Report on Carcinogens Background Document for X Radiation & Gamma Radiation and Neutrons

18 June 2003

FOREWORD

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of all substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (DHHS) has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP) who prepares the Report with assistance from other Federal health and regulatory agencies and non-government institutions.

Nominations for listing in or delisting from the RoC are reviewed by a formal process that includes a multi-phased, scientific peer review and multiple opportunities for public comment. The review groups evaluate each nomination according to specific RoC listing criteria. This Background Document was prepared to assist in the review of the nomination of Ionizing Radiation. The scientific information in this document comes from publicly available, peer reviewed sources. Any interpretive conclusions, comments or statistical calculations, etc. made by the authors of this document that are not contained in the original citation are identified in brackets [ ]. If any member(s) of the scientific peer review groups feel this Background Document does not adequately capture and present the relevant information they will be asked to write a commentary for this Background Document that will be included as an addendum to the document. In addition, a meeting summary that contains a brief discussion of the respective review group's review and recommendation for the nomination will be added to the Background Document, also as an addendum.

A detailed description of the RoC nomination review process and a list of all nominations under consideration for listing in or delisting from the RoC can be obtained by accessing the NTP Home Page at http://ntp-server.niehs.nih.gov. The most recent RoC, the 10th Edition, was published in 2002 and may be obtained by contacting the NIEHS Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov (800-315-3010).

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
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Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Executive Summary

Introduction

Ionizing radiation is radiation that has sufficient energy to remove electrons from atoms, creating ions. The result of this ionization is the production of negatively charged free electrons and positively charged ionized atoms. Ionizing radiation can be classified into two groups: photons (gamma and X rays) and particles (alpha, beta, and neutrons). Ionized atoms (free radicals), regardless of how they are formed, are much more active chemically than neutral atoms. These chemically active ions can form compounds that interfere with the processes of cell division and metabolism. The degree of damage suffered during exposure to ionizing radiation depends upon the type, intensity, energy, duration, and chemical form of radiation. The amount of energy deposited per unit of path length in the material of interest by ionizing radiation is called the 'linear energy transfer' (LET) and is given in units of energy per unit length (e.g., keV/µm). Although gamma rays, X rays, and neutrons are all ionizing forms of radiation, they differ in energy transfer. Photons (gamma rays and X rays) and electrons are considered low-LET (except for the very lowest energy electrons). Low-LET radiations tend to have more tortuous tracks in matter and have more widely dispersed energy deposition patterns. Neutrons and alpha particles, protons, and other heavy charged particles are high-LET radiations. High-LET particles tend to slow down in straight lines, leaving dense energy deposition tracks.

X radiation and gamma radiation

<u>X rays.</u> X rays are high-energy photons produced by the interaction of charged particles with matter. X rays are produced effectively by the rapid deceleration of charged particles (often electrons) by a high atomic number material. X rays and gamma rays have essentially the same properties but differ in origin. X rays are emitted from processes outside the nucleus, while gamma rays originate inside the nucleus. X rays are generally lower in energy and are less penetrating than gamma rays. The energy distribution of X rays is continuous with a maximum at an energy about one-third that of the most energetic electron. As photons interact with matter, their spectral distribution is further altered in a complex manner due to the transfer of energy.

<u>Gamma rays.</u> Like visible light and X rays, gamma rays are weightless packets of energy called photons. Gamma rays often accompany the emission of alpha or beta particles from a nucleus. They have neither charge nor mass and are very penetrating. One source of gamma rays in the environment is naturally occurring potassium-40. Artificial sources include plutonium-239 and cesium-137. Gamma rays can easily pass through the human body or be absorbed by tissue, thus constituting a radiation hazard for the entire body. Gamma rays resulting from radioactive decay consist of monoenergetic photons with energies as high as several MeV (megaelectron volt) in energy. Due to the scattering and absorption within the radioactive source and the encapsulating material, the emitted photons have a relatively narrow spectrum of energies.

Neutrons

Neutrons are electrically neutral particles that, together with positively charged protons, make up atomic nuclei. The number of neutrons defines the isotope of an element. Neutrons have mass and energy and may be produced by humans with machines such as a cyclotron. The neutron decays to a proton by beta emission. As uncharged particles, neutrons do not interact with atomic electrons in the matter through which they pass, but they do interact with the nuclei of the atoms present. The nuclear force, which leads to these interactions, is very short ranged, which means that neutrons have to pass close to a nucleus for an interaction to take place. Because of the small size of the nucleus in relation to the atom as a whole, neutrons will have a low probability of interaction, and could thus travel considerable distances in matter. Neutrons are capable of generating a much denser ion path and damage to human tissue than electrons. Interactions of neutrons with biological material may result in the production of gamma radiation, protons, and alpha particles.

Alpha and beta particles

Alpha and beta particles, which also are identified as part of ionizing radiation, are not included with these nominations but may be reviewed separately in the future for possible listing in the Report on Carcinogens.

Human Exposure

Exposure to ionizing radiation comes from a variety of natural (environmental exposure) and anthropogenic sources, including exposure for military, medical, and occupational purposes.

X radiation and gamma radiation

Environmental exposure. Environmental exposure to X and gamma rays results from terrestrial sources, particularly the radioactive nuclei chemically bound in the upper 25 cm of the earth's crust and in building construction materials. Radioactivity also has been released into the environment from nuclear accidents, primarily from the largest nuclear accident to date that occurred in Chernobyl, Ukraine in 1986. The worldwide annual per capita dose for residual radioactivity from Chernobyl was estimated to be 0.002 millisievert (mSv) in 2000, down from a maximum of 0.04 mSv in 1986. Environmental exposure also can come from nuclear power generation.

Occupational exposure. Occupational exposure to X and gamma rays affects approximately 5 million workers worldwide with most being employed as coal miners or other underground miners in non-coal mines. Other occupationally exposed workers include medical workers, nuclear industry workers, and airline crews. The Nuclear Regulatory Commission limits the occupational dose to 5 rem/year [1 rem = 0.01 Sv].

Medical uses. Exposure to medical radiation occurs for a large portion of the population of more developed countries, such as the United States, that have a high level of medical care. However, medical exposures are very small compared to the exposure to natural

sources of radiation with an annual collective dose of about 2 x 10^6 person-Sv/year for medical procedures compared to 14 x 10^6 person-Sv/year from background exposures. Medical exposures also differ from other exposures to radiation since the exposed individual receives a direct benefit from the procedures, which include diagnostic radiology and radiation therapy.

Military uses. Major past exposures to X and gamma radiation have resulted from military uses of atomic weapons with the detonation of two atomic bombs over Hiroshima and Nagasaki, Japan in 1945 and additional atmospheric testing of nuclear weapons that were carried out between 1945 and 1980. Survivors of the bombings of Hiroshima and Nagasaki were exposed to approximately 300 mSv on average while the local population near the nuclear test site in Nevada was estimated to have received an average dose of about 3 mSv.

Neutrons

Exposure to neutrons derives from many of the same sources as those causing exposure to X and gamma radiation. However, neutron exposure from the atomic bombs at Hiroshima and Nagasaki, Japan is now considered to have contributed only 1% to 2% of the total dose of ionizing radiation. Similarly, medical uses of neutrons are very limited currently, and occupation exposure to neutrons in the nuclear industry accounts for only about 3% of the total annual effective dose to nuclear plant workers. Occupational exposure to neutrons can occur for aircraft crews and for oil-field workers when the later use neutron radiation for well logging. Most environmental exposure to neutrons is from cosmic radiation, which has been estimated to result in an annual effective dose of 80 to 200 μSv at sea level.

Dosimetric methods

A variety of dosimetric methods are used for monitoring X and gamma rays in environmental and medical settings. X and gamma ray detectors include gas detectors, scintillators, and semiconductors. Individual personnel monitors of many types are in use, including film badges, thermoluminescent dosimeters, optically stimulated luminescence technology, and self-reading pocket dosimeters. Monitoring methods for neutrons are divided into detectors of slow neutrons and fast neutrons. Detectors of slow neutrons include proportional counters using ^{10}B or ^{3}He, scintillators with ^{6}Li or ^{10}B, ionization chambers lined with ^{235}U, and semiconductors attached to a ^{6}Li or ^{10}B radiator. Detectors of fast neutrons may be based on tissue-equivalent ionization chambers, recoil proton techniques, capture reactions or moderated detectors. Rem meters and neutron spectrometry, either proton recoil based or “Bonner sphere,” also can be used to detect fast neutrons. A wide variety of personnel monitors for neutrons are in use, e.g., nuclear emulsions, thermoluminescent detectors, track-etch detectors, electronic pocket dosimeters, activation detectors, and bubble detectors. Exposure to ionizing radiation also may be measured through the use of biological indices that may be either *in vivo*, i.e., measurement of radioactivity in the human body, or in vitro, i.e., measurement of radioactivity in urine, excreta, or other material taken from the body.

Human Cancer Studies

X radiation and gamma radiation

IARC concluded in 1999 that all radiation studies taken together present a consistent body of evidence for carcinogenicity of X radiation and gamma radiation in humans. IARC's conclusion is corroborated by the newly published studies reviewed here. Recently published studies of second cancer occurrences after radiation treatment for first cancers further supported the A-bomb survivor results concerning differences in latency by type of cancer (higher risk of hematopoietic cancers appears in the first 10 years of follow-up compared to higher risks of solid cancers with increasing follow-up) and by age at exposure (higher risk for thyroid cancer after irradiation in childhood and for breast cancer after irradiation in adolescence and during the reproductive years). Described below are the conclusions reached concerning which organ sites are to be considered radiosensitive and at what dose levels specific organs are affected.

It is largely undisputed that leukemia and cancers of the thyroid, breast, and lung are associated with radiation exposure, and that these associations have been found at doses as low as 0.2 gray (Gy). The risk, however, depends to some extent on the age at exposure with exposure during childhood being mainly responsible for higher leukemia and thyroid cancer risks and exposure during reproductive age for breast cancer. As recently suggested by some studies, lung cancer risk may be more strongly related to exposure later in life. Associations between radiation and cancers of the salivary glands, stomach, colon, bladder, ovary, central nervous system, and skin have been reported but are less well quantified. An exhaustive review by Ron (1998) noted that the relative risks (RR) for these cancer sites at 1 Gy exposure generally range from 1 to 2.5 for these sites. Some recent studies added additional evidence for cancers at these sites being caused by radiation exposures, i.e., by medical treatment with radiation (Garwicz *et al.* 2000, Bhatia *et al.* 2002, Kleinerman *et al.* 1995, Brenner *et al.* 2000, Ron *et al.* 1999, Lichter *et al.* 2000, Yeh *et al.* 2001), or by occupational low and protracted doses as reported for a large Canadian worker cohort (Sont *et al.* 2001). The first large study of sarcomas conducted by Yap *et al.* (2002) added angiosarcomas to the list of radiation-induced cancers occurring within the field of radiation at high therapeutic doses. In the IARC report, associations of ionizing radiation exposures with cancers of the liver, esophagus, and, to a lesser extent multiple myeloma and non-Hodgkin's lymphoma, were considered inconsistent. Two recent studies, one conducted in a worker population (Gilbert *et al.* 2000) and another among A-bomb survivors (Cologne *et al.* 1999), suggested that liver cancers can be caused by radiation at doses above 100 mSv (in the worker population especially with concurrent exposure to radionuclides), and a linear dose-response relationship for external radiation and liver cancers was calculated for the A-bomb survivors (RR = 1.81; 95% CI = 1.32 to 2.43 per 1 Sv liver dose). A recent study by Modan *et al.* (2000) added some evidence that radiation exposure during childhood may affect the incidence of lymphomas and melanomas.

Finally, chronic lymphatic leukemia, Hodgkin's disease, cancers of the cervix, prostrate, testis, and pancreas have rarely been related to radiation, although a recent large worker

cohort study (Sont *et al.* 2001) suggested otherwise for the latter two cancer types (testis and pancreatic cancers).

Neutrons

There are no adequate epidemiological data available to evaluate the carcinogenicity of neutrons in humans.

Studies in Experimental Animals

X radiation and gamma radiation

X rays and gamma rays are clearly carcinogenic in all the species tested (see Table below for tumor sites that have been observed in animal studies), although tissues differ in their susceptibility to both radiation qualities i.e., low- and high-LET radiations. The degree of susceptibility for the induction of benign and malignant tumors is species-, strain-, age- and sex-dependent. Exposures in the early prenatal stages do not appear to increase cancer rates, but exposures in the later stages may do so. The question of whether parental irradiation increases the susceptibility of offspring to radiogenic cancer is controversial, and conflicting results have been obtained in different experiments.

Neutrons

Low-energy neutrons, such as fission neutrons, are significantly more potent carcinogens than low-LET radiations, such as X or gamma rays. There are some differences in the effects among radiations of different quality, but none of the differences have been sufficient to reject the assumption made in risk estimation for radiation protection purposes, namely, that the effects of radiations of different LET differ quantitatively but not qualitatively. There is no conclusive evidence of a signature alteration that might distinguish tumors induced by high-LET radiations from those induced by low-LET radiations. Tumors sites induced in experimental animals following exposure to neutron radiation are summarized in the Table below.

Summary of tumors sites observed in experimental animals following exposure to X rays, gamma rays, or neutrons

Tumor Site	Test Animal Type of Radiation														
	Mouse			Rat			Rabbit			Dog			Monkey		
	X	γ	N*	X	γ	N*	X	γ	N*	X	γ	N	X	γ	N*
Bone			✓		✓			✓	✓			✓	✓		✓
Brain/Nervous System												✓	✓		✓
Colon													✓		✓
Epithelial Tissues			✓												
GI Tract	✓												✓		
Harderian Gland	✓	✓	✓												
Heart												✓			
Hemithorax												✓			
Kidney													✓		✓
Leukemia	✓	✓	✓								✓				
Lymphoma	✓	✓	✓								✓				
Liver	✓	✓	✓			✓									✓
Lung	✓	✓	✓			✓						✓			
Mammary		✓	✓	✓	✓	✓									
Multiple Myeloma													✓		
Ovary	✓	✓	✓												
Pituitary		✓	✓												
Skin			✓					✓	✓						
Soft Tissues			✓												
Spinal Cord												✓			
Thyroid				✓							✓		✓		✓
Vascular System			✓								✓		✓		✓

* N = Neutrons

Genetic and Related Effects

X radiation and gamma radiation

Human *in vivo* studies. Studies in humans exposed to ionizing radiation following A-bomb detonations and various radiation accidents and occupational exposures clearly show that low-LET radiations induce chromosomal alterations and gene mutations in somatic cells. The induction of genetic alterations in germ cells is less clear-cut. Studies of males exposed as a consequence of the Chernobyl accident suggest that X rays and gamma rays may induce transmissible minisatellite mutations in male germ cells.

Animal *in vivo* studies. Studies in normal mice, transgenic mice, and rhesus monkeys have demonstrated that X and gamma rays induce mutations, chromosomal aberrations, micronuclei and DNA strand breaks in somatic cells. X rays and gamma rays induce genetic damage in germ cells of mice, including dominant lethal mutations, recessive visible mutations, and recessive lethal mutations.

Human and animal *in vitro* studies. Evidence of chromosomal aberrations of various types is well documented and constitutes the primary effect of ionizing radiation exposure. In human cells, ionizing radiation also induces mutations, micronuclei, and DNA strand breaks in somatic cells. Studies in animal somatic cells have shown that ionizing radiation induces mutations, polyploidy, chromosomal instability, DNA damage, and cell transformation. Irradiation of human sperm resulted in chromosomal aberrations and micronuclei, which were observed following fertilization of hamster oocytes.

Mechanistic concerns. Double-strand DNA breaks and some base damage, quite possibly at multiply damaged sites (or sites of clustered damage), appear to be most important for the induction of chromosomal alterations and point mutations. These genetic end-points are largely the consequence of misrepair during one of several known DNA repair processes, although errors of DNA replication can occur for DNA damage remaining at the time of replication. A number of cellular components and functions are involved in ensuring efficient and accurate repair. Mutations in one or more of these processes will result in increased sensitivity to the induction of genetic damage.

Neutrons

Human *in vivo* and *in vitro* studies. Studies of individuals accidentally or medically exposed to neutron radiation show that induced chromosomal aberrations can persist for decades, and some *in vitro* studies show genomic instability in progeny of irradiated human cells. Many *in vitro* studies consistently demonstrate that neutron radiation induces chromosomal aberrations in human peripheral lymphocytes more effectively than gamma radiation. Human data do not show statistically significant effects of parental exposure on chromosomal abnormalities and mutations in subsequent generations.

Animal *in vivo* and *in vitro* studies. DNA damage, chromosomal aberrations, genomic instability, gene mutations, and cell transformations occurred in mammalian cells exposed to neutrons *in vitro*. Germ-line instability in mice has persisted for at least two generations following irradiation. Somatic cell mutations have been detected at the *hprt* locus and in *ras* oncogenes, and various cytogenetic effects, including chromosomal aberrations, sister chromatid exchanges, and micronuclei, have been reported in irradiated mice. Reciprocal translocations in male germ cells were reported in rhesus monkeys and marmosets.

