occur at sites distributed throughout the target genes (Grosovsky and others 1988; Miles and Meuth 1989; Nelson and others 1994). In contrast, spontaneous point mutations tend to occur preferentially at particular sites in the genes. Radiation leads to more transversion and frameshift mutations than are found spontaneously, but large intergenic mutants occur spontaneously at a substantial frequency.

The determination of quantitative dose-effect relationships is more difficult in the ease of mutation than in the ease of chromosomal aberrations but the measurements that have been made indicate a curvilinear relationship for sparsely ionizing radiation, in general, and a linear relationship at low doses (Cox and others 1977). Recent molecular-biology techniques are providing more insight into the mechanisms that lead to mutations after radiation. Although DNA DSBs and complex damage are clearly implicated with recombinational repair processes, the precise mechanisms remain to be elucidated.

### CONCLUSIONS

Considerable progress is being made toward understanding the mechanisms that lead to the formation of chromosomal aberrations and the induction of mutations. It appears that DNA DSBs and complex damage are the critical lesions, but there is no consensus. Both end points can readily be associated with carcinogenesis, so it is relevant that dose-effect relationships for both end points are curvilinear with a strong indication of linearity at low doses. Both end points also show similar behavior with respect to decreasing dose rate and radiation quality. It is also relevant that measurements of dicentric yields at doses less than 20 mGy do not, and probably will not, provide experimental data that will define the shape of the dose-effect relationship at low doses. The shape of the dose-effect curve will need to be inferred from a deeper understanding of the mechanisms involved in the formation of aberrations and the induction of mutations.

# 4

# ANIMAL STUDIES

Experimental studies of radiation carcinogenesis in animals have been used to develop biologic principles applicable to human risk estimates and to the development and testing of mechanistic models. Long-term animal studies conducted from the 1950s through the 1980s provided a substantial amount of quantitative information on dose-response relationships for a number of radiation-induced tumors alter gamma irradiation and on the influence of dose rate and fractionation on these relationships (NCRP 1980; UNSCEAR 1988, 1993). Studies have also focused on the carcinogenic effects of fission-spectrum neutrons (NCRP 1980, 1989; UNSCEAR 1988). These studies were essentially complete before 1990 and were, for the most part, available to the previous BEIR committee.

The major conclusions derived from the studies were as follows:

- The dose-response relationship for cancer induction after gamma irradiation could generally be described by a linear-quadratic function.
- Lowering the dose rate resulted in a diminution of the carcinogenic effects at high total doses as a result of a reduction in the quadratic component; the dose-response relationship was linear over a wide range of total doses.
- The linear slope of the response at low doses was similar to that for the linear portion of the linear-quadratic function after high-dose-rate exposures.

Based on studies of the irradiation of animals with neutrons a linear dose-response relationship was observed for the induction of most tumors at doses of 0.0 to 0.2 Gy; it was followed by a plateau or bending over of the curve at higher doses. Reducing the dose rate either had no effect on the dose-response relationship in the low-dose range or, in some instances, it increased the response per unit of dose. The differences in shape of the dose-response curve for cancer induction by gamma rays and neutrons resulted in the assignment of rather high relative-biologic-effectiveness (RBE) values for cancer induction at low doses. All the above data are consistent with biophysical models of radiation effects applicable to a variety of other end points, including radiation-induced cell-killing, induction of chromosomal aberrations, and radiation-induced mutation. These models predicted linear-quadratic dose-response relationships and reduced effectiveness per unit dose of low-LET radiation at low doses and low dose rates (Kellerer and Rossi 1972; Ullrich and Storer 1979).

Because of their consistency with projections from biophysical models of radiation effects, the combination of dose response and dose-rate data for tumor induction obtained from animal studies and data on various end points in animal and human cells provide substantial support for the application of a dose and dose-rate effectiveness factor (DDREF) in the estimation of cancer risks in human populations at low doses and low dose rates (UNSCEAR 1988; NRC 1990; ICRP 1991).

The high RBEs for neutrons at low doses (also predicted on the basis of biophysical models) observed in animal studies was important in the modification of quality factors used in risk estimates for neutrons (ICRP 1963). The neutron data are also likely to be important in the future analysis of data on atomic-bomb survivors, inasmuch as a portion of the dose that they received was from neutrons, but the contribution is still being evaluated.

After analysis of the results of long-term studies, it was recognized that understanding of radiation risks at low doses would not be improved by attempting to measure the effect at low doses on animals, but rather would require a better understanding of the underlying mechanisms. As a result, experimental studies of carcinogenesis since the last BEIR report have focused on mechanisms and on the cellular and molecular events involved in neoplasia. Over this time, the understanding of molecular events involved in the carcinogenesis process, in general, has increased dramatically. It is now clear that cancer development entails alterations in multiple genes that are involved in the regulation of progression through the cell cycle, cell growth and differentiation, and cell death, and in genes that are involved in the maintenance of genomic fidelity. A number of investigators have now demonstrated that alterations in genes that control genomic fidelity can play a major role in the early events leading to cancer by conferring a mutator phenotype on the affected cells (Loeb 1991, 1997). Cells with alterations in other critical genes later arise as a result of clonal selection.

The long latent periods and the complexity of the neoplastic process have been formidable obstacles in identifying specific radiation effects that initiate the sequence of events in cancer development at the cellular and molecular level. However, some generalizations can be made. Both in vitro and in vivo studies have amply demonstrated that radiation acts principally at the level of initiation of the carcinogenic process and is considerably less effective in promoting already-initiated cells or in influencing the progression of neoplasia (Han and Elkind 1982; Hill and others 1987, 1989; Bowden and others 1990). Mechanisms by which radiation initiates carcinogenesis are still poorly understood. It is generally accepted that the carcinogenic effects of radiation are related to its clastogenic and mutagenic effects, but no causal relationship between changes in specific genes and the development of radiation-induced cancer has been established. In fact, initiation frequencies derived from recent studies that used in vivo/in vitro models for radiation-induced cancer (with initiation frequencies around $10^{-2}$ initiated cells per Gy) are not compatible with a target whose size is limited to a specific gene or even a family of several genes (Kennedy 1985; Gould and others 1987; Selvanayagam and others 1995).

Rather, those frequencies indicate that the cellular target for the initiation of carcinogenesis after irradiation constitutes a substantial fraction of the entire genome. Such results have led to new approaches in the exploration of possible mechanisms of radiation carcinogenesis. A major focus of current research is on the role of radiation-induced genetic instability in carcinogenesis.

## CONCLUSIONS

Over the next few years, two closely linked approaches using animal models of carcinogenesis are likely to contribute to the understanding of the mechanisms of radiation-induced cancer. Researchers conducting this new generation of animal studies are taking advantage of the current rapid development of molecular genetics. A number of laboratories have begun to use genetically engineered mice with alterations in specific genes to determine the influence of these genes (such as ATM, BRCA1, and BRCA2) on susceptibility to radiation-induced cancer. At the same time, other laboratories are focusing on the inherent differences in susceptibility to radiation-reduced cancer among different mouse strains and beginning to dissect genes involved in controlling susceptibility. Both approaches should yield useful information on susceptible subpopulations and might into the underlying lesions and the processing of these lesions, which initiate carcinogenesis after exposure to ionizing radiation. Progress on both fronts should be substantial over the next 4-5 years and results of relevance to risk estimates are expected to be available for an important BEIR VII phase-2 study.

# 5

# RADIOBIOLOGIC PRINCIPLES AND RISK MODELING

Risk models serve the primary purpose of representing radioepidemiologic data, but they must also conform with established radiobiologic principles. Current models and the resulting risk estimates are in apparent or real conflict with such principles, in two main aspects that have led to a lack of transparency and to continued controversy. The two main aspects are linearity vs. curvilinearity and the dose dependence of RBE. Both are closely related to the problem of threshold versus linear nonthreshold dependence on dose for radiation-related excess cancer rates.

## LINEARITY VERSUS CURVILINEARITY

One of the major disagreements between radiobiologic observations and current risk models concerns the shape of the dose-response curve for gamma radiation. In the majority of radiobiologic observations after photon irradiation—from chromosome and cell-inactivation studies to findings on animal tumors—dependence on dose is found to be curvilinear; often it is described as a linear-quadratic dependence. In contrast, at low to moderate doses (<2 Gy) there is no apparent deviation from linearity in the excess rates of solid cancer among atomic-bomb survivors, the primary source of risk estimates (Thompson and others 1994; Pierce and others 1996). As a consequence, dose proportionality is often used in current models.

The International Commission on Radiological Protection (ICRP) has concluded that linear dependence on dose is inconsistent with radiobiologic findings. In its derivation of nominal risk coefficients for photons, ICRP has therefore used the dose and dose rate effectiveness factor (DDREF) to account for a postulated curvilinearity in dose or for an assumed dose-rate effect. The value 2 was adopted both for solid cancers and for leukemia in ICRP Publication 60 (ICRP 1991). It was stated that that was the highest value consistent with the data on solid cancers, and that for leukemias the value was fully consistent with the observations of atomic-bomb survivors. However, there have been no actual numerical evaluations.