The genetic effects induced by neutron radiation are qualitatively similar to the effects of X rays and gamma rays, but there are some quantitative differences. Several investigators have identified some potential cytogenetic fingerprints of neutron radiation based on these quantitative differences. These include the ratios of simple translocations to insertions (I-ratio), complete exchanges to incomplete rejoinings (S[I]-ratio), and dicentrics to interstitial deletions (H-ratio). In general, chromosomal aberrations, mutations, and DNA damage are induced more efficiently; DNA lesions are more severe and repaired less efficiently; and there are higher proportions of complex aberrations compared to low-LET radiation.

Other Relevant Data

Health effects

Biological effects of ionizing radiation are produced as the energy associated with the radiation penetrates and interacts with the atoms in the tissue. The effects from different types of radiation differ quantitatively but are qualitatively similar. X rays, gamma rays, and neutrons are considered indirectly ionizing radiations because they most frequently cause ionization of water molecules with production of reactive products that may produce modifications of DNA molecules. These reactive products include free electrons, ionized water molecules, hydroxyl ions, hydrogen free radicals, hydrogen ions, hydroxyl radicals, and, in the presence of molecular oxygen, hydrogen peroxide, hydroperoxy radicals, and hydroperoxy ions. When reactions of these products with living cells produce unrepaired damage, deterministic (health effects in which the severity is dependent on the dose) and stochastic effects (health effects in which the severity is independent of the dose but the probability is dependent of the dose, e.g. genetic effects and cancer) may result.

Early effects of ionizing radiation are deterministic effects that relate primarily to cell death and vary with the radiosensitivity of cell populations. The prodromal syndrome comprises a set of acute symptoms of gastrointestinal and neuromuscular symptoms that are seen as the initial response to whole-body irradiation. Increasing doses are associated with decreased survival time and with primary lethal effects that range from the hematopoietic syndrome through the gastrointestinal syndrome to the central nervous system syndrome. Neutrons have a higher relative biological effect compared to low-LET radiation.

Radiation-sensitive disorders

Certain genetic disorders predispose affected individuals to radiation sensitivity and cancer. These disorders include ataxia-telangiectasia (A-T), Nijmegen breakage syndrome, Mre11 deficiency, and ligase IV deficiency. Mutations of the A-T gene have been associated with breast and prostate cancer, head and neck cancer, lymphoma, and leukemia.

Potential mechanisms of carcinogenesis

Several mechanisms by which ionizing radiation could cause cancer have been proposed. Ionizing radiation may induce DNA damage directly, resulting in single-strand breaks, double-strand breaks, modifications of deoxyribose rings and bases, intra- and interstrand DNA-DNA cross-links, and DNA-protein cross-links. Epigenetic mechanisms that result in alteration in the expression of genomic information also have been proposed. These proposed mechanisms include radiation-induced genomic instability, induction of mutations by cytoplasmic irradiation, and “bystander effects,” which are based on mutational events occurring in cells that do not directly receive exposure to ionizing radiation.

Abbreviations

Bq	Becquerel
C kg^{-1}	Coulomb per kilogram of air
Ci	Curie
CLL	Chronic Lymphatic Leukemia
ENU	Ethylnitrosourea
EPD	Electronic Pocket dosimeters
EV	Electron-volt
GM	Geiger-Mueller counter
Gy	Gray
HIDA	N-substituted-2,6-dimiethyl phenyl carbamoylethyl iminodiacetic acid (hepatic iminodiacetic acid);
HMPAO	Hexamethyl propyleneamine oxime
HPGe	High purity germanium
IC	Ionization chamber
J	Joule
Kerma	Kinetic energy released in matter
LET	Linear Energy transfer
MAA	Macroaggregated albumin
MDP	Methylene diphosphonate
MNU	Methylnitrosourea
PC	Proportional Counter
R	Roentgen
RBE	Relative biological effectiveness
RSD	Radiation Sensitive disorders

SI	Standard International units
SRPD	Self-Reading Pocket dosimeters
Sv	Sievert
TEPC	Tissue-Equivalent Proportional Counter
TLD	Thermoluminescent Detector

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1 Introduction

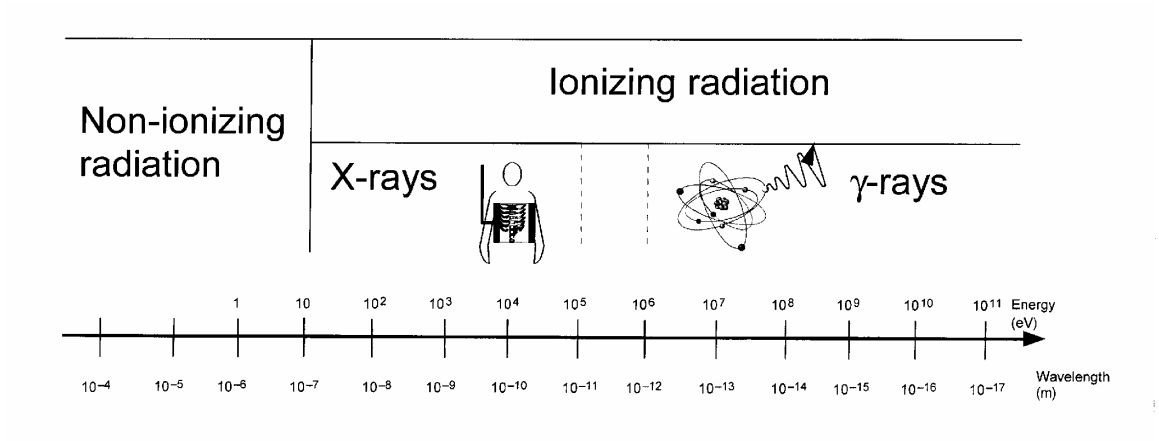
X radiation and gamma radiation was nominated for possible listing in the Report on Carcinogens by the National Institute of Environmental Health Sciences (NIEHS) based on the conclusions of the International Agency for Research on Cancer (IARC) (2000) that there was sufficient evidence of carcinogenicity in humans for X rays and gamma rays (Group 1) with a large number of epidemiological studies showing a consistent relationship between exposure and carcinogenicity. Neutrons were also nominated for possible listing by NIEHS on the basis of IARC's conclusion that neutrons are carcinogenic to humans (Group 1) based on mechanistic and genotoxic considerations that are supported by sufficient evidence in experimental animals. These are two separate nominations. However, the information for both nominations are discussed in this background document.

1.1 Basic information on ionizing radiation

All matter is composed of atoms bound into molecules by electrons. Ionizing radiation is radiation that has sufficient energy to remove electrons from atoms, creating ions. The result of this ionization is the production of negatively charged free electrons and positively charged ionized atoms. Ionizing radiation can be classified into two groups: photons (gamma and X rays) and particles (alpha, beta, and neutrons) (see Figure 1-1). All types of ionizing radiation can remove electrons, but the various types interact with matter in different ways.

One source of ionizing radiation is the nucleus of unstable (radioactive) atoms. For these atoms to become more stable, the nuclei must emit subatomic particles and high-energy photons (gamma rays). This process is known as radioactive decay. Unstable isotopes of radium, radon, uranium, and thorium exist naturally, while others are formed naturally or by humans in activities such as operation of nuclear reactors. The major types of radiation emitted as a result of spontaneous decay are alpha and beta particles, and gamma rays. X rays arise from processes outside of the nucleus.

Ionized atoms (free radicals), regardless of how they are formed, are much more active chemically than neutral atoms. These chemically active ions can form compounds that interfere with the processes of cell division and metabolism. The degree of damage suffered during exposure to ionizing radiation depends upon the type, intensity, energy, duration, and chemical form of radiation. The underlying assumption in this document is that the effects from different types of radiation differ quantitatively but are qualitatively similar.



Source: IARC 2000.

Figure 1-1. The electromagnetic spectrum

This section presents information on the major types of ionizing radiation, focusing on X rays, gamma rays, and neutrons, and Table 1-1 presents the characteristics of these types of ionizing radiation. The transmission and absorption of ionizing radiation are discussed in Section 6.2.

1.1.1 Photon radiation

Photons are electromagnetic radiation having energy but no mass or charge. As a result, photons are less ionizing than particles but are more penetrating in matter.

1.1.1.1 X rays

X rays are high-energy photons produced by the interaction of charged particles with matter. X rays are produced effectively by the rapid deceleration of charged particles (often electrons) by a high atomic number material. X rays and gamma rays have essentially the same properties, but differ in origin. X rays are emitted from processes outside the nucleus, while gamma rays originate inside the nucleus. X rays are generally lower in energy and are less penetrating than gamma rays. A few mm of lead can stop medical X rays. The energy distribution of X rays is continuous with a maximum at an energy about one-third that of the most energetic electron. As photons interact with matter, their spectral distribution is further altered in a complex manner due to the transfer of energy (BEIR V 1990).

1.1.1.2 Gamma rays

Like visible light and X rays, gamma rays are weightless packets of energy called photons. Gamma rays often accompany the emission of alpha or beta particles from a nucleus. They have neither charge nor mass and are very penetrating. One source of gamma rays in the environment is naturally occurring potassium-40. Artificial sources include plutonium-239 and cesium-137. Gamma rays can easily pass through the human body or be absorbed by tissue, thus constituting a radiation hazard for the entire body.

Several feet of concrete or a few inches of lead are required to stop the more energetic gamma rays.

Gamma rays resulting from radioactive decay consist of monoenergetic photons with energies as high as several MeV. Due to the scattering and absorption within the radioactive source and the encapsulating material, the emitted photons have a relatively narrow spectrum of energies (BEIR V 1990).

1.1.2 Particle radiation

Particles are more highly ionizing than are photons. Excitation and ionization are the primary interactions with matter, and potential for ionization increases as mass and charge increase. The depth of penetration in tissue for particles decreases as mass and charge increase.

1.1.2.1 Neutrons

Neutrons are electrically neutral particles that, together with positively charged protons make up atomic nuclei. The number of neutrons defines the isotope of an element. Neutrons have mass and energy and may be produced by humans with machines such as a cyclotron. The neutron is unstable and decays to a proton by beta emission.

As uncharged particles, neutrons do not interact with atomic electrons in the matter through which they pass, but they do interact with the nuclei of the atoms present. The nuclear force, which leads to these interactions, is very short ranged, which means the neutrons have to pass close to a nucleus for an interaction to take place. Because of the small size of the nucleus in relation to the atom as a whole, the neutrons will have a low probability of interaction, and could thus travel considerable distances in matter.

The human body is composed largely of water, about 60% by weight, which contains many hydrogen nuclei. Elastic scattering of the neutrons with the hydrogen nuclei will cause the protons to recoil violently. Similarly, elastic collisions of neutrons with carbon, oxygen, or other heavier nuclei will cause these to recoil. Because the mass of protons and the other recoiling nuclei is much greater than that of electrons, they generate a much denser ion path resulting in more damage to the tissue. Once neutrons have been slowed down by elastic collisions to thermal energy they are readily captured by some of the reactions described above.

1.1.2.2 Alpha and beta particles

Alpha particles are energetic positively charged particles (similar to helium nuclei, 2 protons and 2 neutrons) that rapidly lose energy when passing through matter, while beta particles (positrons and electrons) are fast-moving positively or negatively charged electrons emitted from the nucleus during radioactive decay. Alpha and beta particles are not part of the nominations under consideration for listing in the Report on Carcinogens.

Table 1-1. Characteristics of different types of ionizing radiation

Radiation	Rest mass[a]	Charge	Typical energy range	Path length: air	Path length: solid	Comments
Gamma rays	–	0	10 keV–3 MeV	–[b]	–[b]	photon from nuclear transformation
X rays	–	0	5 keV–100 keV	–[b]	–[b]	photon from transition of an electron between atomic orbits
Neutrons	1.0086 amu: 939.55 MeV	0	0-15 MeV	–[b]	0–100 cm	free half-life: 10.4 min
Alpha (α) particles	4.0026 amu	+2	4–10 MeV	3–10 cm	25–80 μm	an electron-stripped He nucleus
Negatrons (β-)	5.48×10^{-4} amu; 0.51 MeV	-1	0–4 MeV	0–15 m	0–1 cm	identical to electron
Positrons (β+)	5.48×10^{-4} amu; 0.51 MeV	+1	0-4 MeV	0–15 m	0–1 cm	identical to electron except for sign of charge

Source: ATSDR 1999.

[a]The rest mass (in amu) has an energy equivalent in MeV that is obtained using the equation $E = mc^2$, where 1 amu = 932 MV

[b]Path lengths are not applicable since intensities decrease exponentially

amu = atomic mass unit, KeV = kiloElectron volts; MeV = megaElectron volts

1.2 Nomenclature

The following are definitions of various terms, units, and quantities that will be used throughout this document (these definitions are from IARC 2000, unless another source is provided). Standard International (SI) units are the units typically used today, while traditional units were used in the past and may have been used in older studies cited in this document. Some literature refers to absorbed dose (gray) while other studies refer to effective dose (sievert). For most gamma and x radiation, the absorbed dose and effective dose are the same; thus, one gray is equivalent to one sievert. A summary of the different units and their relationships is presented in Table 1-2.

1.2.1 Ionizing radiation

The term ionizing radiation comprises charged and neutral particles and electromagnetic radiation capable of ionizing matter either directly or by means of secondary products of their interactions with matter. Unless specified otherwise, “photons” in this text refers to the ionizing portion of the electromagnetic spectrum, which encompasses X rays and gamma rays. While energies down to 10 eV may be sufficient to strip loosely bound

orbital electrons, only photons with energies above ~1 keV are typically considered in radiation protection.

Table 1-2. SI and traditional units used in radiation dosimetry, with conversion factors

Quantity	SI unit	Traditional unit	Conversion factor (traditional/SI)	Conversion factor (SI/traditional)
Activity	becquerel (Bq) 1 Bq = 1 nuclear transformation per second	curie (Ci)	1 Ci = 3.7×10^{10} Bq	1 Bq = 2.7×10^{-11} Ci
Absorbed dose	gray (Gy) 1 Gy = 1 J per kg	rad	1 rad = 0.01 Gy	1 Gy = 100 rad
Equivalent dose	sievert (Sv) 1 Sv = 1 J per kg	rem	1 rem = 0.01 Sv	1 Sv = 100 rem
Effective dose	person-sievert (person-Sv)	person-rem	1 person-Sv = the aggregate energy deposited per amount of tissue (in J per kg) for a particular population	1 person-Sv = 100 person-rem
Exposure	coulomb per kilogram of air (C per kg)	roentgen (R)	1 R = 2.58×10^{-4} C per kg of air	1 C per kg = 3,876 R

Source: adapted from IARC 2000.

The amount of energy deposited per unit of path length in the material of interest by ionizing radiation is called the 'linear energy transfer' (LET), and is given in units of energy per unit length (e.g., keV/µm). Although gamma rays, X rays, and neutrons are all indirectly ionizing forms of radiation, they differ in energy transfer.

1.2.1.1 Low-LET radiation, including gamma rays and X rays

Photons (gamma rays and X rays) and electrons are considered low-LET (except for the very lowest energy electrons). Low-LET radiations tend to have more tortuous tracks in matter and have more widely dispersed energy deposition patterns (see Section 6.2.1 and Figure 6-1).

1.2.1.2 High-LET radiation, including neutrons

Neutrons and alpha particles, protons, and other heavy charged particles are high-LET. High-LET particles tend to slow down in straight lines, leaving dense energy deposition tracks (see Section 6.2.1 and Figure 6-2).

1.2.2 Activity

The traditional unit of radioactivity is the curie (Ci) where 1 Ci is equal to 3.7×10^{10} disintegrations per second. The SI unit is the becquerel (Bq). 1 Bq is equal to 1 disintegration per second.

1.2.3 Energy

The SI unit for energy is the joule (J). The energy of ionizing radiation is more commonly expressed in electron-volt (eV) units. One eV represents the energy gained by a single-charged particle, e.g., electron or proton, in a potential differential of 1 V and is equal to 1.6×10^{-19} J.

1.2.4 Dose

The radiation dose and dose rate are related to the damage inflicted on the body and may affect the probability of a stochastic effect such as cancer (IARC 2000). Radiation dose may be expressed as the absorbed dose, equivalent dose, effective dose, or collective dose. These dose measurements are discussed below. The dose rate is the dose per unit time.

1.2.4.1 Absorbed dose

The absorbed dose is the radiation energy absorbed per unit mass of an organ or tissue and is used in studies examining radiation damage to the human body. Dose (D) can be expressed as $D = de/dm$ where de is energy imparted to a finite volume of matter of mass dm. The SI unit of absorbed dose is the Gy and rad is the older unit.

1.2.4.2 Equivalent dose

The absorbed dose alone is not a sufficient indicator of the risk of deleterious effects to humans from ionizing radiation, in particular when delayed effects are of concern. The equivalent dose (H) to an organ or tissue is obtained by weighting the absorbed dose in an organ or tissue by a radiation weighting factor (discussed below). The equivalent dose in tissue (H_T) may be defined as:

$$H_T = \sum_R w_R D_{T,R}$$

Where: w_R = radiation weighting factor for radiation R.