There is an evident contradiction in the use of linear models in risk modeling and the reduction of the resulting values when they are used for risk estimation. Accordingly, a broader approach that incorporates the assumed DDREF directly into the numerical models themselves is required. One way to achieve that is to use models that are linear-quadratic in dose dependence and in the derivation of confidence regions for the resulting linear and quadratic coefficients, $\alpha$ and $\beta$

Figure 1 exemplifies the approach in terms of exploratory computations for solid-cancer and leukemia mortality in Hiroshima (RERF 1994, 1997). A linear-quadratic dependence on dose is used that has the form $ERR = a_r(D_r+RD_n) + bD_r^2$ (1), where $D\gamma$ is the gamma ray organ dose and $D_n$ the neutron organ dose. $R$ corresponds to the limiting relative biologic effectiveness (RBE) of neutrons at low doses and in these computations is assumed to equal 15, which is close to the current radiation weighting factor assumed for fission neutrons. There would be little difference in the results if the quadratic term were taken to be of the form $b(D\gamma. +D_n)^2$.

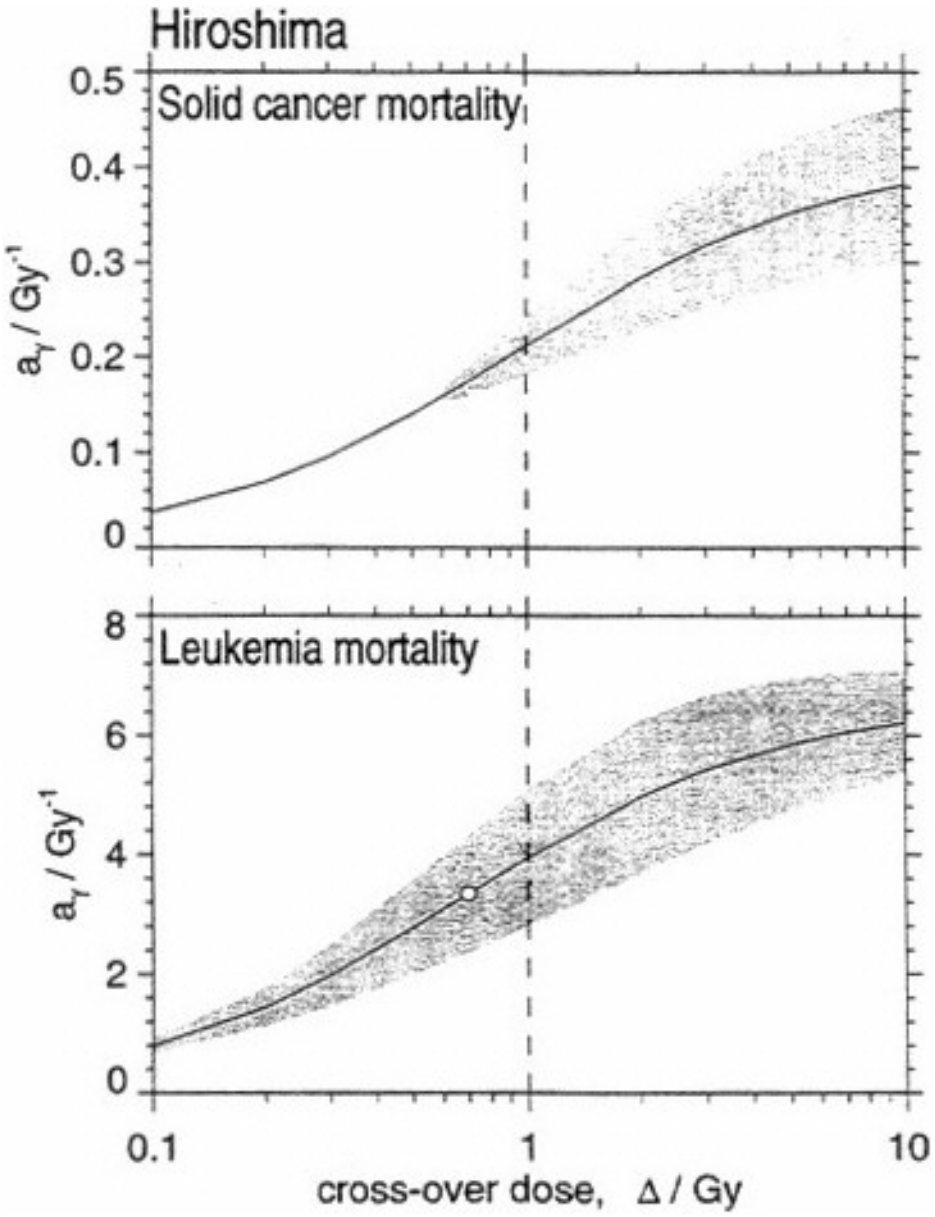



Figure 1. The gray areas give acceptable values (for the 95% confidence level) of parameter combinations aγ and Δ in linear-quadratic fit for all solid-cancer and all leukemia deaths in Hiroshima. aγ: linear coefficient, that is, excess relative risk (ERR) per gray at low doses; Δ: 'crossover dose,' which equals ratio of linear and quadratic coefficient in the dose dependence for γ-rays. Diagrams are given for an assumed maximal RBE of neutrons R = 15. Data on solid cancers fit best to large values of Δ, that is, to linear dependence; but Δ = 1Gy cannot be rejected at the 95% confidence level, and this value corresponds to the DDREF = 2 assumed by ICRP. For leukemias, best estimate is obtained for Δ = 0.7 Gy, and this is fully consistent with DDREF =2.

The parameters $a\gamma$,, $b$, and $R$ are estimated in terms of the familiar regression models. The results are comparatively insensitive to the value of $R$, and the resulting 95% confidence regions of the parameters are therefore given in Figure 1 for the value $R = 15$ which corresponds to current assumptions. Instead of the parameter $b$, the diagram gives the more familiar cross-over dose, $\Delta = a\gamma/b$, that is, the photon dose where the quadratic component is equal to the linear component. Large values of $\Delta$ correspond to nearly linear dependence on dose, while small values belong to strongly curvilinear dependence. The value $\Delta = 1$ Gy corresponds closely to the value DDREF = 2 which has been applied by the ICRP.

For all solid minors taken together, the best fit for this model is obtained for large values of $\Delta$, that is, a linear dependence on dose. The excess relative risk is then 0.4/Gy, but the linear-quadratic dependence with crossover dose $\Delta = 1$ Gy lies within the confidence region of the parameter estimates and corresponds, in agreement with DDREF = 2, to the excess relative risk 0.2/Gy. For leukemia, the best fit is obtained with $\Delta = 0.7$ Gy, and this also agrees with DDREF = 2 relative to the purely linear dependence on dose.

The exploratory computations indicate that the present risk estimates, including the DDKEF = 2, are consistent with the Hiroshima data. The calculations are largely in line with an earlier analysis by Kellerer and Nekolla (1997). They are given here without detailed explanations because they are merely intended to indicate the general direction of more extensive and detailed evaluations of all available data that should be considered by a BEIR VII phase-2 committee. It might also be desirable to explore other options, such as true threshold models rather than the linear-quadratic dependence, for which analogous approaches can be used. An added feature that ought to be included in the modeling, and that can introduce some curvilinearity into the dependence on dose, is the use of newly developed techniques to account for errors in dose estimation.

## THE DOSE DEPENDENCE OF RBE

The analysis of potential curvilinearity is, in the case of the atomic-bomb survivor data, inseparable from the issue of accounting for the RBE of neutrons. In past analyses, it has been common to treat neutrons as a minor contributor to the observed health effects and, in line with this assumption, to use the crude approximation of a simple dose-modifying factor. Instead of total absorbed dose, $D = D\gamma + D_n$, the approach uses a *weighted dose*, $D_w = D\gamma + wD_n$ assuming that the RBE of neutrons is constant. The assumption of a constant RBE of neutrons is, however, directly in conflict with the universal observation—in almost all radiobiological investigations—that the RBE of neutrons assumes its highest values at low doses and decreases at larger doses. The use of the weighted dose $D_w$, in risk modeling is, accordingly, clearly at variance with basic radiobiological principles; it can be justified only as an approximation valid under the assumption that neutrons were minor contributors to the effects, even in Hiroshima.

That assumption remains tentative. If the current dosimetry system, DS86, is accepted, there is little likelihood that Hiroshima data and their comparison with Nagasaki data will permit conclusions on the contribution of neutrons to observed health effects. However, this does not imply that the contribution of neutrons must be minor in those who were exposed to low doses in Hiroshima. If a contrary conclusion was reached earlier in modeling, it was an artifact of using the weighted dose, $D_w$, even when a linear-quadratic dependence on dose was considered. To explain the problem, one needs to write out the linear-quadratic dependence on dose for the ERR. If the relation is formulated in terms of $D_w$, it takes the form:

$$ERR = a D_w + b D_w^2 = a (D_\gamma + w D_n) + b D_\gamma^2 + 2 b w D_\gamma D_n + b w^2 D_n^2. \quad (2)$$

The first two terms in the equation correspond-if one equates $a$ with $a\gamma$ and $w$ with $R$—to equation 1, which is in line with radiobiologic findings and with an RBE of neutrons that decreases with dose. The last two terms, $2\ b\ w\ D\gamma\ D_n + b\ w^2\ D_n^2$, however, are inconsistent with radiobiologic findings since neutrons exhibit a larger linear-, but not a larger quadratic-dose, component. Those terms therefore invalidate any exploration of curvilinearity of ERR with dose, except assumed small values of $w$.