$D_{T,R}$ = absorbed dose in tissue T associated with radiation R.

$\sum_R$ = the sum of all radiation types that impart ionizing tissue in tissue T.

The SI unit for the equivalent dose is the sievert (Sv), where 1 Sv = 1 J/kg.

The radiation weighting factor (formerly the quality factor) reflects the biological effectiveness of the particles that produce damage in the tissue. These numbers are derived from radiobiological experiments examining tumor induction in experimental animals and chromosomal aberrations in human lymphocytes. Radiation weighting factors selected by the International Commission on Radiological Protection (ICRP 1991a) are based on the relative biological effect of certain specific high-LET radiations

compared to that of a reference radiation. The selection is a committee decision rather than a rigorous scientific procedure. Historically, X rays were used as the reference radiation; however, gamma radiation is now frequently used as the reference radiation of choice. Based on some biological endpoints and on the physical characteristics of the deposition of energy, there is evidence that X rays are about twice as effective as gamma rays at very low doses. However, ICRP (1991a) has given a weighting factor (W_R) of 1 to all energies of all photon radiations. Values for radiation weighting factors are given in Table 1-3.

Table 1-3. Radiation weighting factors

Type and energy range	Radiation weighting factor
Photons, all energies	1
Neutrons, energy	–
< 10 keV	5
10–100 keV	10
0.1–2 MeV	20
2–20 MeV	10
> 20 MeV	5
Electrons and muons[a], all energies[b]	1
Protons, other than recoil protons, energy > 2 MeV	5
Alpha particles, fission fragments, heavy nuclei	20

Source: ICRP 1991a.

[a]One of the elementary particles, a member of a category of light-weight particles called leptons which also include electrons and neutrinos.

[b]Excluding Auger electrons (280–2100 eV) emitted from nuclei bound to DNA, which are ejected after excitation by an incident electron beam.

1.2.4.3 Effective dose

The effective dose (E) is the overall biological injury associated with radiation, which takes into account variations in equivalent dose among different organs and tissues. This value is calculated by multiplying the equivalent doses for a number of different organs by tissue weighting factors (discussed below). The effective dose is defined as:

$$E = \sum_T w_T H_T$$

Where: w_T = tissue weighting factor that reflects the contribution of the tissue to the total detriment to human health when the body is uniformly irradiated.
H_T = the equivalent dose in tissue T.

The standard unit for effective dose is the person-sievert (person-Sv), which is equivalent to the aggregate energy deposited per amount of tissue for a particular population.

Tissue weighting factors are based on studies of rates of cancer production in different organ systems after exposure to radiation. These factors are updated periodically and may

be revised in the near future by the International Commission on Radiological Protection (ICRP). Values of different tissue weighting factors are given in Table 1-4.

1.2.4.4 Collective dose

Adequate comparison of the effects from various radiation sources requires information on both individual doses and the number of people exposed. The collective dose is the product of the mean dose of an exposed group and the number of individuals in the group. This terminology is only useful when the individual doses are of the same order of magnitude and occur within a few years' time.

Table 1-4. Tissue weighting factors

Tissue or organ	Tissue weighting factor
Gonads	0.20
Bone marrow (active)	0.12
Colon	0.12
Lung	0.12
Stomach	0.12
Bladder	0.05
Breast	0.05
Liver	0.05
Esophagus	0.05
Thyroid	0.05
Skin	0.01
Bone surface	0.01
Remainder[a]	0.05

Source: ICRP 1991a.

[a]For the purposes of calculation, the remainder is composed of the following: adrenal glands, brain, upper large intestine, small intestine, kidney, muscle, pancreas, spleen, thymus, and uterus.

1.2.5 Exposure

Exposure is an outdated quantity providing a measure of ionizing radiation (limited to photons) in terms of ionization in air. The unit of exposure is the roentgen (R), which is equivalent to ionization of 2.58×10^{-4} coulomb/kg of air. Exposure is not applicable to particulate radiation, photons with energies exceeding 3 MeV, and media other than air.

1.2.6 Kerma

Kerma (kinetic energy released in matter) is the sum of the initial kinetic energies of all charged particles released by indirectly ionizing radiation in a volume element of a given material, divided by the mass of this element. The dimension is energy per unit mass; kerma is therefore a density type quantity. Its use is limited to ionizing radiation, i.e., X rays, gamma rays, and neutrons, and has been used in epidemiological studies of the

survivors of the atomic bombings in Japan. The SI unit of kerma is the gray; an older unit is the rad.

1.2.7 Relative biological effectiveness

A term called the relative biological effectiveness (RBE) has been used to compare the results from various studies involving exposure of biological systems to ionizing radiations of different qualities. RBEs are influenced by dose, dose rate, and fractionation and vary between tissues. The only singular value used to compare carcinogenic effects is the RBE maximum (RBE_m), which is the ratio of the initial slopes of the dose responses of the radiation under study and the reference radiation. The initial slopes are very difficult to determine with accuracy and thus there are very few acceptable estimates of RBE_m. Many of the RBEs in published reports do not meet the rigorous criteria that should be applied if the RBE is to be used to estimate human risk. It is the initial slope of the reference radiation, the alpha component of the linear-quadratic fit, that is particularly difficult to determine with single doses and can be better determined using low-dose-rate irradiation or multiple small doses. These approaches are based on the assumption that the dose response fits a linear-quadratic model. There is an underlying assumption made in the use of RBEs, namely, that the differences in the effects of the radiation under study and the reference radiation are quantitative and not qualitative. This assumption should be re-examined as more information becomes available regarding radiation quality-dependent differences at the molecular and chromosomal level and their repair.

The scientific literature contains a variety of RBE results based on different experiments and outcomes (NCRP 1990). The ICRP uses RBEs as the basis for describing the effectiveness of radiations of differing qualities by “quality factors” (ICRP 1977) and “radiation weighting factors” (ICRP 1991a) (discussed above). These factors are subject to change from time to time as new radiobiological evidence becomes available, or is re-examined by different scientific bodies (see Section 1.2.4.2 and Table 1-3).

2 Human Exposure

All individuals are exposed to ionizing radiation from a variety of natural and anthropogenic sources. Table 2-1 lists the annual per capita effective doses estimated by UNSCEAR (2000) for worldwide exposure to ionizing radiation from all sources.

Table 2-1. Annual worldwide per capita effective doses of ionizing radiation from natural and anthropogenic sources in the year 2000

Source	Worldwide annual per capita effective dose (mSv)	Range or trend in exposure
Natural background	2.4	Exposure may range from 1 to 10 mSv with some populations exposed to 10 to 20 mSv.
Diagnostic medical examinations	0.4	Exposure depends on level of health care; the range of exposures is 0.04 to 1.0 mSv on a per capita basis.
Chernobyl accident	0.002	Higher exposures have been experienced at locations nearest the accident site. The overall exposure has decreased from the maximum value of 0.04 mSv in 1986.
Nuclear power production	0.0002	Exposure has increased as the level of nuclear power production has increased, but improvements in safety procedures tend to decrease exposure.

Source: UNSCEAR 2000.

The largest contribution (82%) for radiation exposure (all types) to the U.S. population is from natural sources, of which radon and its decay products represent approximately two-thirds; the other one-third is from cosmic radiation, terrestrial radiation, and internally deposited radionuclides. The remaining 18% of the total contribution is from anthropogenic sources, such as radiation from medical procedures (15%), consumer products (3%), and other (< 1%), which includes occupational exposures, nuclear fallout, and the nuclear fuel cycle (BEIR V 1990).

This section provides information on the military, medical, occupational and environmental exposures, dosimetric methods and monitoring, and regulations for ionizing radiation (X and gamma radiation, and neutrons). Since the nomination does not include alpha and beta particles, environmental exposure to radon (the largest source of environmental exposure and an alpha-particle emitter) will not be discussed in this section. Radon is considered to be a known human carcinogen and was first listed in the 7th Report on Carcinogens (1994).

2.1 X radiation and gamma radiation

2.1.1 Military exposures

2.1.1.1 Atomic bombs over Hiroshima and Nagasaki

The atomic bombs detonated in Hiroshima and Nagasaki, Japan in 1945 resulted in hundreds of thousands of people being exposed to gamma rays. Dose estimates are available for 86,572 survivors of the bombings of Hiroshima and Nagasaki who were studied as part of the Life Span Study, studies conducted by the Radiation Effects Research Foundation investigating the long-term effects of exposure during the bombings. An average exposure of about 300 mSv was estimated, with the doses decreasing with distance from the bombing center. Gamma ray and neutron doses to the colon were estimated for the 86,572 survivors, with the dose estimates accounting for shielding of the organs by the body and the survivors' orientation, position, and shielding at the time of the bombings (IARC 2000). The collective dose to the colon was estimated at 24,000 person-Sv, and the highest doses to the colon were > 2,000 mSv (see Table 2-2).

Table 2-2. Number of survivors of the atomic bombings of Japan

City	Total	Weighted colon dose (Sv)[a]								
		<0.005	**0.005-0.02**	**0.02-0.05**	**0.05-0.1**	**0.1-0.2**	**0.2-0.5**	**0.5-1.0**	**1.0-2.0**	**3 2.0**
Hiroshima	58,459	21,370	11,300	6,847	5,617	4,504	5,078	2,177	1,070	496
Nagasaki	28,113	15,089	5,621	2,543	921	963	1,230	1,025	538	183
Total	86,572	36,459	16,921	9,390	6,538	5,467	6,308	3,202	1,608	679

Source: Pierce *et al*. (1996, cited in IARC 2000).

[a] Categories defined with a weighting factor of 10 for neutrons. The weighted colon dose was considered to be representative of a more general dose.

2.1.1.2 Nuclear Weapons Testing

Between 1945 and 1980, approximately 520 atmospheric nuclear tests were carried out in the northern hemisphere, with the most intense period of testing between 1952 and 1962. The total collective effective dose of X and gamma rays from weapons testing to date has been estimated at approximately 2.2 x 10^6 person-Sv (IARC 2000). The dose of radiation that people received depended on the distance between their homes and the test sites. Between 1951 and 1975, at a nuclear test site in Nevada, the collective dose of gamma rays to the local population (180,000 persons) was estimated to be approximately 86,000 person-R (the authors used the traditional unit of exposure, the roentgen, because the calculations were made based on historical measurements of the exposure rate) (Anspaugh *et al.* 1990). IARC (2000) reviewed the Anspaugh *et al*. (1990) study and stated that the collective dose to the local population from 1951 to 1962 is equivalent to approximately 500 person-Sv, corresponding to an average dose of about 3 mSv.

2.1.2 Medical exposures

Medical exposures are very small compared to exposure to natural sources of radiation (see Table 2-1). Medical procedures result in an annual collective dose of about 2 x 10^6 person-Sv/year, compared to background exposures of about 14 x 10^6 person-Sv/year (UNSCEAR 2000).

Medical use of ionizing radiation in both diagnosis and therapy has been widespread since the discovery of X rays by Wilhelm Conrad Roentgen in 1895. Advances in the latter half of the twentieth century brought about an increase in the uses of medical radiation, with some techniques, particularly radiotherapy, computed tomography, positron emission tomograpy, and interventional radiation involving fluoroscopy, involving higher doses than for standard diagnostic X rays.

In medical exposures, the exposed individual receives a direct benefit from the procedures, and so the risk/benefit equation is different than for other exposures to artificial radiation. The age, sex, and health status of the population exposed to medical radiation differs from that of the general population: the distribution tends to be skewed towards older age groups (which would reduce the potential carcinogenic risk), but also involves children and adolescents. The approximate distribution by age and sex of recipients of medical radiation in developed countries is shown in Table 2-3.

Table 2-3. Distribution of medical procedures by age and sex

Procedure	Age (years)			Sex	
	0–15	16–40	> 40	Male	Female
Diagnostic radiology, except dental X rays	11	29	60	49	51
Teletherapy	1	11	88	49	51

Source: UNSCEAR 2000.

The exposure to the world's population from medical radiation is much more variable than that from natural background radiation (even though natural background radiation varies considerably between locations), due to the marked difference in the quality of medical care in different cultures. The more developed countries have higher percentages of the population receiving regular medical care, and thus, average and collective radiation exposures are higher (UNSCEAR 1988, 1993, 2000). Health care practice may be divided into four levels on the basis of the number of physicians per 1,000 population: level I, one physician per 1,000 population; level II, one physician per 1,000 to 3,000; level III, one physician per 3,000 to 10,000; and level IV, fewer than one physician per 10,000 persons. In the year 2000, countries with level I health care had about 26% of the world's population, those with level II had 53%, those with level III had 11% and those with level IV had 10%. The approximate numbers of medical radiation procedures performed in countries in each of these categories are shown in Table 2-4.

Table 2-4. Annual frequency of various radiation procedures per 1,000 population

Health care level[a]	I	II	III	IV
Estimated population in millions	1,530 (26%)	3,070 (53%)	640 (11%)	565 (10%)
Diagnostic radiology	920	154	17	29
Dental radiology	309	14	0.2	0.07
Teletherapy	1.5	0.69	0.46	0.05
Nuclear medicine therapy	0.17	0.036	0.021	0.0004

Source: UNSCEAR 2000.

[a]I = 1 physician/1,000 persons; II = 1 physician/1,000–3,000 persons; III = 1 physician/3,000–10,000 persons; IV = < 1 physician/10,000 persons.

Population averages involving medical radiation are not easy to interpret and compare to other exposures, as the individuals exposed have health problems, and this may confound interpretations involving lifespan and cancer incidence. Exposures in medical procedures vary widely. Entrance doses and effective doses from chest X rays are typically less than 1 mGy, whereas doses from radiation therapy are much higher, often near 50 Gy (50,000 mGy) (UNSCEAR 2000).

The following sections present an overview of the major medical uses of X and gamma rays. For further details on these medical applications, see Appendix A.

2.1.2.1 Diagnostic radiology

Diagnostic X rays vary in exposure level but are generally low. In more developed countries, the use of rare-earth screens and fast film has significantly reduced the dose from many procedures. Plain film examinations of the chest and extremities involve relatively low doses (effective doses of perhaps 0.05 to 0.4 mSv), whereas studies involving the abdomen and lumbar spine or pelvis may result in higher doses (effective doses of around 1 to 3 mSv). Approximate doses to skin and effective doses for a number of diagnostic radiology procedures in developed countries are shown in Table 2-5 (UNSCEAR 2000).

Fluoroscopic procedures involve much higher exposures, as the X-ray beam is energized for longer periods of time to allow observation of the movement of material, placement of catheters, and other processes. The typical dose rate to the skin in the primary beam may be around 30 to 50 mGy/min, and the effective dose may reach 1 to 10 mSv. Long interventional procedures (such as coronary angioplasty to widen obstructed blood vessels) may result in skin doses of 0.5 to 5 Gy (500 to 5,000 mGy) and effective doses of as much as 10 to 50 mSv. In some particularly difficult cases involving long exposures, effects in the skin such as epilation and necrosis have been reported.

2.1.2.2 Computed tomograph (CT)

Since the introduction of helical computed tomography (CT) in 1989, CT technology has made significant physical, geometrical, and mechanical advances. In 1998, multi-detector helical scanners were introduced. During this period of technologic development, the

scope of clinical CT applications has expanded from diagnosis, to cancer staging, to CT fluoroscopy, to coronary artery calcium scoring, to treatment planning in radiation oncology, and to co-registration of images in combined PET/CT scanners. Further, collective dose from CT examinations has been increasing since 1989. According to the NRPB of the United Kingdom, CT examinations comprised approximately 2% of all X-ray procedures in 1989 among surveyed installations, but contributed to approximately 20% of the total collective dose. In 1999, the number of CT examinations increased to 4% of X-ray procedures, and their contribution to the collective dose was approximately 40%. Thus, CT dosimetry has become an important subject in both adult and pediatric CT examinations in recent years.

Table 2-5. Approximate mean effective doses from diagnostic radiological procedures in highly developed countries (Health care level I)

Procedure	Average effective dose (mSv) per examination[a]	Average number of examinations per 1,000 population per year
Chest radiograph	0.14	236
Lumbar spine radiograph	1.8	45
Abdominal radiograph	0.53	41
Urography	3.1[b]	12
Gastrointestinal tract radiograph	3.6, 6.4[c]	54, 8.6[c]
Mammography	0.12	25
Radiograph of extremity	0.06	212
Computed tomography, head	0.8–2.6	14
Computed tomography, body	1.5–27	19
Angiography	0.9–23	7.6
Dental X ray	0.016	310
Overall	0.50	942

Source: UNSCEAR 2000.
[a]Doses may vary from these values by as much as an order of magnitude depending on the technique, equipment, and film type and processing.
[b]Source: (UNSCEAR 1993).
[c]For upper and lower GI tract, respectively.

Imaging procedures that do not involve ionizing radiation (ultrasound and magnetic resonance imaging) have increased in popularity in recent decades. Nonetheless, the overall number of procedures employing ionizing radiation has continued to increase. In level I health care countries, the total frequency of diagnostic radiology examinations per 1,000 population increased approximately 12% over the last three decades. The growth in the number of examinations in less-developed countries was greater still (UNSCEAR 2000).

Use of computed tomography has become widely available in many developed countries. In contrast to plain-film radiography, tomographic techniques provide excellent

visualization of soft tissue and good spatial resolution. The scans involve significantly higher doses of radiation (effective doses of perhaps 2.5 to 15 mSv) than plain film techniques. In contrast to plain film techniques, higher exposures always result in better quality images, so care is needed to optimize techniques to obtain the best diagnostic information possible while maintaining radiation doses as low as reasonably achievable, particularly in studies involving pediatric patients (Brenner *et al.* 2001a). The rapid growth in use of computed tomography has resulted in increases in both the average and collective doses from medical diagnosis in many countries. In the U.S., even though computed tomography accounts for less than 10% of procedures, it accounts for over 40% of the absorbed dose (UNSCEAR 2000).

2.1.2.3 Radiation therapy

In radiation therapy, the goal is to deliver high doses of radiation to cancer cells while minimizing doses to normal tissues. For some patients, such as those with limited survival potential, the goal is not to cure the disease but merely to palliate pain.

Radiation therapy may involve use of external beams of radiation, typically high-energy X rays (4 to 50 MeV) and cobalt-60 (^{60}Co) gamma rays. For superficial lesions, electron beams may be used (UNSCEAR 2000). Teletherapy (therapy with proton beams), in which high amounts of energy delivered in the Bragg peak of the radiation deposition curve and lower doses in the overlying tissue, is used for a wide variety of tumors. As seen in Table 2-3, about 90% of teletherapy patients are over age 40; only 1% are children, most of whom have leukemia or lymphoma. Absorbed doses for most teletherapy regimens are in the range of 20 to 60 Gy (20,000 to 60,000 mGy), usually delivered in daily fractions of 2-4 Gy over five weeks. Treatment for leukemia usually involves irradiation of the total marrow, with doses of about 10 to 20 Gy (10,000 to 20,000 mGy) delivered in several fractions (UNSCEAR 2000).

Doses of radiation used in therapeutic nuclear medicine are of course much larger than those used in diagnosis. Radiopharmaceuticals are administered to accumulate in specific tissues, to deliver high absorbed doses, and to kill cells.

2.1.3 Occupational exposure

This section presents information on workers who are occupationally exposed to X rays or gamma rays. Workers in the uranium mining and mill industry, the radiopharmaceutical industry, fuel fabrication, fuel processing, and the luminizing industry will not be discussed here since they are exposed primarily to alpha and beta particle emitters, i.e., radium, uranium, and radon.

According to IARC (2000), approximately 5 million workers worldwide are exposed to natural sources of radiation at levels above background. About 75% are coal miners, about 13% are underground miners in non-coal mines, and about 5% are crews on airlines. See Table 2-6 for the annual occupational exposures of monitored workers worldwide from 1985 to 1989. Most of these exposures are mainly to X rays and gamma radiation.

2.1.3.1 Medical Workers

Workers in the medical profession may be exposed to many different types of radionuclides and radiation. In the early part of the 20th century, radiologists were exposed to high doses of X rays, but today they are exposed to greatly reduced doses. In 1983, X-ray technicians, radiologists, and physicians in the U.S. had average effective doses of 0.96, 0.71, and 0.31 mSv, respectively (NCRP 1989). Teletherapy (see Section 2.1.2.2) involves exposure to gamma and beta rays, although technicians are exposed to lower doses than are patients due to shielding of sources and the limited duration of exposure (IARC 2000). Mostafa *et al*. (2002) measured the exposure of scattered radiation to nurses and other healthcare workers in a trauma intensive care unit. They showed that the level of scattered radiation was less than the allowable exposure of 100 mrem (1 mSv) per year in noncontrolled areas.

Table 2-6. Worldwide occupational exposures to radiation, 1985-1989

Occupational category	Annual average collective effective dose (person-Sv)	Annual average effective dose per monitored worker (mSv)
Mining	1,200	4.4
Milling	120	6.3
Enrichment	0.4	0.08
Fuel fabrication	22	0.8
Reactor operation	1,100	2.5
Reprocessing	36	3.0
Research	100	0.8
Total (rounded)	2,500[a]	2.9
Other occupations		
Industrial applications	510	0.9
Military activities	250	0.7
Medical applications	1,000	0.5
Total (rounded)	1,800[b]	0.6
All applications (rounded)	4,300[c]	1.1

Source: UNSCEAR 1993.

[a]Total of listed items is 2578.4
[b]Total of listed items is 1760
[c]Total of listed items is 4338.4

2.1.3.2 Nuclear industry workers

Workers in commercial nuclear power plants are exposed primarily to gamma radiation. A study in the U.S. in 1984 showed the average equivalent dose for gamma radiation at nuclear power plants for workers was 4.9 mSv, and the annual collective effective dose equivalent was 280 person-Sv (NCRP 1989).

Workers involved in the production of nuclear weapons are exposed to a large number of radionuclides and types of radiation. Workers in reactors are exposed primarily to gamma radiation and beta radiation from fission products and neutron activation products. Fuel reprocessing and separation of weapons materials results in workers being exposed first to gamma radiation from the fission products and then to alpha radiation from plutonium, uranium, and americium during fuel reprocessing (IARC 2000).

A number of studies have examined the collective doses of radiation received by monitored workers in nuclear weapons facilities (see Table 2-7). Of the three U.S facilities studied, a collective dose of 140 to 880 Sv (140,000 to 880,000 mSv), and an average dose of 21 to 36 mSv was reported (Cardis *et al.* 1995).

2.1.3.3 Airline workers

Airline pilots and crew are exposed to both gamma radiation and neutrons (see Section 2.2.3.2). An annual effective dose to aircrews was estimated at 3 mSv (IARC 2000), with an effective dose equivalent for a transatlantic flight estimated to be up to 0.1 mSv (Schalch and Scharmann 1993). In addition, astronauts are exposed to large amounts of radiation from solar flares, the earth's radiation belts, and cosmic radiation. It was estimated that the average radiation doses (type not specified) for the crews of the Apollo missions (5 to 12 days mission) were 0.0016 to 0.0114 Gy (1.6 to 11.4 mGy) and the average doses for the Skylab mission, which lasted 20 to 90 days, were 0.016 to 0.77 Gy (16 to 770 mGy) (ATSDR 1999).

Table 2-7. Collective doses received by monitored workers in nuclear facilities involving exposure to radiation

Facility	No. of workers	Cumulative	
		Collective dose (Sv)	Average dose (mSv)
Sellafield, U.K.	9,494	1,310	138
Atomic Energy Authority and Atomic Weapons Establishment, U.K.	29,000	959	33
Atomic Energy of Canada	11,355	315	28
Hanford, Washington	32,595	877	27
Rocky Flats, Colorado	6,638	242	36
Oak Ridge National Laboratory, Tennessee	6,591	141	21
Total	95,673	3,844	40

Source: Cardis *et al*. 1995.

2.1.3.4 Miners

Coal miners and miners of other minerals are exposed mainly to radon; however, they are included in this discussion because they also are exposed to gamma radiation. The annual effective dose of these workers is estimated to be 8,600 person-Sv, with the vast majority of this exposure assumed to be from radon (IARC 2000).

2.1.3.5 Other occupations

Radioactive materials are used in a number of industrial processes. One industrial process, industrial radiography, i.e., the radiography of welded joints with large sources of gamma radiation, was estimated to result in an average annual effective dose of U.S. workers to gamma radiation of 2.8 mSv in 1985 (NCRP 1989). Another industrial process in which radiation is used is industrial irradiators used to sterilize products or irradiate foods (IARC 2000). Oil-field workers may be exposed to low doses of gamma radiation and neutrons during a process called "well logging" in which gamma ray or neutron sources are used to determine the geological structures in a bore hole. In 1979, the average annual effective dose equivalent of workers in the U.S. to gamma radiation in this process was estimated to be 4.20 mSv (NCRP 1989).

Workers also may be exposed to ionizing radiation in research laboratories. A study of workers at the Los Alamos National Laboratory reported that approximately 8,700 former employees were exposed to external radiation (type and doses not reported) from the mid-1940s to the late 1970s (Breysse *et al.* 2002).

2.1.4 Environmental exposure

This section presents information on human exposure to X rays and gamma rays in the environment. Exposure from sources and processes such as nuclear reactors for power generation, nuclear weapons production, fuel reprocessing, and nuclear waste disposal will not be discussed in detail in this section, since these sources, e.g., radon, uranium, and carbon-14 (^{14}C), emit primarily alpha and beta particles to the environment. However, workers in these facilities may be exposed to gamma radiation and neutrons, and this information is presented in Sections 2.1.3.2 and 2.2.3.1.

2.1.4.1 Natural sources

Radiation is present naturally in the environment from cosmic and terrestrial sources. As shown in Figure 2-1, cosmic radiation contributes approximately 8% of the average population dose of ionizing radiation; however, it only contributes a very small amount of the total exposure to X rays and gamma rays.

Terrestrial radiation contributes 8% of the average population dose of ionizing radiation, the same percentage as cosmic radiation; however, most natural exposure to X and gamma rays is from terrestrial sources. Radioactivity in soil is based on the rock from which it originates. The majority of radioactive nuclei are chemically bound in the earth's crust and not available for human exposure; only the upper 25 cm of the crust provide escaping gamma radiation that results in human exposure (IARC 2000).

Indoor exposure to gamma rays is mainly determined by the building construction materials; indoor exposure is greater than outdoor exposure if earth materials (stone, masonry) have been used (IARC 2000). In the U.S., since wood-frame houses are very common, outdoor dose rates to gamma rays tend to be higher than indoor rates. This is demonstrated by the fact that the absorbed dose rate in air in the U.S. from terrestrial gamma radiation was estimated to be 47 nGy/h [0.000047 mGy/h] outdoors and 38 nGy/h [0.000038 mGy/h] indoors (UNSCEAR 2000).

2.1.4.2 Nuclear accidents

The largest nuclear accident to date worldwide occurred in Chernobyl, Ukraine in 1986. The annual per capita effective dose worldwide in the year 2000 from the Chernobyl accident was 0.002 mSv, which had decreased from a maximum of 0.04 mSv in 1986. The levels are higher at locations nearer the accident site (UNSCEAR 2000). There have been several radiation accidents in the U.S.; the best known was the accident in 1979 at the Three-Mile Island nuclear plant in Pennsylvania. While more than 100,000 people were exposed to high levels of radioactivity as a result of Chernobyl, no radiation injuries resulted from the Three-Mile Island accident (Saenger 1986) where the total dose to the population within a 80-km radius of the reactor was estimated at about 20 person-Sv (Gerusky 1981). Directly comparable data for the radiation exposure to the population around Chernobyl could not be identified, but the much greater magnitude of the Chernobyl explosion is illustrated by the release of 40 million Ci of ^{131}I, 3 million Ci of ^{137}Cs and 50 million Ci of xenon radioisotopes compared to the release from Three-Mile Island of 15 Ci of ^{131}I (Bonte 1988).

2.1.4.3 Nuclear power generation

The majority of the exposure from the generation of electrical energy from nuclear power plants is to alpha and beta particles. However, there also is exposure to gamma rays and X rays; the collective effective dose from gamma rays and X rays was estimated to be about 0.2 person-Sv per year of electrical energy generation. This corresponds to an average dose for the world's population of about 0.1 µSv (0.0001 mSv) (IARC 2000).

2.1.4.4 Consumer products

Consumer products contribute approximately 3% of the average population radiation dose. Ionization-type smoke detectors contain americium-241 (^{241}Am), which emits both gamma rays and alpha particles, incorporated in a metal foil. Current smoke detectors contain less than 40 kBq of ^{241}Am, although in the past detectors with up to 3.7 MBq were used in commercial and industrial facilities (IARC 2000). Television sets emit low energy X rays through a process by which electrons are accelerated and bombard the screen. The total annual dose for an individual watching a color television has been estimated to be 2 to 3 mrad (0.02 to 0.03 mGy) per year (ATSDR 1999). Other consumer products containing ionizing radiation (type of radiation unspecified) include radioluminous clocks and watches, gaseous tritium light devices, and thoriated gas mantles. Miscellaneous consumer products containing sources of ionizing radiation include radioactive attachments to lightning conductors, static elimination devices, fluorescent lamp starters, porcelain teeth, gemstones activated by neutrons, and thoriated tungsten welding rods. All of these products have restrictions as to the maximum

radioactivity allowable in the product and contribute little to the overall population exposure to ionizing radiation (IARC 2000).

2.1.4.5 All sources of radiation

The estimated collective doses from X rays and gamma rays worldwide for a variety of natural and anthropogenic sources are listed in Table 2-8. The largest contribution is from natural sources, followed by medical uses, atmospheric nuclear weapons tests and nuclear power generation.

Table 2-8. Collective doses from X rays and gamma rays worldwide from 1945-1992

Source	Basis of commitment	Collective effective dose from X and gamma rays (million person-Sv)
Natural	current rate for 50 years	120
Medical uses		
Diagnosis	current rate for 50 years	80
Treatment	"	75
Atmospheric nuclear weapons tests	completed practice	2.5
Nuclear power generation	total practice to date	0.2
	current rate for 50 years	2
Severe accidents	events to date	0.3
Occupational exposure		
Medical	current rate for 50 years	0.05
Nuclear power	"	0.12
Industrial uses	"	0.03
Military activities	"	0.01
Non-uranium mining	"	0.4
Total occupational exposure	"	0.6

Source: UNSCEAR 1993, IARC 2000.

2.2 Neutrons

2.2.1 Military exposures

The atomic bombs at Hiroshima and Nagasaki, Japan in 1945 released low levels of neutrons to the environment. It has been estimated that only 1% to 2% of the total dose of ionizing radiation from the bombs was from neutrons (IARC 2000).

2.2.2 Medical uses

Neutrons have very limited use in medical devices. There is some use of neutrons in external beam therapy and boron neutron capture therapy (IARC 2000).

2.2.3 Occupational exposure

2.2.3.1 Nuclear industry workers

Occupational exposure to neutrons occurs primarily in the nuclear industry (IARC 2000). As previously discussed (see Section 2.1.3.2), workers in commercial nuclear power plants are exposed mainly to gamma radiation. Although some workers also are exposed to neutrons, one study showed that less than 3% of the total annual effective dose of nuclear plant workers in the United Kingdom (1946 to 1988) was from neutrons (Carpenter *et al.* 1994). A study in the U.S. in 1984 showed the average equivalent doses of neutrons at nuclear power plants for workers was 5.6 mSv and the total collective dose was 0.038 person-Sv (NCRP 1989).

Workers involved in the production of nuclear weapons may be exposed, during the later stages of weapons production, to low levels of neutrons from alpha particle reactions with light materials. In 1979, 24,787 workers in the U.S. were monitored for exposure to neutrons, and only 326 (1.4%) received neutron dose equivalents greater than 5 mSv (IARC 2000).

A study in the U.S. based on data from 1977 to 1984 showed the average annual effective dose equivalent of neutrons for radiation workers was 1.8 mSv, and the collective effective dose equivalent was 67.5 person-Sv (see Table 2-9).

Table 2-9. Estimated exposure of radiation workers in the U.S. to neutrons.

Employer	No. of exposed individuals	Average annual effective dose equivalent (mSv)	Collective effective dose equivalent (person-Sv)
Department of Energy contractors	25,000[a]	2.6	64
Nuclear power stations	1,100	0.5	0.6
U.S. Navy	12,000	0.24	2.9
Total	38,100	1.8	67.5

Source: IARC 2000.

[a]Total number of workers.

2.2.3.2 Airline Workers

Aircraft crews are exposed to varying amounts of neutrons, depending on the flight route, the aircraft type, and number of flight hours. Studies have estimated average dose equivalents for neutrons for airline crews ranging from 0.6 to 3.6 mSv/year (IARC 2000).

2.2.3.3 Other Occupations

As previously discussed (see Section 2.1.3.5), oil-field workers may be exposed to low doses of neutron radiation during well logging. Annual dose equivalents of neutron exposure from this process have been estimated at approximately 1 to 2 mSv (Fujimoto *et al.* 1985).

2.2.4 Environmental exposure

This section discusses exposure to neutrons from environmental sources.

2.2.4.1 Natural sources

Most exposure to neutrons is from cosmic radiation, the result of cosmic rays entering the solar system and interacting with solar wind, which contains the solar magnetic field. Only the most energetic particles produce effects at ground level (IARC 2000). A small portion of cosmic radiation originates from the sun, with the amount increasing during periods of increased sunspot and solar flare activity. These events run in approximately 11 year cycles; the largest event observed to date occurred in February 1956, during which rates of neutron counts at ground level rose to 3,600% above normal background levels (ATSDR 1999, IARC 2000). The average dose from cosmic radiation increases at higher altitudes; the dose in Denver, Colorado at an altitude of 1,600 meters is approximately twice that received at sea level (IARC 2000).