The use of the realistic equation 1 for estimating the dose contribution from neutrons is, as has been shown (Little and Muirhead 1996; Kellerer and Nekolla 1997), unlikely to change the earlier conclusion (Preston and others 1991) that numerical values of the neutron RBE cannot be derived from data on the atomic-bomb survivors. But the added computations with the meaningful linear-quadratic dependence are desirable because, in contrast with earlier approaches, they can provide an inverse relation between photon and neutron risk estimates that results if various values of the RBE of neutrons are considered (Kellerer and Nekolla 1997). Figure 2 exemplifies, again in terms of explorative calculations for solid-cancer mortality in Hiroshima, the resulting dependence. The computations are constrained to $\Delta \leq 1$ Gy, that is, the solutions are required to correspond to a DDREF of at least 2. The shaded area gives the resulting 95% confidence region for the absolute estimates of lifetime attributable fatality risk of solid cancers for photons and neutrons. The absolute risk coefficients are obtained by appropriate scaling of the excess relative risk $a\gamma$ for photons and $a\gamma\ R$ for neutrons. Values of the implicit parameter $R$ are noted in the graph. Although no value of $R$ can be rejected, there are slightly better fits for large values, as indicated by the standard-error region of the parameter combinations (heavy shading).

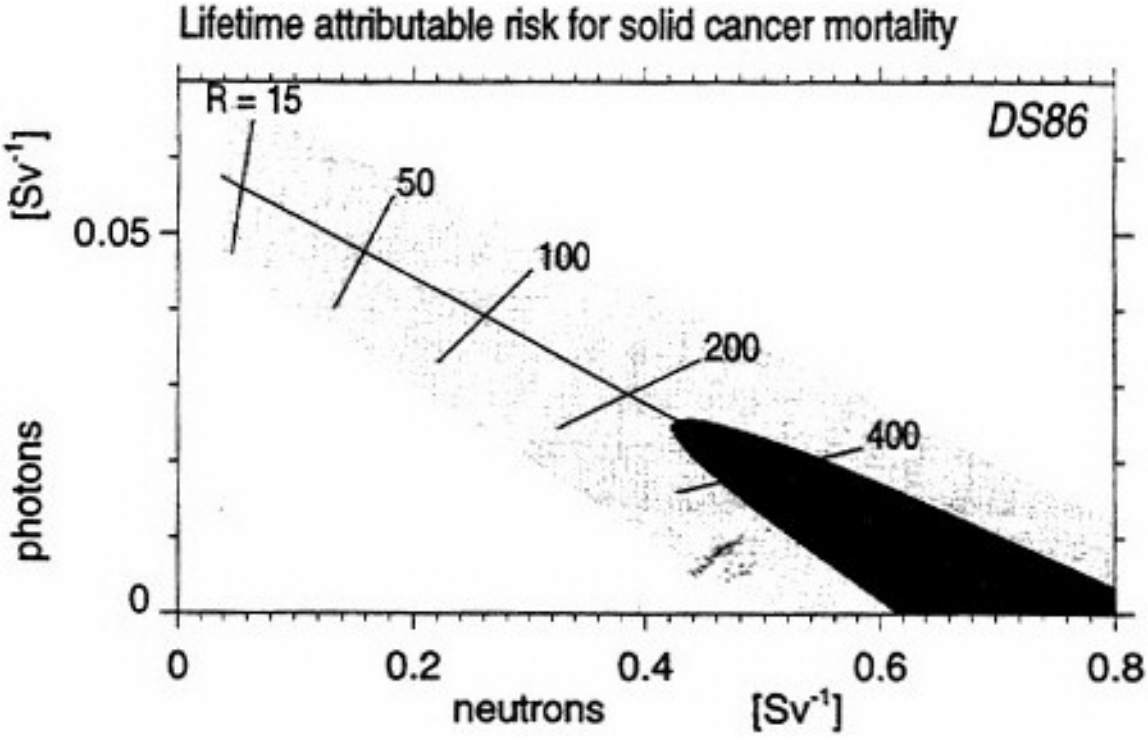



Figure 2. Absolute risk coefficients for mortality obtained from solid-cancer data in Hiroshima in terms of DS86 and the linear-quadratic relation of equation 1. Light and heavily shaded areas show 95% confidence and standard error regions of combinations of photon and neutron risk estimates. The calculations illustrate that the estimates of risk coefficients for protons and neutrons are inversely related; high RBE of neutrons cannot be excluded on the basis of the data but imply reduced risk estimates for photons. The values, R, of the neutron RBE at low dose that are considered here, must be distinguished from the radiation weighting factor, $w_R$, which has been recommended by ICRP for the definition of the effective dose (unit: Sv). The risk coefficient for neutrons in the diagram is given per unit effective dose, taking $w_R$=15 as the ICRP recommendation for the fission neutrons in Hiroshima[1].

If *R* is assumed to equal the radiation weighting factor $w_R$ =15 that corresponds, according to the ICRP recommendations to the fission neutrons in Hiroshima, the current risk estimates are approximated, both for photons and neutrons. For larger assumed values of *R*, the computation provides an upper bound to the absolute risk coefficient for neutrons which would be obtained if the RBE of neutrons were infinite, that is, if the

[1] In these sections, *R* (in the present exploratory computations with a linear-quadratic model), *w* (the constant neutron weight factor in the earlier Radiation Effects Research Foundation calculations), and $w_R$ (the radiation weighting factor that has a fixed ICRP recommended value for a given neutron energy) are a set of closely interrelated and easily confused quantities. *R* (neutron RBE at low doses) is a variable parameter in fitting the data to the linear-quadratic dose dependence. It equals the assumed limiting RBE of neutrons at low doses; at higher doses the neutron RBE is less. *w* is a parameter in other calculations; it equals the assumed constant RBE of neutrons and in view of this essential difference, it might be unwise to use the same symbol for *R* and *w*. Finally $w_R$ is the ICRP estimate of the limiting RBE of neutrons for stochastic radiation effects; it has been given a fixed value for any radiation and is the officially adopted conversion factor from absorbed dose (in Gy) to effective dose (in Sv). ICRP has not defined $w_R$ as the limiting RBE of the radiation in question. It has, instead, defined $w_R$ as the conversion factor from absorbed dose to effective dose, and has assigned certain numerical values that need to be used in regulatory practice.

current dosimetry system (DS86) were valid and if all the excess cancer risk at Hiroshima were attributable to neutrons.

There are, in fact, more-severe restraints that exclude, with DS86, high values of *R*. Although not shown here, the calculations suggest, for larger values of *R*, there is not only a small linear component but also a vanishing quadratic component for gamma rays; this, however, is clearly inconsistent with the observations in Nagasaki, where there can be no doubt about substantial effects of the gamma rays at large doses. A combined analysis of the Hiroshima and Nagasaki data will thus be required and this will involve the consideration of added uncertainties, such as the still unresolved dosimetry of the factory workers in Nagasaki. As stated, the present exploratory computations are merely intended to indicate the general direction of the computational work that could be considered by a BEIR VII phase-2 committee.

Although the results in figure 2 must thus be seen as incomplete, they are important insofar as they indicate the inverse relation between photon and neutron risk estimates. This interrelation is disregarded in present use where the risk estimate for photons is taken to be substantially independent of the assumed RBE of neutrons and the neutron risk estimate is obtained as the product of the unchanged photon risk estimate and the assumed RBE of neutrons.

A scaling factor from excess relative risk to absolute risk has been used here that corresponds—in line with the model chosen by ICRP—to the assumption of a relative risk that does not decrease with time after exposure. More-realistic models will include such decreases and will lead to absolute risk estimates for solid-cancer mortality that are reduced by about a factor of 2 (Kellerer and Barclay 1992; UNSCEAR 1994).

## POTENTIAL IMPACT OF NEUTRONS

The observations on the atomic-bomb survivors are the major basis for the estimation of radiation risks, but they are linked to a dosimetry system, DS86, that is still subject to uncertainties regarding the magnitude of the neutron component in Hiroshima. Activation measurements related to slow neutrons indicate an underestimation of neutron doses with DS86. A tentative modification of the neutron doses uses a correction factor, $c(r)$, that depends on distance, $r$, from the hypocenter

$$c(r) = 0.125 \exp(2.77\, r) \qquad (3)$$

where $r$ is in kilometers (Straume and others 1992).

This correction would decrease the neutron contribution at high doses but substantially increase it in the low-dose region (see Figure 3). There is a possibility that accelerator mass-spectrometry measurements of nickel-63 in exposed copper samples—or the determination of another activation product of high energy neutrons—will, in the near future, provide additional evidence on high-energy neutrons at Hiroshima. In view of

that possibility, it is of interest for a BEIR VII phase-2 committee to consider potential implications of any suggested modifications to DS86 dosimetry for the risk estimates for survivors at Hiroshima and Nagasaki (Kellerer and Nekolla 1997).