The annual effective dose rate from neutrons at sea level and a 50° latitude was estimated to be 80 µSv [0.080 mSv] per year (UNSCEAR 2000). Collective dose equivalents from neutrons have been estimated based on airplane travel rates and estimated neutron exposures. For example, in 1985, an average of 3×10^9 passenger hours in flight was estimated with an annual average rate of approximately 1.6 µSv [0.0016 mSv] per hour of neutrons, resulting in a collective dose equivalent of 5,040 person-Sv of neutrons. By 1997, air travel had grown to 4.3×10^9 passenger hours in flight, resulting in a collective dose equivalent of 7,200 person-Sv of neutrons (IARC 2000).

2.2.4.2 Other sources

There are no anthropogenic releases of neutrons to the environment that would result in significant human exposure.

2.2.4.3 All sources of radiation

UNSCEAR (2000) estimated the average effective dose of neutrons for the world population at 100 µSv [0.1 mSv] per year, with the dose for the population living in the northern hemisphere estimated at 104 µSv [0.104 mSv] per year.

2.3 Dosimetric methods and monitoring

2.3.1 Monitoring methods: X radiation and gamma radiation

The major methods for monitoring exposure to X rays and gamma rays are described in Tables 2-10 and 2-11, below (ATSDR 1999, IARC 2000, Dr. Terry Yoshizumi personal communication).

2.3.2 Monitoring methods: neutrons

The major methods for monitoring exposure to neutrons are described in Tables 2-12 and 2-13, below (ATSDR 1999, IARC 2000, Dr. Terry Yoshizumi personal communication).

Table 2-10. X ray and gamma ray detectors

Detector type	Operating principle	Uses and advantages/disadvantages
Gas Detectors Include ionization chamber (IC), proportional counter (PC), and Geiger-Mueller (GM)	Measures ionization generated by radiation in a gas medium.	X rays are detected by PCs filled with noble gases and beta particles are detected by thin window GMs and PCs.
Scintillators	Detects light (via an electrical signal by a photomultipher tube) emitted from materials excited by interactions with ionizing radiation	Used in photon spectrometry and photon survey instruments. More sensitive to photons than gas detectors.
Semiconductors Most common: silicon, cadmium, telluride and germanium	Measures photon spectra and identifies nuclides in the field.	Best detectors to perform photon and charged particle spectrometry.

Table 2-11. Personnel Monitors for X rays and gamma rays

Detector type	Operating principle	Uses and advantages/disadvantages
Film badges	Consists of a piece of film sandwiched between metal filters and a plastic holder. Radiation exposure results in deposition of metallic silver, and the dose is determined as light transmission that varies inversely with the amount of deposited silver.	Inexpensive and easy to handle. Detection limit 10 mR (2.6 µC per kg).
Thermoluminescent dosimeters	Consists of a photomuliplier tube that measures light emitted when excited electrons return to their normal state. The intensity of light is proportional to the radiation energy absorbed.	Several types are used for personnel monitoring, e.g., lithium fluoride (LiF), calcium fluoride/manganese (CaF_2:Mn), and lithium boron oxide ($Li_2B_4O_7$).
Optically stimulate luminescense technology - aluminum oxide detectors	Measures luminsescent, which is in proportion to the amount of ionizing radiation absorbed.	Monitoring doses to hands and fingers from beta particles and photons. Reusable Detection limit 0.01 mSv (1 mrem)
Self-reading pocket dosimeters Include pocket ion chambers and electronic pocket dosimeters (EPD).	Pocket ion chambers operate on electroscope principle. EPD are usually based on silicon diode detectors.	Pocket ion chambers have low cost but poor reliability. EPD are accurate and reliable.

Table 2-12. Monitoring methods for neutrons

Detector type	Operating principle	Uses and advantages/disadvantages
Detectors of slow neutrons Include proportional counters filled with $^{10}BF_3$ or ^{3}He gas, or containing a liner with ^{10}B; scintillators with ^{6}Li or ^{10}B, ionization chambers lined with ^{235}U, or semiconductors attached to a ^{6}Li or ^{10}B radiator	Uses a few exo -energetic neutron capture reactions that exhibit very high cross-section: (n,α) reaction on lithium-6 (^{6}Li) and boron-10 (^{10}B), (n,p) reaction on helium-3 (^{3}He) and fission on uranium-235 (^{235}U).	Strong neutron signal and very effective discrimination of the photon background.
Detectors of fast neutrons Include recoil proton technique in gas detectors, or in solid or liquid organic scintillators; capture reactions; and moderated detectors and tissue-equivalent proportional counter.	Tissue-equivalent (TE) ionization chambers, filled with TE gas, can be used to determine absorbed dose, which is measured as a function of linear energy transfer. Recoil proton detectors measure secondary protons that are created by neutrons scattering off hydrogen. TEPC measure dose distribution as a function of LET.	Capture reaction detectors have a lower efficiency. TEPC can detects neutrons > ~10 keV
Rem meters	A moderated detector with its size and inner absorber layers adjusted so that its energy response curve mimics as close as possible the fluence-to-dose equivalent conversion factor, $h_{\Phi}(E)$.	Most traditional meters detect neutrons with energies from thermal to ~10 MeV. More recently, the upper energy limit has been extended beyond 100 MeV. Accuracy ± 30%.
Neutron spectrometers Include Proton recoil spectrometry, and mutisphere or “Bonner sphere" spectrometry	Measures the neutron energy spectrum, i.e., fluence, as a function of energy, and calculates the corresponding dose equivalent using fluence-to-dose equivalent conversion factors.	Protein recoil spectrometry can be used only for neutrons with energies above approximately 10 keV and have good energy resolution. Multisphere spectrometer is the only system that covers the energy range from thermal to several tens of MeV but the resolution is course.

Table 2-13. Personnel monitors for neutrons

Detector type	Operating principle	Uses and advantages/disadvantages
Nuclear emulsions	Operates on the same principle as film badges using thicker emulsion layers. Tracks in the emulsions are created by secondary protons generated by neutron recoil on hydrogen atoms in the emulsion and its substrate.	Labor-intensive. Sensitivity energies above ~0.6 MeV.
Thermoluminescent detectors	Uses lithium fluoride (^{6}Li) and lithium boron isotopes (^{10}B), which exhibit high cross sections for the (n,α) reaction.	Unsuitable for detection of fast neutrons.
Track-etch detectors	Passage of a charged ionizing particle through a dielectric material results in energy deposition and damage to molecules along the track. Tracks are visible by etching using a hydroxide or an acid.	Personnel dosimetry. Can detect neutrons with energies > ~0.1 MeV.
Electronic pocket dosimeters	Radiator materials can increase sensitivity by generating secondary charged particles.	Personnel dosimetry. Silicon diodes are sensitive to fast neutrons.
Activation detectors	Uses different materials with different energy thresholds for neutron activation to obtain a crude estimate of the neutron spectrum.	Used as supplemental detector for accidents when the dynamic range of the regular dosimeter is exceeded. Not suitable for routine personnel dosimetry.
Bubble detectors	Uses droplets of superheated liquid, which become heated and evaporate and form a bubble after interaction with neutrons. The signal (bubbles or gas volume) is proportional to the neutron dose equivalent.	Used as supplemental detectors. Not suitable for routine personnel dosimetry.

2.3.3 Patient exposure and dosimetry in the medical setting

External irradiation of patients occurs in diagnostic radiography, fluoroscopy, and radiation therapy. In diagnostic radiology, patient dose considerations are usually secondary to obtaining necessary clinical information. Further, the range of dose to specific organs may vary many orders of magnitude due to the difference in patient size and shape, the difference in manufacturers' imaging system designs, and the range of technical exposure parameters employed with each imaging system. In fluoroscopy, the actual dose distribution to which a patient is exposed is often difficult to determine even when all technical parameters are known since the beam direction may vary during the procedure. In radiation therapy, a high degree of accuracy in dose is required in clinical delivery of dose to a target organ, i.e., a 5% uncertainty in dose may be crucial to the clinical results.

Patient dose in diagnostic radiology is estimated using combinations of local physical measurements and previously tabulated data presented in tables and graphs. A number of organizations have published handbooks for estimating absorbed organ doses for reference adults and children, including the Center for Radiological Health, Germany's National Research Center for Environment and Health, Institute for Radiological Protection, and the United Kingdom's National Radiological Protection Board (NRPB).

In external radiotherapy, linear accelerators or teletherapy (^{60}Co) units are employed. In the U.S., however, teletherapy (^{60}Co) units are being replaced with modern linear accelerators. Modern external radiotherapy employs 3-D conformal radiotherapy (3DCRT). Conformal radiotherapy refers to a method for planning and treating target volumes based on 3-D images (CT) which aims to produce a high dose area of radiation that conforms to the shape of the target. The treatment planning software incorporates dose measurements taken in a water phantom with an ionization chamber. In recent years, the Metal Oxide Semiconductor Field Effect Transistor (MOSFET) was used for *in-vivo* verification of surface dose (Butson *et al.* 1996).

2.4 Biological indices of exposure

Biological indices currently in use for measuring exposure to ionizing radiation consist of two types of bioassay programs: *in-vivo* analysis (direct bioassay) and *in-vitro* analysis (indirect bioassay). *In-vivo* analysis is the measurement of radioactivity in the human body utilizing external counting equipment for the detection of radiation in the body. Typical equipment includes a whole body or chair counter with normally one or more sodium iodide (NaI) scintillation crystals with photomultiplier tubes.

In-vitro analysis is the estimation of radioactivity in the human body based on measurements of radioactivity in urine, excreta, or other materials taken from the body and on a biokinetic model of the radionuclide in body tissues and organs. Urine sample analysis is a quick way to determine whether a person has been exposed to radioactive material (ATSDR 1999) (see Table 2-14 for some of the most common methods used for *in vivo* and *in vitro* analysis). In addition, concentrations of radioactive materials in air in work areas may be measured by air sampling techniques (see Section 2.3) and can be used to derive internal doses.

Table 2-14. Common analytical methods for *in vivo* and *in vitro* analysis of radioactivity

Sample matrix	Preparation method	Device used
Whole body, portion of body, or organ (X or gamma radiation)	position individual in front of detector with area of interest shielded from extraneous radiation.	multichannel analyzer with NaI detector for up to a few gamma-emitters, a germanium detector for any number of gamma-emitters, or a planar germanium detector for alpha-emitters that also emit X rays.
Urine, blood or feces	put any solids into solution; do chemical separation if multiple radioactive elements are present; deposit thin layer on a planchet or mix with liquid scintillation cocktail	Geiger-Mueller counter for high-energy beta or gamma-emitters; multichannel analyzer for gamma-emitters
Personal monitoring: external radiation dose (beta and gamma radiation)	heat dosimeter to produce thermoluminescence develop film none	thermoluminescent dosimeters film badge electronic dosimeter
Contamination monitoring: surfaces, skin, clothing, shoes (beta and gamma radiation)	none	Geiger-Mueller counter

Source: ATSDR 1999.

2.5 Regulations

Regulations have been set for ionizing radiation by a number of U.S. governmental agencies including the U.S. Department of Energy, Department of Transportation, Environmental Protection Agency, Food and Drug Administration, Nuclear Regulatory Commission, and Occupational Safety and Health Administration. See Appendix B for the specific regulations for X rays, gamma rays, and neutrons.

2.6 Summary

Exposure to ionizing radiation results from a variety of natural and anthropogenic sources. The largest radiation exposure (all types) to the U.S. population and worldwide results from natural background radiation with a per capita effective dose of 2.4 mSv. The remaining exposure is from anthropogenic sources, with medical procedures and consumer products accounting for most of the exposure. Occupational exposures, nuclear fallout, including that from the Chernobyl accident, and nuclear power production make up less that 1% of the total radiation exposure. While the naturally occurring alpha emitter radon and its decay products constitute the major source of natural radiation, exposure to X rays and gamma rays and neutrons occurs from a number of sources.

Major past exposures to X and gamma radiation have resulted from military uses of atomic weapons with the detonation of two atomic bombs over Hiroshima and Nagasaki,

Japan in 1945 and additional atmospheric testing of nuclear weapons that were carried out in the northern hemisphere between 1945 and 1980. The survivors of the bombings of Hiroshima and Nagasaki were exposed to approximately 300 mSv on average while the local population near the nuclear test site in Nevada was estimated to have received an average dose of about 3 mSv.

Exposure to medical radiation occurs for a large portion of the population of more developed countries, such as the U.S., that have a high level of medical care. However, medical exposures are very small compared to the exposure to natural sources of radiation with an annual collective dose of about 2 x 10^6 person-Sv/year for medical procedures compared to 14 x 10^6 person-Sv/year from background exposures. Medical exposures also differ from other exposures to artificial radiation since the exposed individual receives a direct benefit from the procedures, which include diagnostic radiology and radiation therapy.

Occupational exposure to X rays and gamma rays affects approximately 5 million workers worldwide with most being employed as coal miners or other underground miners in non-coal mines. Other occupationally exposed workers include medical workers, nuclear industry workers, and airline crews.

Environmental exposure to X rays and gamma rays results from terrestrial sources, particularly the radioactive nuclei chemically bound in the upper 25 cm of the earth's crust and building construction materials. Radioactivity also has been released into the environment from nuclear accidents, primarily from the largest nuclear accident to date that occurred in Chernobyl, Ukraine in 1986. The worldwide annual per capita dose for residual radioactivity from Chernobyl was estimated to be 0.002 mSv in 2000, down from a maximum of 0.04 mSv in 1986. Other sources of environmental exposure include nuclear power generation and consumer products containing gamma-ray emitters.

Exposure to neutrons includes many of the same sources as those causing exposure to X and gamma radiation. However, exposure to neutrons from the atomic bombs at Hiroshima and Nagasaki, Japan are now considered to have contributed only 1% to 2% of the total dose of ionizing radiation. Similarly, medical uses of neutrons are very limited currently, and occupational exposure to neutrons in the nuclear industry account for only about 3% of the total annual effective dose to nuclear plant workers. Occupational exposure to neutrons can occur for aircraft crews and for oil-field workers (when neutron radiation is used for well logging). Most environmental exposure to neutrons is from cosmic radiation, which has been estimated to result in an annual effective dose of 80 μSv at sea level and a 50° latitude.

A variety of dosimetric methods are used for monitoring of X rays and gamma rays in environmental and medical settings. X ray and gamma ray detectors include gas detectors, scintillators, and semiconductors. Individual personnel monitors of many types are in use, including film badges, thermoluminescent dosimeters, optically stimulated luminescence technology, and self-reading pocket dosimeters. Monitoring methods for neutrons are divided into detectors of slow neutrons and fast neutrons. Detectors of slow neutrons include proportional counters using ^{10}B or ^{3}He, scintillators with ^{6}Li or ^{10}B,

ionization chambers lined with ^{235}U, and semiconductors attached to a ^{6}Li or ^{10}B radiator. Detectors of fast neutrons may be based on tissue-equivalent ionization chambers, recoil proton techniques, capture reactions or moderated detectors. Rem meters and neutron spectrometry, either proton recoil based or "Bonner sphere," also can be used for detection of fast neutrons. A wide variety of personnel monitors for neutrons are in use, e.g., nuclear emulsions, thermoluminescent detectors, track-etch detectors, electronic pocket dosimeters, activation detectors, and bubble detectors. Exposure to ionizing radiation also may be measured through the use of biological indices that may be either *in vivo*, i.e., measurement of radioativity in the human body, or *in vitro*, i.e., measurements of radioactivity in urine, excreta, or other material taken from the body.

3 Human Cancer Studies

3.1 X radiation and gamma radiation

X radiation and gamma radiation have been classified by IARC (2000) as carcinogenic to humans (Group 1) based on *sufficient evidence* for carcinogenicity in humans and *sufficient evidence* in experimental animals. A number of new studies of human populations have been published since the IARC review and will be described below (see section 3.1.2). These studies include recent publications of new results from the A-bomb survivor cohorts, some new or updated cohort studies performed in the nuclear industry, and studies examining the effects of medical irradiation including radiation treatment for adult and childhood cancers. The latter include breast, testis, prostate, and cervical cancers, Hodgkin's disease, acute lymphocytic leukemia, all childhood and adolescent cancers; medical non-cancer treatment, i.e., therapeutic irradiation for infertility, menstrual disorders, acne and other skin disorders, and adenoid hypertrophy; medical diagnostic irradiation; and a study of veterans exposed during nuclear testing in the Pacific. Note that some of the literature refers to absorbed dose (gray) while other studies refer to effective dose (sievert). For most gamma and X radiation, the absorbed dose and effective dose are the same; thus, one gray is equivalent to one sievert.

Criteria similar to those employed by the IARC working group to select recently published studies were applied for this review. In the studies chosen by IARC and for this current review, exposure measures are based on directly measured or well-characterized estimates of radiation dose, which in many cases allowed for dose-response analyses. In addition, these studies included sufficiently large numbers of subjects to evaluate site-specific and/or total cancers. Thus, this section concentrated on reviewing well-described and sufficiently large cohort and case-control studies with appropriately long follow-up periods after exposure that employed radiation dose estimates measured or modeled at the individual level for analysis. This section did not include case-reports and case-only series or studies that did not contain adequate description of exposure levels received by individuals or studies with inadequate average follow-up time after exposure. For example, none of the recent studies evaluating cancer occurrences in medical radiologists/medical radiation technicians or flight crews met the criterion of adequate exposure assessment. Overall, the studies previously reviewed by IARC and newly published since the IARC review provide substantial human data for the evaluation of the carcinogenicity of X rays and gamma radiation. All of the reviewed studies taken together present a consistent body of evidence for evaluating the carcinogenicity of X and gamma radiation in humans at a wide range of dose levels.

3.1.1 IARC evaluation

While the carcinogenic effects of ionizing radiation have been studied extensively in a wide variety of human populations and exposure settings, IARC (2000) based its conclusions for causal associations primarily on the evidence provided by epidemiological high dose studies of 1) survivors of the atomic bombings in Japan and 2) patients exposed to radiation for medical reasons. IARC considered and reviewed epidemiological studies of populations exposed at lower doses of radiation but

determined that they were not informative enough to allow inclusion in the evaluation of cancer risks in humans.

IARC underscored in its evaluation the fact that an association between radiation and cancer has been found consistently in many different populations throughout the world exposed at different times and in different countries. Furthermore, follow-up after radiation exposure in general has been long. Since in many studies the dose of radiation received by individuals was estimated with considerable accuracy, dose-response relationships could be evaluated and were found for a number of cancers and dose ranges. The sensitivity of tissues to the carcinogenic effects of ionizing radiation differs widely, but dose-response relations have been shown for leukemias, thyroid cancer following irradiation in childhood, breast cancer, and a combined category of all cancers. Cancers that appear to be readily inducible by X rays and gamma rays also include some gastrointestinal tumors, including those of the stomach and colon; however, cancer is induced only rarely or at relatively high doses at some sites including bone, soft tissue, uterus, skin and rectum. Some cancers, such as chronic lymphocytic leukemia, are not caused by exposure to X rays or gamma rays. Dose-dependent cancer risk after exposure to X rays or gamma rays is modified by a number of factors including the age at which exposure occurs, the length of time over which the radiation is received and the sex of the exposed person. The level of cancer risk also varies with time since exposure. While there is some variation in the level of risk for specific cancers seen in epidemiological studies of populations exposed to X rays and gamma rays, the IARC working group concluded that the consistency of the association, the strength of the association, and the dose-response relationships all provide strong evidence that X rays and gamma rays cause cancer in humans.

IARC (2000) reviewed studies of X and gamma radiation from four types of exposures: military use, medical use, occupational exposure, and environmental exposure. Their review focused on studies with large numbers of subjects, documented exposures, and the evaluation that bias or confounding factors influenced results minimally; they did not consider case reports. Epidemiologic studies included in the IARC review were conducted in the following human populations: Atomic bomb survivors; personnel involved in the Chernobyl accident and nuclear weapons testing; occupational exposures of workers such as radium dial painters, radiologists, underground miners, nuclear workers and aircraft personnel; and exposure of medical patients including radiation treatment for malignant diseases (cancers and patients undergoing bone-marrow transplantation) and benign diseases (ankylosing spondylitis, gynecological disorders, peptic ulcers, benign breast disease, tinea capitis, enlarged thymus, enlarged tonsils, hemangioma) and patients undergoing diagnostic procedures (tuberculosis fluoroscopy). Practically all of the studies are cohort studies with long follow-up periods (some 40 years and more) with recorded or estimated radiation doses ranging from very low doses in environmental and occupational settings to very high doses experienced by patients medically treated with radiation.

3.1.1.1 Military exposures

Atomic Bombs over Hiroshima and Nagasaki

The most important studies of military exposures involve A-bomb survivors. These studies are collectively called the Life Span Study (see Section 2.1.1.1); they were conducted by the Radiation Effects Research Foundation investigating the long-term effects of exposures to radiation during the bombing of Hiroshima and Nagasaki, Japan in 1945 (Mabuchi *et al.* 1994, Thompson *et al.* 1994, Preston *et al.* 1994, Tokunaga *et al.* 1994, Pierce *et al.* 1996, cited in IARC 2000). These studies are considered a singularly important source of information, because they involved a very large population (86,572 subjects with dose estimates) consisting of men and women of a wide range of ages who received various doses. Dose estimates were well characterized for individual study subjects and they included long-term follow-up; thus achieving complete ascertainment for both cancer mortality (1950 to 1990) and cancer incidence (1958 to 1987) for those residents who remained in the two cities. Limitations of these data include that all subjects are Japanese exposed during wartime, and host and environmental factors may have modified their risk for cancer. Also, the mortality follow-up cohort was not established until 1950 (five years after the bombing) and the cancer incidence follow-up did not take place until 1958. These delays may have resulted in an initial selection bias for estimation of cancer risks, especially leukemia, shortly after exposure. Furthermore, while radiation exposure was predominantly due to gamma radiation – with the contribution of neutrons estimated to be 1% to 3% of the dose – there is still some uncertainty about dose estimates calculated for Hiroshima (Gold 1999).

The A-bomb studies allowed evaluation of cancer risk by site. The first cancer linked to radiation in this cohort was leukemia, and its estimated excess relative risk (ERR) is by far the highest: ERR per Sv = 4.4 (95% CI = 3.2 to 5.6). Furthermore, the risk increases with increasing dose over the range of 0 to 2.5 Sv, and the largest excess was seen in the early years of follow-up, especially for those survivors exposed as children. For those exposed as adults the excess risk was generally lower than for children; however, the risk persisted throughout follow-up. For solid tumors, an increasing risk also was seen with increasing dose over the range of 0 to 2.5 Sv, but the temporal pattern differed from that for leukemia, including a longer minimal latent period. Furthermore, most of the excess deaths for those exposed under the age of 30 years occurred in the last 5 years of the 40-year follow-up. The risk of breast cancer showed the largest ERR (1.6; 95% CI = 1.1 to 2.2) for solid tumors and a strong linear dose-response that was remarkably age-dependent, with a decrease in risk with increasing age at exposure. Similarly, a strong age-dependence was seen for thyroid cancers, with no radiation effects observed for subjects exposed at an age older than 14 years, while those exposed as children experienced an ERR of 4.7 (95% CI = 1.7 to 11). Other cancers clearly linked to radiation exposures in these A-bomb survivor mortality and incidence studies were those of the salivary glands, stomach, colon, liver, ovary, and urinary bladder, and non-melanoma skin cancer. Evidence was equivocal for cancers of the esophagus, gall bladder, kidney, and nervous system and for non-Hodgkin's lymphoma and multiple myeloma. Cancers with little evidence for an association with radiation exposure in this cohort include those of the oral cavities (except salivary glands), rectum, pancreas, uterus, and prostate and

Hodgkin's disease, but the IARC committee concluded that the possibility of associations with these cancers cannot be excluded on the basis of the A-bomb studies alone.

Nuclear weapons testing

The U.S. Atomic Energy Commission in collaboration with the Department of Defense conducted 19 operations (test series), involving 230 detonations (shots) from 1945 until the signing of the Limited Nuclear Test Ban Treaty in 1963 (Thaul *et al.* 2000). The testing occurred at the Nevada test site and the Pacific Proving ground (Enewetak and the Bikini Atolls, southwest of Hawaii) and involved exposure to over 200,000 military and civilian personnel. Several large studies on subsets of the population from a specific test series, Operation Hardtack (Watanabe *et al*. 1995, cited in IARC 2000) and Operation Crossroads (conducted by the National Academy of Sciences) (Johnson *et al*. 1997, cited in IARC 2000) or from a group of five test series (The Five Series Study conducted by the National Academy of Sciences involving Operations Greenhouse, Upshot-Knothole, Castle, Redwing, and Plumbob) did not find an increased leukemia risk but the IARC working group noted as weaknesses of these studies insufficient dosimetry and exposure to generally low doses of radiation. A later publication of the Five Series Study as well as a study on the highest exposed individuals from the 19 test series is discussed in Section 3.1.2.1. Studies of populations living downwind from the Nevada site (in which more than 100 atomospheric weapons tests were conducted between 1951 and 1958) reported a weak association for acute leukemias excluding chronic lymphatic leukemias (CLL) (odds ratio [OR] = 1.7; 95% CI = 0.94 to 3.1 for those exposed to ≥ 6 mGy) (Stevens *et al.* 1990, cited in IARC 2000). Dose estimates, however, were solely derived from residency information.

In the USSR about 118 atmospheric weapons tests were conducted between 1948 and 1962 in northeastern Kazakhstan. It was estimated that most local residents were exposed to an effective dose of 100 mSv. A study published in 1994 suggested that proximity of the residency to the testing sites (Zaridze *et al.* 1994, cited in IARC 2000) increased the rate of acute leukemias among children under 15 years of age.

Studies of military personnel participating in weapons tests included a large group of United Kingdom soldiers in Australia and islands of the Pacific Ocean and found an increased risk for leukemia among those participating in the testing compared to non-participating military personnel (RR = 1.8; 95% CI = 1.0 to 3.1) (Darby *et al.* 1988, Darby *et al.* 1993, cited in IARC 2000) and a much smaller study conducted in New Zealand confirmed the increased risk for leukemia (RR = 5.6; 95% CI = 1.0 to 42) (Pearce *et al.* 1997, cited in IARC 2000) but found no increased risk for all cancer mortality.

Plutonium weapons production in the former USSR led to contamination of the Techa River and exposure of the population living in its vicinity. Inhabitants of the riverside village were most likely exposed to both internal and external radiation, i.e., mainly gamma radiation and internal gamma and beta radiation. A study following mortality among residents from 1950 to 1989 (641,304 person-years of exposure to 0.005 to >1 Sv) reported an increased mortality rate from leukemia (21 excess cases) and solid tumors (30

excess deaths) related to both types of radiation (Kossenko *et al.* 1997, cited in IARC 2000).

3.1.1.2 Medical uses

Radiotherapy for Cancer Treatment

One form of cancer treatment is high-dose radiotherapy, and large cohorts of primary cancer survivors (up to 200,000 patients in some studies) have been followed over long periods to compare the second cancer incidence and mortality with that in patients with the same disease but treated by other means. IARC (2000) reviewed the most informative of well over 100 studies of patients treated with therapeutic doses of radiation, that is, those studies that included assessments of radiation dose. They summarized results linking exposures to radiation to increased risk of developing subsequent cancers.

Malignant diseases studied include cervical cancer, Hodgkin's disease, breast, ovarian and testicular cancer, and malignant childhood cancers (brain, thyroid, soft tissue sarcoma, leukemia; bone-marrow transplants). Treatments consisted of and resulted in the delivery of high local doses of X rays and gamma rays to the affected and adjacent organs. Some problems of interpretation arose due to high-dose cell killing effects and potential confounding due to concomitant treatment with chemotherapeutic agents that can cause cancer.

After treatment of primary cancers of the cervix, increased risks for second cancers were observed for many organs close to or within the field of radiation except for the colon, liver and gall-bladder, melanoma, Hodgkin's disease, multiple myeloma, chronic lymphatic leukemia, corpus uteri and ovary, and breast (note: ovarian ablation during radiotherapy is a factor complicating the interpretations of these results) (Boice *et al.* 1985, Boice *et al.* 1988, cited in IARC 2000). Increased incidence of cancers of the bladder, rectum, lung, pancreas, esophagus, small intestine, vagina, and stomach occurred first after 10 years of latency (and remained elevated for up to 40 years of follow-up) and after exposure to doses in the order of grays to the proximal organs; no excess was found for organs of distant sites exposed to fractions of grays. Small increases in acute and non-lymphocytic leukemia were observed; but at the high doses used to treat these cancers, it seems possible that the bone marrow of the pelvis was destroyed.

Follow-up studies after treatment of young patients with Hodgkin's disease found a dose-response relationship for those treated with radiotherapy, i.e., an increasing risk for leukemia with doses to the red bone marrow above 10 Gy (Kaldor *et al.* 1990a, cited in IARC 2000) and for lung cancers (Kaldor *et al.* 1992, cited in IARC 2000), and an excess risk for thyroid cancers among those who received high doses to the cervical lymph node areas (Hancock *et al.* 1991, cited in IARC 2000). Increased risks for acute non-lymphocytic leukemia also were found among breast cancer survivors treated with radiotherapy at mean doses of 7.5 Gy to the bone marrow (Curtis *et al.* 1992, cited in IARC 2000). A U.S. study of women in Connecticut who survived a first breast cancer by at least 5 years and were treated with radiation reported an increased risk for the contralateral breast among those women who were under 45 years of age at the time of treatment (Boice *et al.* 1992, cited in IARC 2000). Yet, a large Danish and a considerably smaller Canadian study found no increased risk to the contralateral breast after radiation

treatment (Storm *et al.* 1992, Basco *et al.* 1985, cited in IARC 2000). In both studies the majority of women were perimenopausal or postmenopausal at time of treatment for the first breast cancer, and neither study reported an effect in the subgroup of premenopausal women. Rather, results suggested that risks were slightly increased in women treated at an older age (> 50 to 55 years at first diagnosis and at doses > 100 cGy), but wide confidence intervals precluded conclusions, and the authors stated that they did not have the statistical power to show effects for subgroups. Other studies reported increased risks for second lung cancers and soft-tissue sarcomas occurring inside the radiation field (Inskip *et al.* 1994, Karlsson *et al.* 1996, cited in IARC 2000).

Studies of patients in Europe and North America treated for ovarian cancer showed no increased risks for leukemia (Kaldor *et al.* 1990b, Travis *et al.* 1999, cited in IARC 2000); bone marrow doses were high (18.4 Gy) and likely to have caused bone marrow sterilization. Bladder tumor risk was increased, but possibly due to the small case sample (n = 63) the confidence intervals included the null value (RR = 1.9, 95% CI = 0.77 to 4.9) (Kaldor *et al.* 1995, cited in IARC 2000). Studies of men treated for testicular cancers reported increased risks for stomach, bladder, and pancreas cancers and acute leukemia after radiotherapy (Travis *et al.* 1997, cited in IARC 2000).

Radiotherapy for Childhood Cancers

The most informative studies of children irradiated for childhood cancers are those of the Late Effects Study group (Tucker *et al.* 1984, 1987a, 1987b, 1991, cited in IARC 2000) and several United Kingdom (Hawkins *et al.* 1987, Hawkins *et al.* 1992, Hawkins *et al.* 1996, cited in IARC 2000) and French (de Vathaire *et al.* 1989, 1999, cited in IARC 2000) groups. These studies reported that childhood cancer survivors experienced increased occurrences of any second cancer and, if treated at very high doses, of cancers of the bone (> 10 Gy); cancers of the brain (> 5 Gy); cancers of the breast in girls (average dose 40 Gy to the chest region); and cancers of the thyroid at medium (average dose 7 Gy) but not high doses (average dose 13 Gy). Equivocal results were reported for leukemia (the IARC committee noted that high doses to the thyroid and to the bone marrow may have resulted in stem cell killing rather than carcinogenic transformation). Furthermore, radiation doses > 5 Gy increased the risk of osteo- and soft tissue sarcomas in a dose-response fashion in genetically predisposed individuals (with hereditary forms of primary retinoblastoma).

Radiotherapy for Benign Disease

Studies also have been conducted among patients treated with somewhat smaller doses of X rays or gamma rays for benign diseases such as ankylosing spondylitis, gynecological disorders, peptic ulcers, and benign breast disease (in adults) and tinea capitis, enlarged thymus, enlarged tonsils, and hemangioma (in children), in which case one expects less of a cell-killing effect, good survival after treatment, and minimal confounding from concomitant (chemotherapeutic) treatment. Studies of middle-aged women treated for benign breast disease showed an increased breast cancer risk at low-to-medium doses, including a dose-response relationship and an inverse relation to age at exposure (mostly for pre-menopausal exposure) (Baral *et al.* 1977, Mattsson *et al.* 1993, Mattsson *et al.* 1995, Mattsson *et al.* 1997, Mettler *et al.* 1969, Shore *et al.* 1986, cited in IARC 2000).

Risk for developing any other cancer also was slightly increased, with the largest risks exhibited by the colon, stomach, and lung, but not for leukemia.