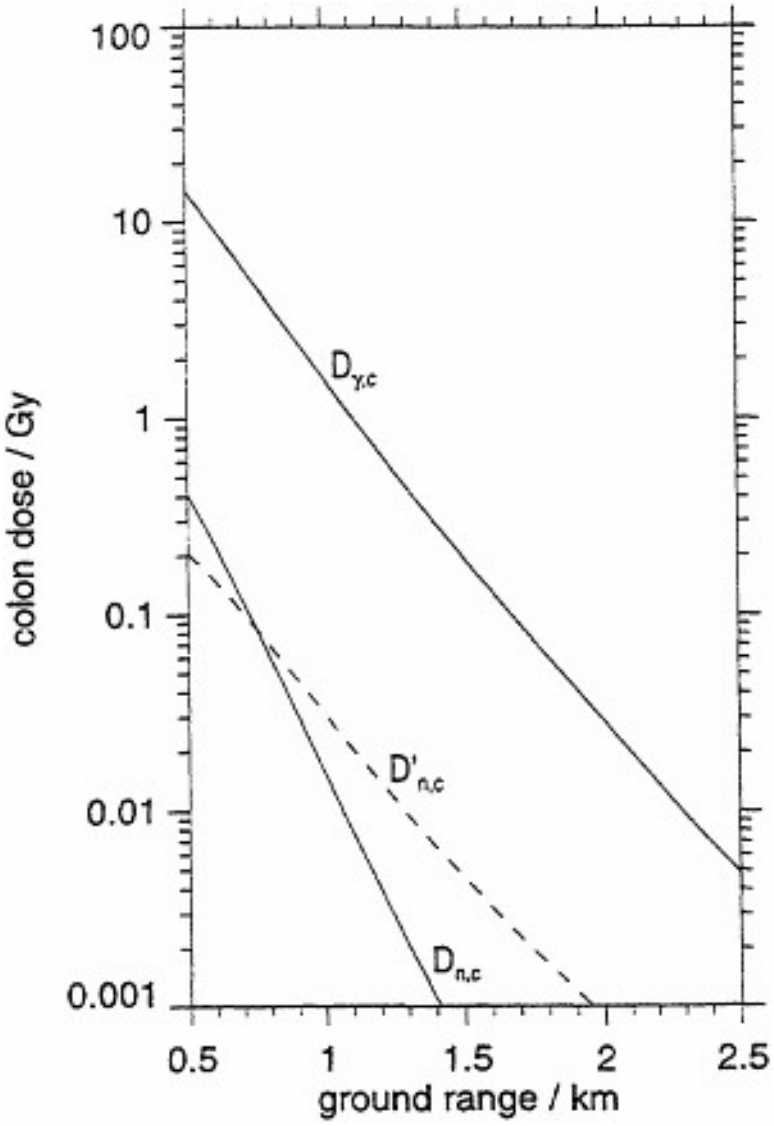



Figure 3. Dependence of the gamma ray and the neutron colon dose on distance from hypocenter in Hiroshima according to DS86 (solid lines) and neutron dose tentatively modified according to Straume and others (1992) (broken line). For conversion of kerma to organ dose, see Kellerer and Nekolla (1997).

Computations for solid-cancer mortality in terms of any modified dosimetry could provide new estimates of photon-and neutron-risk coefficients. Examples are shown in figure 4, which is analogous to figure 2.

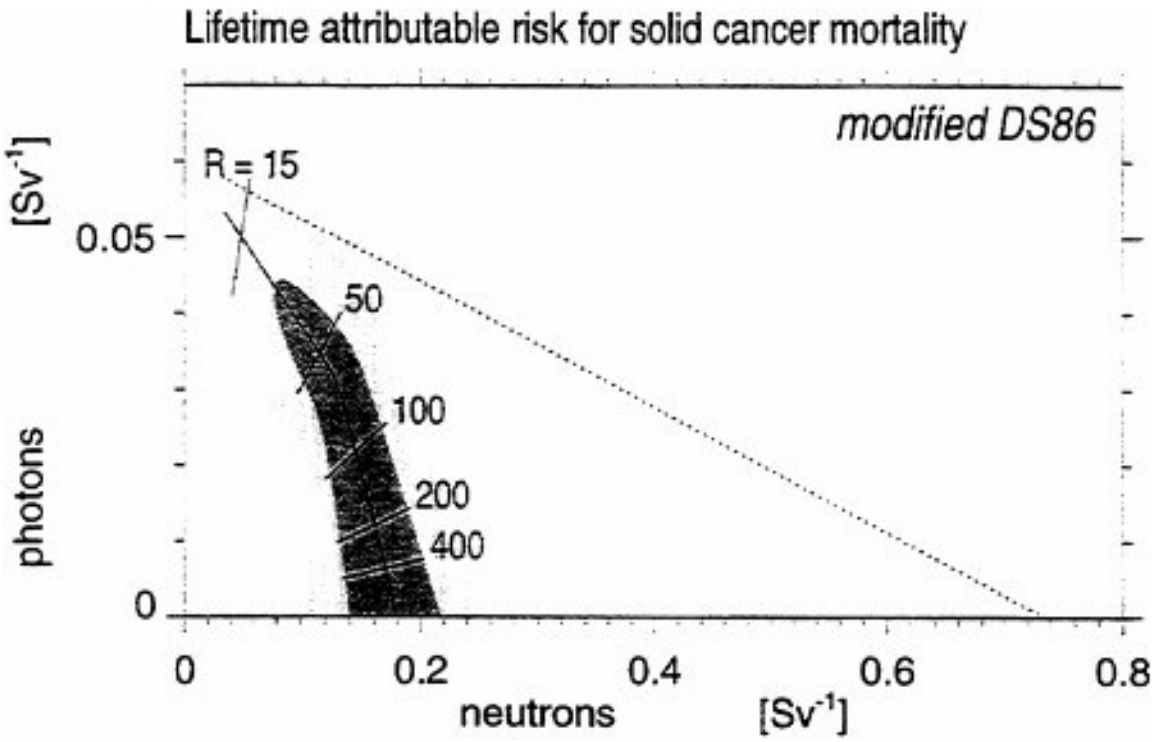



Figure 4. Absolute risk coefficients for mortality obtained from solid-cancer data in Hiroshima in terms of a tentatively modified DS86 and the linear-quadratic relation of equation 1. Light and heavily shaded areas show 95% confidence and standard error regions of combinations of photon and neutron risk estimates. The calculations illustrate that the estimates of risk coefficients for protons and neutrons are inversely related; high RBE of neutrons cannot be excluded on the basis of the data but imply reduced risk estimates for photons.

The results show that a modified dosimetry could exclude risk coefficients for neutrons that are substantially larger than now assumed. The broken line corresponds to the results shown in figure 2. See legend to figure 2 with regard to the difference between the parameter $R$ and the radiation weighting factor $w_R$, that is used in the definition of the effective dose of neutrons (in Sv).

With the dosimetry as modified here, roughly the same risk estimates are obtained for both photons and neutrons if a low neutron RBE, between 10 and 20, is assumed. Nevertheless, there are potentially major implications of any modified neutron dosimetry. The inverse relation between the risk coefficients for gamma rays and neutrons shows that very high values of neutron RBE would be consistent with the data. But they would correspond primarily to a largely reduced linear component in the dependence of dose due to photons, rather than an enhanced effectiveness of the neutrons. In fact, with this tentative modification, the solid-cancer data from Hiroshima would be consistent with a vanishing linear component for photons but a substantial quadratic component, that is, an effectiveness of gamma rays at larger doses in line with the Nagasaki observations. The maximal risk coefficient for neutrons, however, would differ little from the one now assumed.

### CONCLUSIONS

If a revised neutron dosimetry confirms the trend that is indicated by the available activation measurements for Hiroshima, the solid-cancer data from Hiroshima could cease to be proof of a finite risk coefficient for photons. That would be a major change in the evidence supporting or refuting the linear non-threshold hypothesis, and it would add importance to the data either from Nagasaki, where the neutron doses are smaller and are unlikely to be affected by dosimetric reassessment, or from other studies where photons alone were involved.

The exploratory computations referred to here are tentative, not only because any changes in the neutron dosimetry that may be required are still uncertain, but also because they use the assumed correction factors only in a summary fashion (for details, see Kellerer and Nekolla 1997). In spite of the limitations of the computations, they demonstrate clearly the potentially important implication of new findings on the neutron doses in Hiroshima and they show the need for more-detailed numerical analyses.

# 6

# MATHEMATICAL MODELS OF RADIATION CARCINOGENESIS

One recent development in radiation-protection research that has implications for risk assessment is the use of mathematical models of the cancer process based on a multistage mechanism. The concept of the multistage process of cancer dates back to the early part of the century, but it was in the 1950s that approaches to modeling the process gained momentum with the models developed by Armitage and Doll (1954, 1957) and the multiple-mutation approaches of Fisher (1958) and Burch (1960). Knudson (1971) derived a two-stage explanation of retinoblastoma in children from a study of the occurrence of sporadic unilateral tumors and familial bilateral tumors, which led to the concept of antioncogenes or tumor-suppressor genes (Knudson 1985, 1991). Support for the concept of tumor-suppressor genes came also from cell-fusion experiments; fusion of a tumor cell with a normal cell was found to suppress the malignant phenotype (Harris 1971; Stanbridge 1976). In the meantime, the retinoblastoma (*Rb*) gene has been identified, and the molecular biologic implications of the analysis of retinoblastoma have been verified (Knudson and others 1976; Cavenee and others 1983, 1985; Dunn and others 1988; Benedict and others 1988, 1990). Tumors arise from a biallelic mutation of the *Rb* gene in accordance with the two-stage model. Children with bilateral tumors carry an inherited mutation in the *Rb* gene in all their cells, so a spontaneous mutation in the normal *Rb* gene in a retinoblast cell leads to the tumors, but the rare sporadic unilateral tumors are associated with two spontaneous mutations in a retinoblast cell. Many other tumor-suppressor genes have been identified in recent years, and several reviews document these developments (Marshall 1991; Weinberg 1991; Skuse and Ludlow 1995).