Radiotherapy for peptic ulcers (average dose of about 15 Gy to the stomach) significantly increased the relative risk (compared to patients not receiving radiotherapy) for mortality from all cancers combined, stomach cancer, pancreatic cancer, lung cancer, and leukemia (Griem *et al.* 1994, cited in IARC 2000). Benign gynecologic diseases such as uterine bleeding were treated with radiation, with a median dose to the uterus of 5.2 to 32 Gy resulting in 0.5 to 1.2 Gy to the bone marrow. These patients were found to be at higher risk for all cancer mortality, especially for the colon and rectum, uterus and other female organs, urinary bladder, and leukemia, but not lymphoma or Hodgkin's disease; results for multiple myeloma were equivocal (Inskip *et al.* 1990a, 1990b, 1993, Darby *et al.* 1994, Ryberg *et al.* 1990, cited in IARC 2000). Furthermore, when the ovaries received more than 5 Gy, fewer deaths from breast cancer were observed than expected. Treatment with radiation for infertility in a small cohort of women did not result in subsequent increases of cancer (Ron *et al.* 1994, cited in IARC 2000). Ankylosing spondylitis patients received X-ray treatment to the (mostly lower) spine. Studies of such irradiated patients compared to non-irradiated patients showed increased mortality from all cancers and leukemias except CLL in tissues most likely to have been exposed during radiotherapy such as the esophagus, lung, bladder, kidney, colorectal, bone, and connective and soft tissue; effects also were observed for the prostate, non-Hodgkin's lymphomas and multiple myelomas, but not for stomach or breast cancers (Darby *et al.* 1987, Weiss *et al.* 1994, 1995, Damber *et al.* 1995, Johansson *et al.* 1995, cited in IARC 2000).

Studies of children treated for tinea capitis (ringworm of the scalp) in Israel and New York reported a scatter dose to the thyroid of about 0.10 Gy (Ron *et al.* 1988, 1989, 1991, Shore *et al.* 1976, 1984, cited in IARC 2000). An increase in thyroid cancers and tumors of the central nervous system were reported for Israeli children only, while in both cohorts non-melanoma skin cancers were increased. Children treated before the age of six months for an enlarged thymus (0.69 Gy to the breast and 1.4 Gy to the thyroid) experienced an increase in risk for thyroid cancers in a dose-response manner and an increased risk for skin cancers; treated female infants developed breast cancer more often than untreated siblings (RR = 3.6; 95% CI = 1.8 to 7.3) (Shore *et al.* 1980, Shore *et al.* 1985, Shore *et al.* 1993, Hildreth *et al.* 1985, Hildreth *et al.* 1989, cited in IARC 2000). The treatment of children under age 2 for skin hemangioma resulted in estimated mean doses to the breast of 0.29 Gy and slightly increased the breast cancer risk among women and resulted in an excess risk of cancer of the thyroid 19 years after exposure (estimated thyroid dose 1.1 Gy); no increase in leukemia risk was observed (Lindberg *et al.* 1995, Karlsson *et al.* 1997, 1998, Lundell and Holm 1995, Lundell *et al.* 1996, 1999, Fürst *et al.* 1988, 1989, cited in IARC 2000). Studies also suggested a dose-response relationship for brain tumors with an ERR of 2.7 per Gy (95% CI = 1.0 to 5.6) (Lundell and Holm 1995, cited in IARC 2000). Patients treated for enlarged tonsils (average dose to the thyroid of 0.6 Gy) and cervical tuberculous adenitis were found to be at increased risk for thyroid cancers at higher doses (Favus *et al.* 1976, Schneider *et al.* 1985, 1993, Fjalling *et al.* 1986, cited in IARC 2000). In a pooled analysis (Ron *et al.* 1995, cited in IARC 2000) of all children irradiated for benign diseases and A-bomb survivors exposed during

childhood, an ERR of 7.7 (95% CI = 2.1 to 28.7) per Gy for developing thyroid cancers was estimated; the increased risk was strongly dependent on the age at exposure (highest for children under age 5 at exposure) and suggested a dose-response relationship.

Diagnostic procedures

Frequent X-ray monitoring (fluoroscopy) of therapeutically collapsed lungs of adult tuberculosis patients resulted in mean doses of 1.02 Sv to the lung and 0.79 to 0.89 Sv to the chest. Two large Canadian and one U.S. study with long follow-up times of such patients found no increased risk from lung cancers but reported an excess risk for breast cancer mortality and a dose-response relationship (Howe 1995, Miller *et al.* 1989, Howe and McLaughlin 1996, cited in IARC 2000); in both studies, the excess relative risk for breast cancers decreased sharply with age at irradiation, and no risk was observed when patients were > 40 years of age when first exposed (Hrubec *et al.* 1989, Boice *et al.* 1991b, Little and Boice 1999, cited in IARC 2000). The U.S. study also reported an increased risk for esophageal but not for lung cancers or leukemias (Davis *et al.* 1989, cited in IARC 2000). Other studies attempting to estimate effects from diagnostic X rays provided only limited information due to either a lack of dosimetry, potential for biases, or dependence on generally very low doses of radiation exposure (chronic myeloid and monocytic leukemia) (Preston-Martin *et al.* 1989, cited in IARC 2000); thyroid cancers (Inskip *et al.* 1995, cited in IARC 2000)). A large case-control study conducted in the U.S. reported a link between the number of diagnostic X rays and leukemia or non-Hodgkin's lymphoma (Boice *et al.* 1991a, cited in IARC 2000). However, these findings did not hold up for these two cancer groups when the last 2 years prior to diagnosis were excluded, but a dose response was found for multiple myeloma in the same study. Similarly inconclusive were several case-control studies of thyroid cancers in which exposure was assessed via interview (Wingren *et al.* 1993, Hallquist *et al.* 1994, Wingren *et al.* 1997, Ron *et al.* 1987, cited in IARC 2000).

Women who received multiple X-ray examinations for scoliosis as children were found to be at a somewhat higher risk of developing breast cancer even though the dose to the breast was estimated to have been relatively low (0.13 Gy) (Hoffman *et al.* 1989, cited in IARC 2000). The authors could not control for reproductive confounding factors that may have played a role.

Whether prenatal exposure to low doses of X rays are causally associated with childhood cancers is subject to controversy among researchers, but the medical profession has nevertheless acted on the assumption that the association is causal and has replaced X ray with ultrasound procedures during pregnancy. Early studies of UK children known as the Oxford Survey of Childhood Cancers and a U.S. study suggested increased risks (Stewart *et al.* 1958, Bithell and Stewart 1975, Knox *et al.* 1987, Muirhead and Kneale 1989, MacMahon 1962, cited in IARC 2000). Some follow-up studies were either unable to find effects or found effects that critics attributed to biases such as recall bias of mothers with children who have cancer (Monson and MacMahon 1984, Inskip *et al.* 1991, Boice and Inskip 1996, Boice and Miller 1999, cited in IARC 2000). The IARC working group also raised the question whether it would be biologically plausible for exposures occurring right before birth to induce a number of embryonic tumors. Nevertheless, Doll

and Wakeford (1997, cited in IARC 2000) recently estimated the excess risk associated with prenatal exposure to radiation to be 6% per Gy.

3.1.1.3 Occupational exposures

Medical Workers

Studies of early radiologists in the U.S., Britain, and China (Doll 1995, Miller 1995, cited in IARC 2000) exposed to high doses (in the order of many Gy) of radiation provided evidence that such exposures caused leukemias and other cancers such as skin cancers, pancreatic cancers, and multiple myeloma among members of this profession (Lewis 1963, Matanoski *et al.* 1975a, 1975b, cited in IARC 2000). However, studies of other medical personnel have been limited due to a general lack of dosimetry information for the early years of radiation device use. A large study of radiological technologists from the U.S., who had been certified between 1926 and 1980, reported an increased risk of breast cancer (standardized mortality ratio [SMR] = 1.5; 95% CI = 1.2 to 1.9) among those who were employed before 1940 (radiation exposure was likely highest during the pre-war period) and who worked for more than 30 years (Doody *et al.* 1998, cited in IARC 2000); however, breast cancer risk was found not to be associated with a surrogate measure of exposure in a nested case-control study conducted within this cohort (Boice *et al.* 1995, cited in IARC 2000).

Workers in the Chernobyl clean-up

About 600,000 to 800,000 workers ('liquidators') are believed to have participated in the clean-up after the Chernobyl accident in the restricted 30-km zone around the plant and in contaminated areas of Belarus and the Ukraine. A small portion (36,000) was professional radiation workers from other nuclear plants, but the majority were military reservists, construction workers, and others. IARC stated that most scientific papers published to date only compared mortality and/or morbidity rates of cancer in liquidators to those of the general population and reports of increased rates of leukemia among some of the liquidators but not in others. These results have been difficult to interpret (Cardis *et al.* 1996, Buzunov *et al.* 1996, Okeanov *et al.* 1996, Ivanov *et al.* 1997a, Rahu *et al.* 1997, cited in IARC 2000). Uncertain dosimetry also made a study of emergency workers difficult to interpret; this study did not report an increased leukemia risk more than two years after first exposure among those exposed in 1986 (at assigned doses in excess of 0.25 Gy) (Ivanov *et al.* 1997a, cited in IARC 2000).

Nuclear Industry Workers

A combined study (Carpenter *et al.* 1994, cited in IARC 2000) of three UK nuclear worker cohorts covering 40,761 externally monitored employees between 1946 and 1988 with a mean follow-up of 24 years reported a statistically significant association between cumulative gamma radiation dose and leukemia, skin cancer including melanoma, and ill-defined and secondary neoplasms. Muirhead *et al*. (1999, cited in IARC 2000) published results from a study based on the National Registry of Radiation Workers in the UK with a follow-up from 1976 to 1992 that included 124,743 workers from several facilities (including all of those in Carpenter *et al*. 1994, cited in IARC 2000); the ERR for leukemia reported in this pooled analysis was smaller than that for the Carpenter *et al.* study (ERR per Sv = 2.55; 90% CI = -0.03 to 7.16; n = 89 vs. 4.2 per Sv; 95% CI = 0.4 to

13.4; n = 60), but, in addition, they observed an increased risk for multiple myeloma (ERR per Sv = 4.1; 90% CI = 0.03 to 14.8; n = 35).

A combined analysis of workers from several sites in the U.S., including the Hanford nuclear site (Gilbert *et al.* 1993a, cited in IARC 2000), the Oak Ridge National Laboratory (Wing *et al.* 1991, cited in IARC 2000) and the Rocky Flats nuclear weapons site (Wilkinson *et al.* 1987, cited in IARC 2000), with 19 years of average follow-up and an average dose of 2.7 mSv did not find an increased excess risk for leukemias among these workers. Instead, an increased mortality risk for esophageal and laryngeal cancer and Hodgkin's disease was observed. These findings were interpreted as most likely due to chance given the large number of statistical tests performed. The IARC working group noted that the study results were dominated by data from the Hanford cohort and that there was considerable heterogeneity in cancer outcomes between the cohorts, raising questions about the appropriateness of data pooling for these cohorts. A study of Los Alamos National Laboratory workers also reported an association between radiation dose and cancer mortality of the esophagus, brain, and Hodgkin's disease, but not for leukemia or all cancer sites combined (Wiggs *et al.* 1994, cited in IARC 2000). Finally, Frome *et al.* (1997, cited in IARC 2000) reported that Oak Ridge nuclear plant workers exposed to higher doses of radiation experienced excess risks of dying from all cancers (ERR per Sv = 1.45; 95% CI = 0.15 to 3.48) and lung cancers (ERR per Sv = 1.68; 95% CI = 0.03 to 4.94) but not from leukemia.

A cohort study of workers from the Mayak nuclear complex found an increased mortality for all cancers and leukemias (Koshurnikova *et al.* 1996, cited in IARC 2000); the mean cumulative external dose among exposed workers was high (1 Gy), and the excess relative risk for leukemia was estimated to be 1.3 per Gy (no CI reported). Later reports (Tokarskaya *et al.* 1997, Koshurnikova *et al.* 1998, cited in IARC 2000) evaluating lung cancer risk in these plutonium workers did not find an effect for gamma radiation dose, but the IARC working group pointed out uncertainty in dosimetry and the potential for possible selection and confounding biases in these studies.

IARC (Cardis *et al.* 1995, cited in IARC 2000) conducted a combined study that partially overlapped with the above mentioned combined studies and included data from workers monitored for external radiation in diverse nuclear facilities in Canada (Gribbin *et al.* 1993, cited in IARC 2000), the UK (Carpenter *et al.* 1994, cited in IARC 2000), and the U.S. (Gilbert *et al.* 1993b, cited in IARC 2000) and covered more than 2 million person-years and 3,976 deaths from cancer. The study reported an excess relative risk for leukemia other than CLL of 2.2 per Sv (90% CI = 0.1 to 5.7; n = 119), but found no excess risk for cancer deaths for any of 31 specific sites examined except multiple myeloma (ERR per Sv = 4.2; 90% CI = 0.3 to 14; n = 44).

Study of radiation workers from various occupations

Ashmore *et al*. (1998, cited in IARC 2000) published a study of 206,620 Canadian radiation workers in industrial and medical occupations, who were included in a National Dose Registry established in 1951 and followed until 1983. The authors reported an increased risk for death from all cancers in men (ERR per 10 mSv = 3.0; 90% CI = 1.1 to 4.9) but not in women and a dose-response relationship was observed for lung cancer in

men (ERR per 10 mSv = 3.6; 90% CI = 0.4 to 6.9) but for no other cancers. The IARC working group suggested that this study may have suffered from confounding and ascertainment bias; also, this study suffered from a relatively short mean follow-up of approximately 14 years.

3.1.1.4 Environmental exposures

Natural sources

A number of mostly ecologic studies of natural radiation have been conducted in Europe, Asia, and the U.S. that compared cancer incidence or mortality from populations living in areas with different background levels of radiation. According to IARC, these studies were unlikely to be able to observe an effect of background radiation on cancer, since the effects were likely to be small in comparison with those due to other causes, and the studies were limited by difficulties in obtaining outcome data in a standardized fashion for large populations and areas. Overall, the study results were equivocal and insufficient to draw any clear conclusions given the above mentioned caveats and study limitations.

The Chernobyl accident

A study of the population of Kaluga *oblast*, the region nearest to Chernobyl, found no statistically significant trends for cancer incidence or mortality in 1981-1995 except for thyroid cancer in women (Ivanov *et al.* 1997b, cited in IARC 2000). (The dramatic increase in childhood thyroid cancer after exposure to radioactive iodine from the accident is discussed in the IARC monograph on alpha and beta ionizing radiation.) The European Childhood Leukemia-Lymphoma Incidence Study did not see a geographic pattern for childhood leukemia between 1980 and 1991 that would suggest an influence on disease rates due to Chernobyl radiation fall-out (Parkin *et al.* 1993, Parkin *et al.* 1996, cited in IARC 2000). Similarly, Swedish (Hjalmars *et al.* 1994, cited in IARC 2000) and Finnish (Auvinen *et al.* 1994, cited in IARC 2000) population-based studies did not observe increased rates of childhood leukemia for this period, but Petridou *et al.* (1996, cited in IARC 2000) reported that in Greece, *in utero* exposure due to contamination after the Chernobyl accident may have led to an increased rate of leukemia in infants younger than 1 year of age. A German study of *in utero* exposures found an overall increased leukemia risk after the accident for children during their first year of life, but no clear association with periods of estimated highest *in-utero* exposure after the accident (Michaelis *et al.* 1997, Steiner *et al.* 1998, cited in IARC 2000).

Populations living around nuclear installations

A number of studies have been conducted among populations living near nuclear facilities, and some have reported clusters of childhood leukemia (Black 1984, Forman *et al.* 1987, Viel *et al.* 1995, Pobel and Viel 1997, Bithell *et al.* 1994, cited in IARC 2000), but other studies subsequently conducted in the UK (Cook-Mozaffari *et al.* 1989, Kinlen *et al.* 1991, Kinlen 1993, cited in IARC 2000) or in other countries did not find or dismissed such associations (McLaughlin *et al.* 1993, Hill and Laplanche 1990, Hattchouel *et al.* 1995, Michaelis *et al.* 1992, Jablon *et al.* 1991, cited in IARC 2000). All of these studies were severely limited by the ecologic nature of exposure assessment as well as factors such as population migration and parental occupation, especially parental employment in nuclear facilities [note: the influence of paternal preconceptional exposure to radiation on childhood cancers was considered questionable by the IARC working

group]. Studies of the population around the Three-Mile Island nuclear plant in the U.S. found no relation to cancer incidence with the radiation released (Hatch *et al.* 1990, Jablon *et al.* 1991, cited in IARC 2000) or reported inconsistent results (Fabrikant 1981, Wing *et al.* 1997, cited in IARC 2000).

3.1.2 New studies released after the IARC review was published

The studies on military exposures, medical uses, and occupational exposures to X rays and gamma rays reviewed below were, for the most part, published after the IARC (2000) review. A summary table for these studies is included in Appendix C. No new studies on environmental exposures published since the IARC review were found.