As a consequence of the analysis of the occurrence of retinoblastoma, Moolgavkar and Knudson (1981) proposed a two-stage model with clonal expansion of "intermediate" cells for human carcinogenesis. The model has been shown to provide a qualitative description of the age-dependent incidence of all human cancers for both children and adults (Moolgavkar and Venzon 1979; Moolgavkar 1983) and has been applied to the epidemiology of carcinomas of the breast and lung (Stevens and Moolgavkar 1979; Moolgavkar and others 1979, 1980, 1989, 1993) and radon-induced lung tumors in rats (Moolgavkar and others 1990). The mathematical nature of the model has been investigated (Moolgavkar and others 1988; Moolgavkar and Luebeck 1990; Moolgavkar 1992; Heidenreich 1996; Heidenreich and others 1997, in press) The model, or slight modifications of it, is gaining increasing use for the analysis of radiation-induced cancer in both epidemiologic and animal studies (Kai and others 1993; Leenhouts and Chadwick 1994a,b, 1997; Little 1995; Venema and others 1995; Holt 1997; Moolgavkar 1997), although some workers continue to take the Armitage-Doll multistage model into account (Little and others 1992, 1994; Chen 1993; Little and Charles 1994; Little 1995, 1996).

## THE TWO-MUTATION MODEL

Although the modifications of the two-mutation model lead to some quantitative differences in analyses, a global description that covers the essence of the model can be used to gain an insight into the cancer process and derive some generally applicable implications. A schematic representation of the two-mutation model based on the developments of Moolgavkar and Knudson (1981) is given in figure 5.

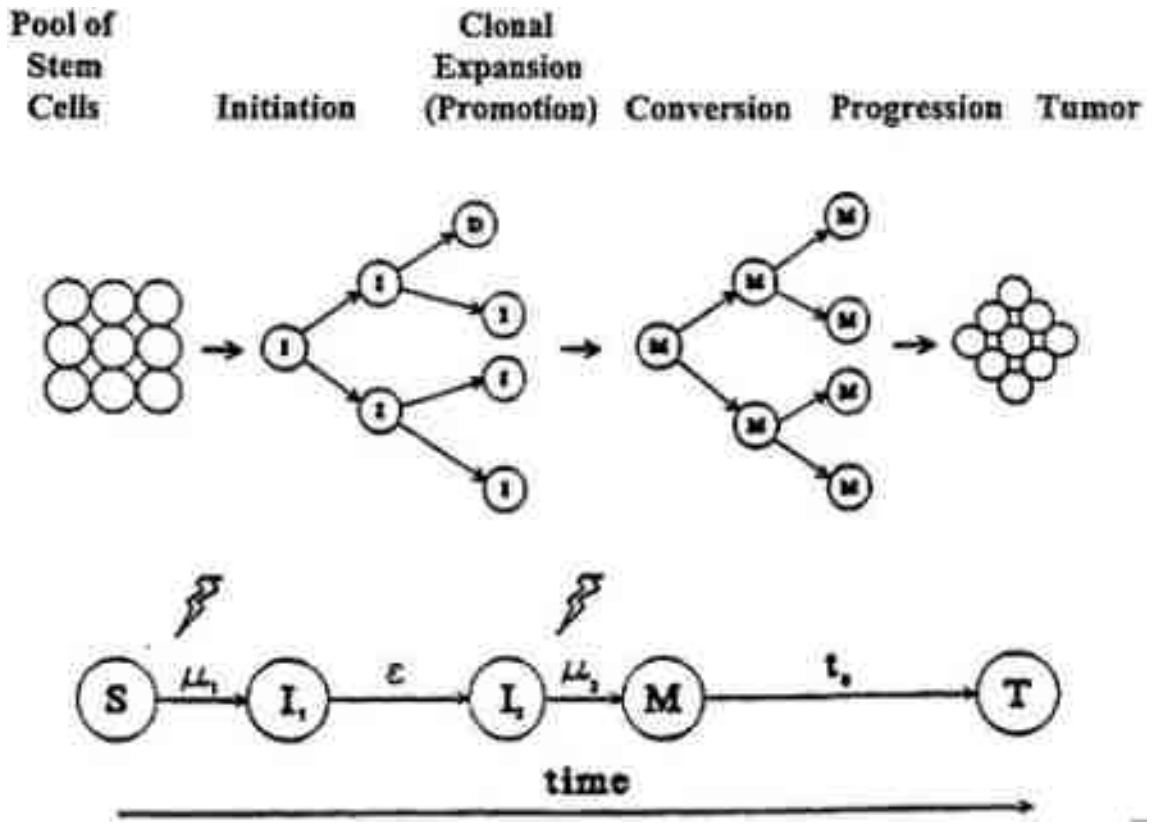



Figure 5. Moolgavkar and Knudsen two-stage model with clonal expansion of "intermediate" cells for human carcinogenesis. Adapted with permission from Chadwick and Leenhouts (1995).

The model includes two mutational steps, $\mu_1$ (initiation) and $\mu_2$ (conversion), which convert a normal stem cell via an intermediate stage to a malignant cell, which can then grow out (progression) into a detectable tumor. An important aspect of the model is that it incorporates cellular turnover of the stem cells, the intermediate cells (I), and the malignant cells (M), as well as taking cell death (D) and differentiation info account. Thus, an intermediate cell can divide in a non-linear, exponential-like, clonal expansion (promotion) to create, with the passage of time ($\varepsilon$), an increasing number of potential target cells for the second mutation. To increase the biologic plausibility of the model, a lag time($t_0$), often held constant, is invoked for the time from the generation of the first malignant cell to the detection of the tumor, although this should not be confused with latency. The model thus incorporates the various steps associated with the development of cancer, such as initiation, promotion, conversion, and progression; but the terminology and characterization of these steps are not unambiguously identified in the cancer literature.

The mathematical equations that can be derived from the model to describe the age-dependent incidence of a specific cancer do not always have an explicit solution, but by calculating the number of cells in each compartment—that is, the stem cells, the intermediate cells, and the malignant cells—for small intervals in an iterative process

starting from zero, or birth, we can derive the age-specific incidence. The calculations rely on assumptions about the starting conditions at time zero, such as the number and time-dependent expansion in the number of stem cells, the levels of the mutation rates, and the expansion rate of the intermediate cells. Spontaneous cancer is inherently assumed to develop with time as a consequence of two "spontaneous" mutations occurring in the initiation and conversion steps, and analysis of age-specific incidence curves for a given unexposed population can be used to define the background against which a radiation effect must be determined. Radiation is assumed to be able to induce mutations in both steps, although the relative importance of radiation in each step is not defined a priori and radiation-induced killing of stem cells, intermediate cells, and malignant cells is also taken into account. In this way, dose-effect relationships determined in cellular radiation biology are introduced into the model, and lead to the simultaneous calculation of the age-specific incidence and the dose-dependent incidence of a cancer. The model can be used for both acute and very protracted (such as lifetime) exposures; radiation-induced mutations increase the mutation rates, $\mu_1$ and $\mu_2$, instantaneously in the case of an acute dose or for the duration of exposure for a chronic dose. Thus, in accordance with cellular radiation biology a linear-quadratic dose-effect relationship might be used for the cellular effects of an acute exposure in general, with a linear dose-effect relationship for chronic and very protracted exposures; in addition, the cellular RBEs of different kinds of radiation can be taken into account. Radiation is normally not assumed to act as a promoter except at very high acute doses, when cell depletion might stimulate increased division of stem cells and intermediate cells.

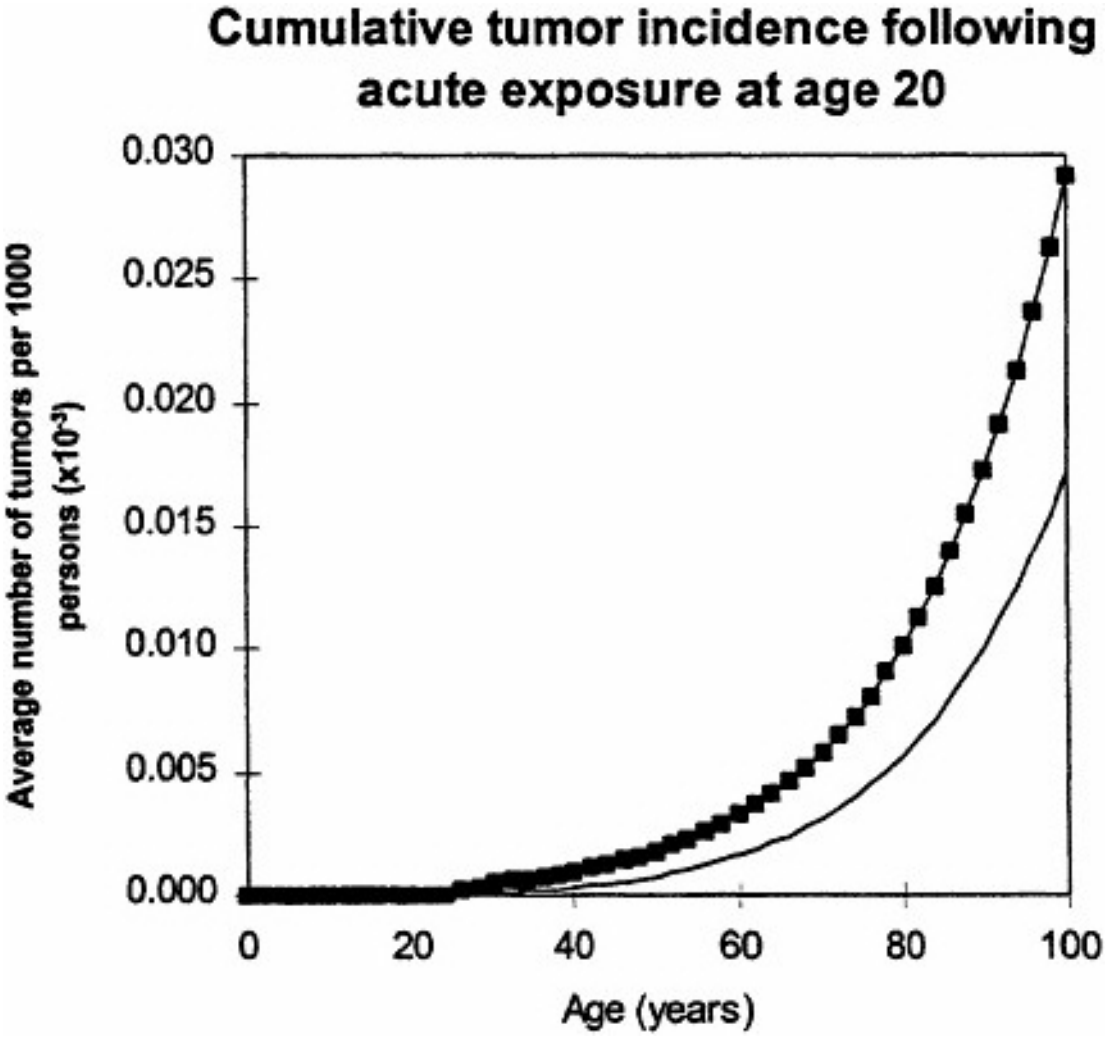
A consequence of radiation increasing the mutation rates in both the initiation and conversion steps of the sample model previously described and the interdependence of one mutational step on the other for the development of cancer, is that in most cases, a radiation-induced mutation in one step will interact with a "spontaneous" mutation in the other step on the path to cancer. That actually implies that the level of the radiation effect is related to the "spontaneous" cancer incidence. It also means that at low doses radiation will usually be a cocarcinogen, inasmuch as a second mutation induced "spontaneously" will be needed to complete the process, and only rarely will it be a complete carcinogen.

Thus, models might be used in the analysis of epidemiologic cohort studies that have good age-specific incidence data for both the cohort and the control populations. It is less suited to the analysis of epidemiologic case-control studies. Its use for the analysis of results of animal experiments is often hindered by the fact that many animal tumors are not directly lethal. Unless animals are sacrificed throughout the experiment, the tumors are not detected until the animals are moribund and age-specific incidence data are not normally available.

## IMPLICATIONS OF THE MODEL FOR RISK ESTIMATION

The two-mutation model—which is, as Knudson (1991) points out, "a minimal model for carcinogenesis," is also remarkably effective and has several implications for radiation risk. The model, which calculates simultaneously the age-specific incidence and the dose-dependent incidence of cancers over the whole lifetime, can be used for acute exposures (Little and Charles 1994; Moolgavkar 1997), very protracted exposures such as those of uranium miners (Moolgavkar and others 1993), and lifetime exposures such as to indoor radon. The model has therefore been used by some investigators to provide a basis for lifetime extrapolations of radiation risk. A BEIR VII phase-2 committee should examine all relevant models and consider how appropriate models might contribute to risk assessment.

If an acute exposure affects the first mutation step (initiation), the model predicts that the risk will resemble a relative-risk projection; if the exposure affects the second mutation (conversion) the model predicts that the risk will resemble an absolute-risk projection (figure 6).



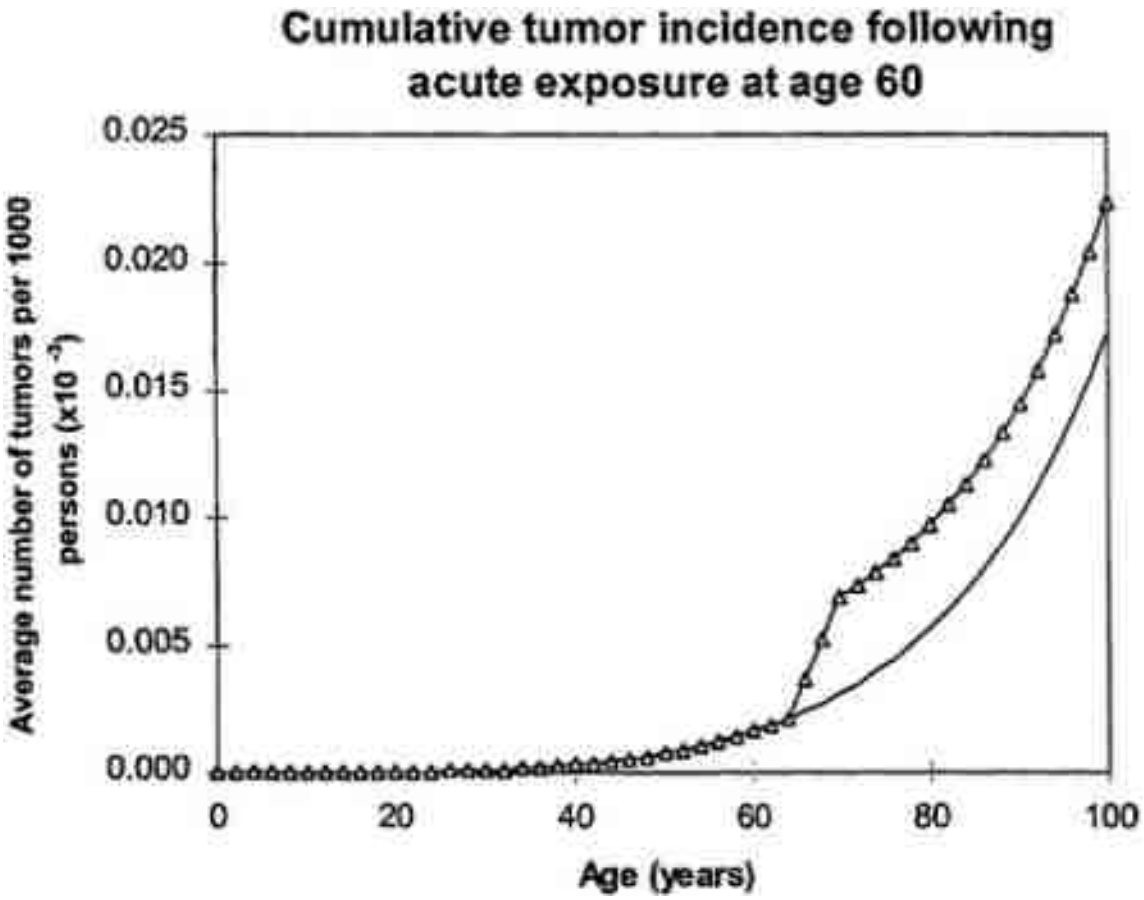



Figure 6. Upper panel: calculated age dependence of tumor incidence after acute exposure at age 20 (■) compared with spontaneous incidence (continuous line) resembles relative risk.

Lower panel: calculated age dependence of tumor incidence after acute exposure at age 60 (Δ) compared with spontaneous incidence (continuous line) resembles absolute risk. Adapted with permission from Chadwick and Leenhouts (1995).

In all calculations, it was assumed that cellular radiation sensitivity was co tam throughout lifetime and that the radiation sensitivity was equal for both mutational steps.

If the exposure is over a lifetime, the model predicts that the risk will resemble a relative-risk projection (figure 7), which implies that, in this case, exposure at an early age is the defining factor (Leenhouts and Chadwick 1994a; Chadwick and Leenhouts 1995).

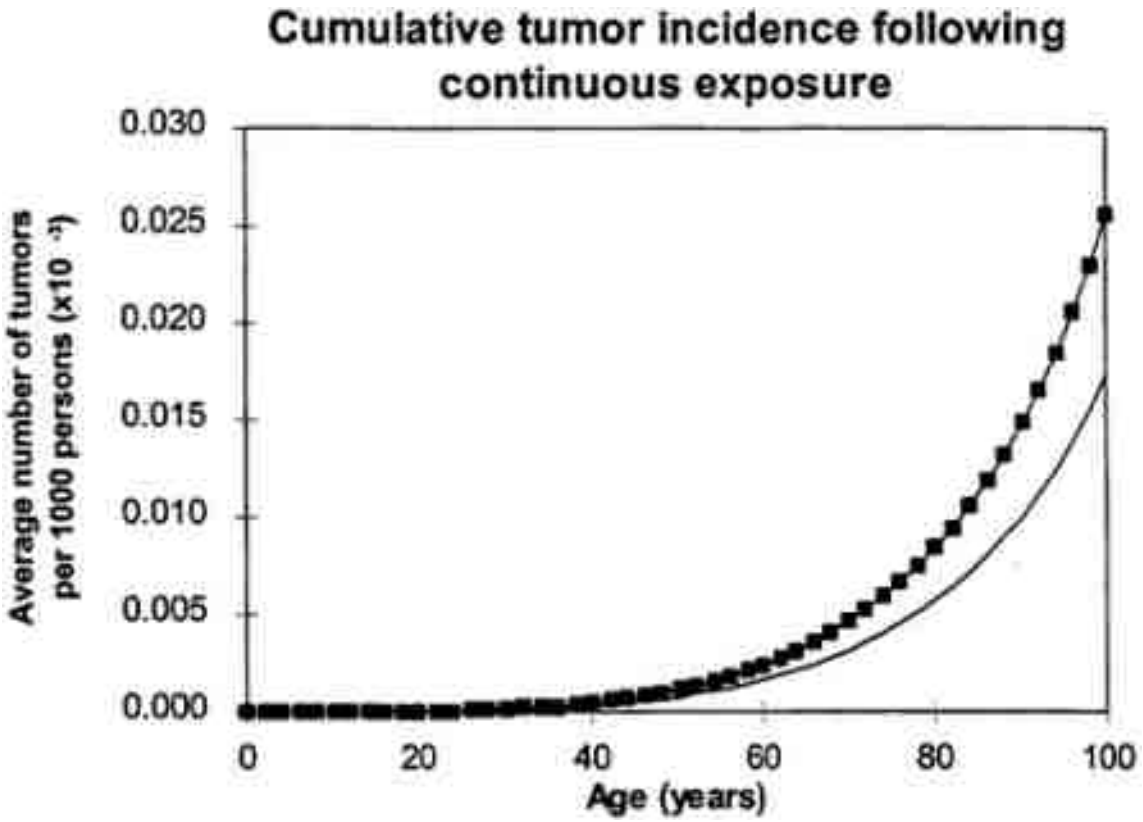



Figure 7. Calculated age dependence of tumor incidence after protracted lifetime exposure (■) compared with spontaneous incidence (continuous line) resembles relative risk. In all calculations, it was assumed that cellular radiation sensitivity was constant throughout lifetime and that the radiation sensitivity was equal for both mutational steps. Adapted with permission from Chadwick and Leenhouts (1995).

Inherent in the model is the interplay between the radiation-induced mutations and the spontaneous mutations and the fact that, at low doses, the radiation will always be a cocarcinogen. Because the background cancers must arise from "spontaneous" mutations, and there is interplay between the radiation-induced mutations and the "spontaneous" mutations, the model predicts that the risk following exposure to radiation depends on the background incidence of cancer associated with both acute and protracted exposures (figure 8).

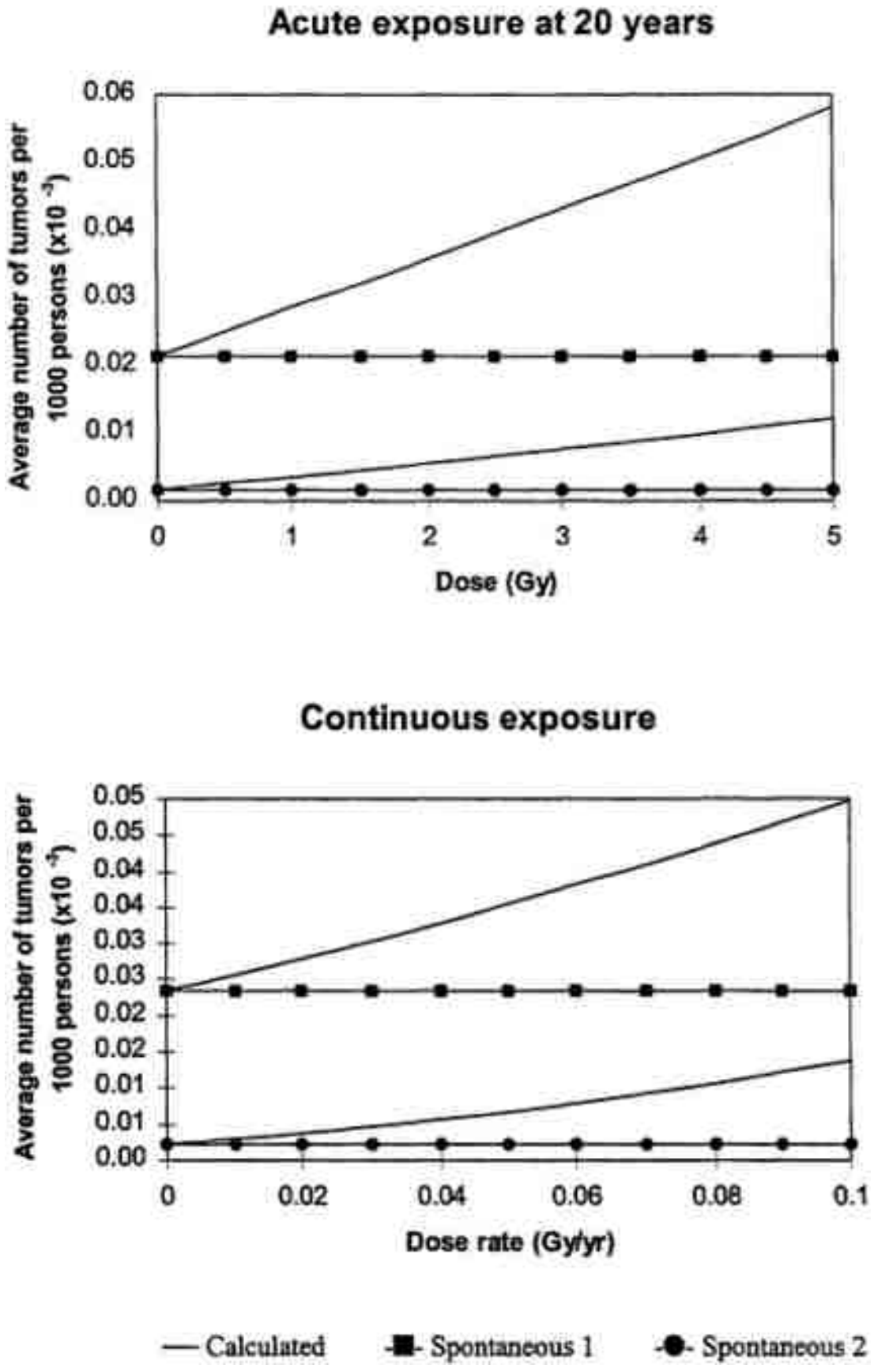



Figure 8. Upper panel: calculated low-dose incidence at end of life after acute, low-dose-rate exposure at age 20 for two levels of spontaneous cancer incidence as a function of lifetime dose. Initial slope increases as spontaneous-cancer incidence increases. All calculations assume

constant cellular radiation ensitivity throughout lifetime and equal radiation sensitivity for both mutational steps. Lower panel: calculated low-dose incidence at end of life after protracted lifetime exposure for two levels of spontaneous-cancer incidence as a function of average annual dose rate. Initial slope increases as spontaneous-cancer incidence increases; both curves exhibit slight upward curvature. In all calculations, it was assumed that cellular radiation sensitivity was constant throughout lifetime and that the radiation sensitivity was equal for both mutational steps. Adapted with permission from Chadwick and Leenhouts (1995).

For example, the probability that a particular radon exposure leads to a lung cancer in a nonsmoker is lower than the probability that it leads to a lung cancer in a smoker, even though the relative risk in nonsmokers is higher. With respect to cancers that are extremely rare, the model predicts that radiation will have little chance of inducing a cancer except after protracted exposure to very high doses (for example, bone cancers in radium-dial painters). An important consequence of this dependence of risk following exposure to radiation on spontaneous-cancer incidence is that different cancer types with different spontaneous incidences cannot be grouped for analysis. This implication of the model provides a theoretical basis, which can be tested, for the extrapolation of radiation risk for a specific cancer across populations (such as, breast cancer from Japan to America) on the basis of the background level of the cancer.

In the comparison of exposure conditions (such as age at first exposure, length of exposure, and radiation effect on second mutations), the model implicitly predicts an "inverse dose-rate effect" that is more related to an exposure-time effect but, in any case is independent of any considerations of inverse dose-rate effect at the cellular level. It seems likely that the inverse dose-rate effect revealed by the model is the one responsible for the indications of such an effect found in some epidemiologic studies (such as studies of uranium miners).

The model provides a basis for the study of the combined effect of two carcinogenic agents by adding the mutagenic contributions of the two agents to the spontaneous mutation rate in both the initiation and the conversion steps. In addition, the model offers the possibility of including the effect of agents that are thought to be promoters rather than mutators.

The model implies that the shape of the dose-effect relationships for induction of the more commonly occurring cancers will reflect the shape of the cellular dose-effect relationships for the induction of chromosomal mutations and that the modifying effects of dose rate and radiation type at the cellular level will be reflected in the changes in the shape of the cancer-induction curves (Leenhouts and Chadwick 1994b). In the case of very rare cancers, the model implies that induction by radiation will be rare and have a curvilinear dose-effect relationship.

## CONCLUSIONS

Recent developments in the application of the two-mutation model of carcinogenesis to the analysis of radiation epidemiologic and animal studies have suggested differing approaches and different insights into the action of radiation. A BEIR VII committee should critically examine the status of models that might be relevant to risk assessment. Multistage models might provide a tool that can be used to relate the molecular investigations on radiation mechanisms at the cellular level to the epidemiologic studies of exposed populations. The models also might provide a basis for extrapolating radiation effects to low doses and low dose rates and across populations which could be useful and meaningful for risk assessment. It must always be borne in mind that any model is a simple representation of the facts and, while the use of the two-mutation model of carcinogenesis might suggest different insights into the action of radiation, considerable care must be taken in applying such models to experimental data and in interpreting the results of such analyses.

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# Information on Committee Members

**RICHARD B. SETLOW** (Chairman) is a Senior Biophysicist and the Associate Director for Life Sciences at the Brookhaven National Laboratory. He received his Ph.D. in Physics from Yale. Shortly thereafter he began Biophysics research on the effects of ionizing radiation and ultraviolet radiation on proteins and DNA. This work led to investigations of radiation effects on viruses and microbial cells. After teaching Physics and Biophysics for a number of years he moved to the Biology Division of the Oak Ridge National Laboratory where he and his colleagues discovered Nucleotide Excision Repair—the first DNA repair mechanism that worked in the dark. His subsequent experiments dealt with the effects of radiation on human cells and the variances among humans, as measured on lymphocytes, of the responses and repair of chemical, ultraviolet and ionizing radiation damage to DNA. His present research deals with the spectral regions that induce malignant melanomas in fish-a surrogate for humans. Dr. Setlow was President of the Biophysical Society in 1969-1970. He is a member of numerous scientific societies including the National Academy of Sciences and the American Society for Cancer Research. For a number of years he was a member and the chairman of the Scientific Advisory Board of the National Center for Toxicological Research and is now a member of the Science Board of the FDA. He was the chairman of two committees that recently issued National Academy Press Reports — *Mortality of Veterans in the Crossroads Nuclear Test* (1996) and *Radiation Hazards to Crews of Interplanetary Missions: Biological Issues and Research Strategies* (1996). Dr. Setlow is the recipient of the Finsen Medal from the International Association for Photobiology and the Enrico Fermi Award from the Department of Energy.

**KENNETH H.HADWICK** is the Head of Sector with the Radiation Protection Research Unit, Directorate General for Science, Research and Development, European Commission. He graduated with a B.Sc. in Physics from Liverpool University, UK and earned a M.Sc. in Radiation Biophysics from the University of London, UK. He was awarded a Ph.D. by the University of Utrecht in the Netherlands. He worked in research on cellular radiation biology for over twenty years and is co-author of over seventy articles and a book on theoretical radiation biology. Dr. Chadwick is currently Head of Sector responsible for the management of Radiation Biology and Health Effects research contracts in the Nuclear Fission Safety Programme of the European Commission in Brussels. Dr. Chadwick is a Fellow of the Institute of Physics, UK; a Fellow of the Society for Radiological Protection, UK; a Member of the Radiation Research Society, USA; and a Member of the European Physical Society.

**PHILIP C. HANAWALT** is the Howard and Jessie Watkins University Professor in the Department of Biological Sciences at Stanford, with a joint appointment in the Dermatology Department, Stanford Medical School. He earned his Ph.D. in Biophysics at Yale University and has received an honorary Sc.D. from his alma mater, Oberlin College. He has served as Director of the Biophysics Graduate Program and as Chair of the Department of Biological Sciences at Stanford. He has trained 25 Ph.D. students and over 60 postdoctoral research fellows from over 20 countries. He has served as President

of the Environmental Mutagen Society and on the Board of Directors of the American Association for Cancer Research. He has served as a managing editor for *Mutation Research* and is currently a member of the Board of Reviewing Editors for *Science*. He serves on numerous advisory boards including that of the Fogarty International Center (NIH), the Office of Environmental Health Hazard Assessment (California EPA), and the toxicology advisory committee for the Burroughs-Welcome Fund. Dr. Hanawalt's specialty is photobiology and DNA repair, having co-discovered excision-repair and more recently the pathway of transcription-coupled repair. He has received an outstanding investigator award from the National Cancer Institute and in 1989 was elected to the National Academy of Sciences. He is presently a member of the Board on Radiation Effects Research in the National Research Council.

**GEOFFREY R. HOWE** is Professor of Public Health and Head, Division of Epidemiology at Columbia University School of Public Health. Dr. Howe earned his Ph.D. degree from the University of Leicester. He was formerly professor at the University of Toronto and Director of the National Cancer Institute of Canada Epidemiology Unit. He is a member of the National Commission for Radiological Protection and measurements and served as consultant or member on the last three BEIR Committees. He is a member of the editorial boards of *Cancer Causes and Control* and *Cancer Epidemiology, Biomarkers and Prevention* . Dr. Howe's radiation studies include those of tuberculosis patients exposed to low-LET radiation, miners exposed to radon, nuclear workers, and the National Cancer Institute sponsored studies of the consequences of the Chernobyl accident. He has acted as an adviser to the World Health Organization with respect to Chernobyl studies and serves on the Department of Energy's scientific review group for the latter's joint American/Russian radiation studies.

**ALBRECHT M. KELLERER** is the Director of the Radiobiological Institute of the University of Munich and of the Institute of Radiation Biology of the GSF, National Research Center for Environment and Health. He was formerly professor of radiation biophysics at Columbia University in New York, and subsequently professor and chief of the Institute for Medical Radiation Research at the University of Wurzburg. Dr. Kellerer's research specialties include microdosimetry, radiation risk assessment, and radiobiology. Dr. Kellerer is a member of the German National Commission for Radiation Protection, chairman of its committee for risk assessment and a member of committees of the ICRU and the International Commission on Radiological Protection. Dr. Kellerer is also the managing editor of the *Journal of Radiation and Environmental Physics*.

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**NANCY L. OLEINICK** is Professor and Director of the Division of Radiation Biology in the Department of Radiation Oncology, Case Western Reserve University School of Medicine, Cleveland, Ohio, and Director of the Radiation Biology Program of the CWRU/Ireland Cancer Center. She earned her Ph.D. in Biochemistry from the University of Pittsburgh. Dr. Oleinick's research specialties are in radiobiology and photobiology. One long-term research interest concerns the role of chromatin structure and the nuclear matrix in determining the microheterogeneity of radiation damage to DNA, DNA repair, and the cellular responses to ionizing radiation. Recent research has also focused on the cellular and molecular effects of photosensitization related to photodynamic therapy. Dr. Oleinick has served on the Editorial Boards of *Radiation Research, International Journal of Radiation Biology*, and *Photochemistry and Photobiology*, on the US National Institutes of Health Radiation Study Section, and on the Board of Scientific Counselors, Division of Cancer Etiology, National Cancer Institute. She is currently President-Elect of the American Society for Photobiology. Dr. Oleinick has been a member of numerous other review panels considering radiation effects, including the Presidential Advisory Committee on Human Radiation Experiments and the Veterans' Advisory Committee on Environmental Hazards.

**ROBERT L. ULLRICH** is the Vincent P. Collins Distinguished Professor in Radiation Oncology Research and Director of the Biology Division in the Department of Radiation Oncology at the University of Texas Medical Branch. Dr. Ullrich earned his Ph.D. from the University of Rochester School of Medicine and Dentistry. He was formerly head of the Radiation Carcinogenesis Unit at the Oak Ridge National Laboratory. He is a member of the Board of Directors of the National Council on Radiation Protection and Measurements and a Member of the Council of the Radiation Research Society. He received the Research Award of the Radiation Research Society in 1987. Dr. Ullrich has served on the National Research Council Panel on Space Radiation Effects, a number of National Institutes of Health advisory committees, and on the International Commission on Radiological Protection Task Group on estimates of radiation-induced cancer at low doses.

# List of Acronyms

| | |
|---|---|
| AP | apurinic-apyrimidinic |
| AT | ataxia telangiectasia |
| ATM | the protein mutated in ataxia telangiectasia |
| BEIR | Biological Effects of Ionizing Radiations (refers to a series of studies conducted by committees of the National Research Council). |
| BrdU | bromodeoxyuridine |
| BRER | Board on Radiation Effects Research (of the National Research Council) |
| DDKEF | dose and dose-rate effectiveness factor |

| | |
|---|---|
| DNA | deoxyribonucleic acid |
| DNA-PK | DNA-dependent protein kinase |
| DNA-PKcs | 470-kDa catalytic subunit of DNA-dependent protein kinase |
| DS86 | the current dosimetry system used to estimate doses to survivors of the atomic-bombs in Hiroshima and Nagasaki |
| DSB | double-strand break |
| ERR | excess relative risk |
| FISH | fluorescent in situ hybridization |
| high-LET | high-linear energy transfer |
| ICRP | International Commission on Radiological Protection |
| LET | linear energy transfer |
| low-LET | low-linear energy transfer |
| MDS | multiply damaged site |
| NAS | National Academy of Sciences |
| NCRP | National Council on Radiation Protection and Measurements |
| NHEJ | nonhomologous end joining |
| NIH | National Institute of Health |
| NRC | National Research Council |
| PCC | prematurely condensed chromosome |
| PCNA | proliferating-cell nuclear antigen |
| Rb | retinoblastoma |
| RBE | relative biologic effectiveness |
| RERF | Radiation Effects Research Foundation (located in Hiroshima and Nagasaki, Japan; where studies of the health effects in the atomic-bomb survivors are conducted) |
| RNA | ribonucleic acid |
| SSB | single-strand break |
| TCR | transcription-coupled repair |
| UNSCEAR | United Nations Scientific Committee on the Effects of Atomic Radiation |
| UV | ultraviolet |
| UVC | ultraviolet C |

# ACKNOWLEDGMENTS

The committee members would like to express their appreciation to Drs. Ernest Beutler, Sara Darby, R.J. Michael Fry, Samuel Hellman, Dade Moeller, J. Edward Rall and Charles Stevens for their participation in the National Academy of Sciences' review of this manuscript. We would also like to thank Doris Taylor, Peggy Johnson, and Dennis Gleeson for their technical and administrative assistance during the phase-1 study.