3.1.2.1 Military exposures

New A-bomb survivor results

Pierce and Preston (2000) published a paper that employed the Radiation Effects Research Foundation data for A-bomb survivors in Hiroshima and Nagasaki, Japan to evaluate cancer risks of low radiation doses focusing solely on those A-bomb survivors who received doses less than 0.5 Sv and had been within 3,000 m of the hypocenter of the bombs. Solid cancer incidence data were collected from 1958 to 1994 and included 7,000 cancer cases among 50,000 survivors in this low dose and distance range. The incidence data provided by a tumor registry set up especially for this cohort are thought to generate more accurate data than the cancer mortality data employed in similar previous analyses that evaluated radiation effects at low dose ranges in this cohort (Pierce *et al.* 1996). In this A-bomb survivor cohort, about 35,000 persons presenting 5,000 cancer cases received doses in the range of 0.005 to 0.2 Sv, a range that is of primary interest for radiation protection policy in populations. Thus, the authors state that their results provide useful risk estimates for doses as low as 0.05 to 0.1 Sv, and their estimates of effect show that linear extrapolation computed from the wider dose range of 0 to 2 Sv or 0 to 4 Sv does not overestimate risk estimates. Furthermore, they reported a statistically significant risk in the range of 0 to 0.1 Sv, and the upper confidence limit for any possible threshold was suggested to be at about 0.06 Sv. They also provided some evidence that modification of the neutron dose would not markedly change their conclusions and that it is appropriate to suggest that solid cancer rates increase about 5% for a dose of 0.10 Sv. More generally they noted that the analyses of the mortality as well as the incidence data together suggest that solid cancer radiation risks persist even 50 years after exposure and that, given sex and age at exposure, an acute radiation exposure increases normal age-specific solid cancer rates by a dose-dependent factor throughout life.

Cologne *et al.* (1999) examined the radiation risk for primary liver cancers in the same cohort of atomic bomb survivors. Earlier studies of liver cancer in this cohort had been based solely on death certificates, which can lead to inaccuracies due to frequent metastases from other sites to the liver. A comprehensive pathology review of known or suspected liver neoplasms in the cohort between 1958 and 1987 generated a total of 518 incident, first primary cases of mostly hepatocellular carcinoma. The relative risk due to radiation exposure was estimated to be linear (RR = 1.81 per Sv weighted liver dose; 95% CI = 1.32 to 2.43). While males and females had similar relative risk, the radiation-

related excess incidence was substantially higher in males due to a three-fold higher background liver cancer incidence in male survivors. Age at exposure was found to be important; there was no excess risk observed for those exposed before age 10 or after age 45, and most of the excess risk was evident in those who were exposed between the ages of 10 and 30. Whether this was due to a difference in sensitivity or possible confounding by other factors could not be addressed retrospectively, but the authors speculated that a male-specific age-at-exposure effect may be related to testosterone (known to be related to hepatocellular carcinomas in humans and shown to modify the carcinogenic effects of aflatoxins) modifying the carcinogenic effect of radiation during male adolescence. The authors also pointed out that cholangiocarcinoma and hemangiosarcoma cases were rare and, thus, may not be associated with whole-body radiation exposure (different from the internal alpha-particle-emitting radiological contrast medium Thorotrast).

Exposure due to atmospheric nuclear testing

As mentioned in Section 3.1.1.1, there have been several studies evaluating military personnel who participated in the U.S. atmospheric nuclear weapons testing program from 1945 to 1962. The National Academy of Sciences reexamined the data of the initial 1985 study on five test series conducted by Robinette *et al*. (1985 as cited by Thaul *et al*., 2000) because the Defense Nuclear Agency (DNA), which had provided data for that report, revealed that it had incorrectly identified many members of the participant cohort. The 1985 report used dose data provided by the DNA Nuclear test Personnel Review Program, which attempted to assign each individual participant an estimate of the radiation dose received. The working group of the Five Series Study evaluated this data and concluded that the dose data were not appropriate for epidemiologic analysis because among other reasons, the there was a lack of consistency in estimating dose and that there appeared to be an overall tendency to overestimate both external and internal doses. Thus, the 2000 Five Series Study did not use dosimetry data but used test participation in the analysis. The study reported a non-significant increase in leukemia mortality in test participants compared to referents (Hazard Ratio 1.15 [0.93-1.43]. A significant increased risk of leukemia deaths was found for participants at land test series (Nevada) compared to land series referents (Hazard Ratio =1.9 [1/04-2.13])). Limitations of this study include mortality ascertainment, limited power due to small sample size, and lack of information on lifetime radiation exposure and inadequate dosimetry.

Dalager *et al.* (2000) conducted a study of personnel (from the 19 test sites) who received ionizing radiation doses that met or exceeded a dose of 5 rem (50 mSv) in a 12-month period. Participants were on average exposed to 0.6 rem (6 mSv) of gamma radiation and many received no measurable dose. Dalager *et al.* (2000) conducted a cancer mortality study in a subpopulation of veterans who received the highest gamma radiation doses (≥ 5 rem [≥ 50 mSv]; n = 1,010; 94 died of cancer) and compared their cancer mortality with that of a group of Navy veterans who received a minimal radiation dose (≤ 0.25 rem [≤ 2.5 mSv], n = 2,870; 149 died of cancer) as participants of the HARDTACK I series (see Section 3.1.1.1). Mortality follow-up was from April 1958 through December 1996 and identified a total of 814 deaths among the 3,880 cohort members. The authors reported that mortality from all cancers (RR = 1.29; 95% CI = 0.97 to 1.72; 94 exposed to ≥ 5 rem) and from all lymphopoietic cancers (RR = 3.72; 95% CI = 1.28 to 10.83; 11

exposed to ≥ 5 rem) was elevated in the group exposed to ≥ 5 rem (≥ 50 mSv) compared with controls. Respiratory tract cancers were the largest contributor to the solid cancer death excess among exposed veterans (RR = 1.41; 95% CI = 0.91 to 2.18; 39 exposed to ≥ 5 rem). Relative risks for exposed (5 rem [50 mSv] or greater) compared to unexposed Navy only personnel who were very similar in age, rank, and assignment but differed in radiation dose were comparable to the estimates reported for all exposed vs. non-exposed veterans and, thus, suggested that confounding due to other risk factors may not have been a major problem in this study. Although the follow-up time was long, the total numbers of cancers observed in this cohort was small and, thus, the statistical power to examine cancer subgroups was low. The National Academy of Sciences had concluded that the database for determining radiation dose was not suitable for epidemiologic studies.

3.1.2.2 Medical uses

Secondary cancers after medical treatment for a primary cancer in adults

Studies of second cancers after medical radiation treatment for a first cancer commonly have employed cohort or nested case-control designs in which survivors who were treated with radiation for a primary cancer were compared to survivors who were treated by other means such as surgery or chemotherapy. As mentioned above, while these studies are powerful tools for examining high radiation dose cancer risks, the interpretation of the results is hampered by problems related to the potential for high-dose cell killing effects, confounding due to concomitant treatment with chemotherapeutic agents that can cause cancer, and a potential for genetic susceptibility to cancer in subgroups of these patients. Furthermore, in most of these studies, researchers were able to estimate radiation dose from treatment records but were unable to collect data on other risk factors potentially confounding the radiation estimates since lifestyle factors are commonly not recorded in medical records. However, these studies justifiably assumed that patients treated with radiation most likely have risk factor profiles similar to patients treated by other means such as chemotherapy or surgery. A general disadvantage of some of these cancer treatment cohorts is their relatively small size, allowing for the examination of only total secondary cancers or the most common cancer types, but this shortcoming may be outweighed by the advantage of being able to employ a more comprehensive assessment of radiation dose based on treatment protocols available for members of such cohorts.

In recent years, studies were published that used the U.S. population-based Surveillance, Epidemiology, and End Results (SEER) cancer registry data to examine radiation treatment effects subsequent to a diagnosis of a primary cancer reported to SEER (Brenner et al. (2000), Huang et al. (2001), Yap et al. (2002)). The SEER cancer registry covers approximately 10% of the U.S. population and, because it started to collect data in 1973, followed a very large number of patients over a relatively long period after first cancer diagnosis and medical treatment with radiation. Thus, it provides exceptional statistical power to examine the occurrence of rare second cancers and a relatively long follow-up period. A further strength of studies relying on SEER or other cancer treatment cohorts is that when comparing the second cancer risks in patients treated with radiotherapy to those treated with chemotherapy or surgery, one largely avoids problems associated with underreporting of second malignancies since both treatment groups

would be subjected to similar surveillance after the first cancer occurred. The biggest limitation of using SEER data is that radiation dose estimation has to be based on the assumption that most cancers of a certain type reported to SEER were treated relatively consistently according to standard medical radiation protocols, since researchers do not have access to actual radiation treatment protocols as they do for other smaller studies of cancer patients treated at one or more facilties.

Some authors (Ng et al. (2002), Galper et al. (2002), Bhatia et al. (2002)), examining the occurrence risk of second cancers at specific treatment facilities, employed the SEER data to calculate the expected number of cases based on the rates for primary cancers of a specifc type reported to SEER. They compared these expected numbers to the observed numbers of second cancers occurring in their own patient population treated with radiation; thus, these researchers combined the two advantages (i.e., reviewing medical records to estimate radiation dose levels in their own patient populations and relying on the statistical power provided by the large SEER cohort for the calculation of effect estimates or the "externally standardized incidence rate ratios"). However, when using SEER rates for primary cancers to calculate expected numbers, these authors had to assume that other unmeasured risk factors do not differ between their own and SEER patients and, in addition, that risk factors are similar in both types of patients (those diagnosed for a primary cancer and those diagnosed for a secondary cancer). This latter assumption may not be justified, however, if a first cancer diagnosis and treatment is followed by significant changes in lifestyle, such as quitting smoking, that may decrease the occurrence risk of certain secondary cancers. The most likely effect of a change in behavior after first cancer would be to reduce effect estimates for radiation treatment since the expected number of cancers based on pre-cancer diagnosis behavioral risk factor distributions might be too high.

Brenner *et al.* (2000) employed the 1973 to 1993 prostate cancer incidence data from the SEER Program cancer registry to compare all second malignancies occurring subsequent to a primary diagnosis of prostate carcinoma in 51,584 men who received radiotherapy (3,549 subsequently developed second malignancies) with 70,539 men (5,055 subsequently developed a second primary cancer) who underwent surgery for prostate cancer without radiotherapy. For those patients for whom exposure to radiation therapy was recorded in the SEER database, information about the prevalent treatment techniques prior to 1993 (^{60}Co irradiation) was used to estimate doses. The dose to the lung from radiotherapy was estimated to be ~ 0.6 Gy, compared to ~ 6 Gy to the pelvic region (bladder and rectum), and ~ 2 Gy to the kidneys. The mean survival time after prostate carcinoma diagnosis was 4.2 years in the radiotherapy group and 4.4 in the surgery group. Radiotherapy for prostate carcinoma was associated with an overall small increase in the risk of solid tumors (RR = 1.06; 95% CI = 1.01 to 1.11) relative to treatment with surgery only. Among patients who survived for > 5 years, the increased relative risk for all solid cancers reached 15% (RR = 1.15; 95% CI = 1.06 to 1.24), and increased further to 34% for patients surviving > 10 years (RR = 1.34; 95% CI = 1.14 to 1.57).

Brenner *et al.* (2000) also reported that the most significant contributors to the increased cancer risk in the irradiated group were carcinomas of the bladder (10 years after treatment RR = 1.77; 95% CI = 1.14 to 2.63), rectum (10 years after treatment RR = 2.05;

95% CI = 1.09 to 3.92), and lung (10 years after treatment RR = 1.42; 95% CI = 1.05 to 1.93), and sarcomas within the treatment field (all year RR = 1.85; 95% CI = 1.15 to 3.01); that is, increased cancer risks were observed in close proximity to the radiation treatment field. Interestingly, no increased risk of leukemia was observed (RR in the first 5 years after treatment = 1.05; 95% CI = 0.76 to 1.44). The authors reported that due to treatment techniques used over the majority of time covered by this study, the dose to the lung was estimated to have been nearly two orders of magnitude less than the doses received by the bladder and rectum, i.e., organs in proximity to the irradiated field, but nevertheless these lower doses produced a comparable size increase of lung cancers. They proposed that this might be due to the fact that risks at high doses are attenuated by the effect of cell killing and that cancer may be more easily induced at lower doses of radiation in cells actively dividing (such as lung tissue cells). Finally, they pointed out that when they compared smoking rates in a small group of patients treated with radiation to those treated with surgery, smoking behavior did not seem to bias their lung cancer results.

Using data from the SEER registries, Yap *et al.* (2002) evaluated the secondary development of sarcoma (n = 263) in 274,572 cases diagnosed with primary breast cancer between 1973 and 1997. Radiation treatment information was based on the information provided to the SEER database only. Comparing the cumulative incidence of 87 sarcoma cases who had received radiation therapy with 176 sarcoma cases treated without radiation therapy, the authors showed that while the occurrence of secondary sarcomas one year or more after primary diagnosis of breast cancer in general was low (cumulative incidence at 15 years post diagnosis 3.2 per 1,000 (standard error [SE] = 0.4) with radiation therapy vs. 2.3 per 1,000 (SE = 0.2) without radiation treatment; $P = 0.001$), angiosarcomas accounted for 56.8% of those sarcomas occurring within the field of radiation (25 out of 36 in-field angiosarcomas and all but 2 occurred within 3 to 7 years from the diagnosis of the primary breast cancer). Only 5.7% of angiosarcomas occurred in cases that did not receive radiotherapy (cumulative incidence of 0.9 per 1,000 for angiosarcoma at 15 years post diagnosis with and 0.1 per 1,000 without radiation treatment). Thus, they showed that radiotherapy in the treatment of breast cancer was associated with an increased risk of subsequent sarcoma, specifically angiosarcoma in or adjacent to the radiation field. The median latent period for developing a sarcoma was 6 years (range 1 to 21 years). This is an important and informative study of sarcoma occurrence because most previous studies were based on no more than 1 to 5 cases with the exception of a few larger studies that identified a total of 11 to 19 sarcomas; a recent review by Marchal *et al.* (1999) identified a total of 52 cases of angiosarcoma after irradiation for breast cancer in the world literature. Thus, although Yap *et al.* (2002) were not able to estimate the radiation dose received by these women, this is an important study due to its exceptionally large size and because it shows one specific type of sarcoma occurring within or adjacent to the field of radiation at a much higher rate than in patients treated by other means.

Huang *et al.* (2001) identified 194,798 women diagnosed with invasive primary breast carcinoma (excluding those with distant metastasis) between 1973 and 1993 in SEER, and ascertained subsequent cases of thyroid carcinoma. Among 48,495 women treated with radiation for breast cancer, 28 women subsequently developed thyroid carcinoma.

Furthermore, among 146,303 women not treated with radiation, 112 women subsequently developed thyroid carcinoma. The distribution of thyroid carcinoma histologies in both the radiation treatment (RT) cohort and the non-RT cohort was similar to that in the female general population. Overall, there was no increase in the risk of thyroid carcinoma in either the RT cohort or the non-RT cohort compared with the general population; (standardized incidence ratio [SIR] = 1.1; 95% CI = 0.8 to 1.6 for the RT cohort and SIR = 1.2; 95% CI =1.0 to 1.4 for the non-RT cohort). When the RT-cohort was compared with the non-RT cohort, the RR of thyroid carcinoma was 1.0 (95% CI = 0.7 to 1.5).

Galper *et al.* (2002) compared 1,884 patients with clinical Stage I or II primary breast cancer treated with gross excision and/or ≥ 60 Gy (median 63) to the primary tumor site (breast) between 1968 and 1987 at Brigham and Women's hospital in Boston to determine the incidence of all secondary cancers by specific location among long-term survivors (median follow-up of 10.9 years, range 0.2 to 27.9). Radiation dose estimates were derived from medical records. The women treated with radiation at Brigham and Women's received more than 60 Gy of total dose to the tumor bed. In addition, 57% of the women also received supraclavicular/axillary radiation (median dose 45 Gy, range 20 to 60), while 28% of the women received systemic chemotherapy. The expected numbers of cancers were calculated based on the SEER rates for non-breast malignancies. By 8 years of follow-up, 432 patients (23%) had developed distant metastases, 295 patients (16%) a local/regional recurrence, and 159 (8%) a contralateral primary. In this cohort, 147 patients (8%) developed a second primary malignancy compared with 127.7 expected according to SEER (i.e., a relative increase of 15% (P = 0.05)). Furthermore, within the first 5 years after treatment of the primary cancer, the observed and expected rates of all second cancers were identical (47 vs. 46.9), but after 5 years, 24% more second cancers were observed than expected (100 vs. 80.8, P = 0.02). In younger patients (< 50 years of age at breast cancer diagnosis), the excess of observed cancer rates was larger than in older patients (43% vs. 7% increase).

Lung cancers were observed in 33 women representing an excess of 52% above expected (P = 0.01), and most of these lung cancers occurred > 5 years after treatment (n = 23) and in women who were > 50 years old at the time of breast cancer diagnosis (n = 27). Furthermore, a somewhat larger percentage of patients that developed lung cancer had received third-field radiation. The observed incidence of ovarian cancer was significantly greater than expected in patients < 50 years of age, but was not different than expected in patients ≥ 50 years old (7 vs. 1.96, P = 0.004 and 5 vs. 5.3, P = 0.61, respectively). The number of colorectal cancers and lymphomas was not increased in comparison to the expected number (22 vs 23.4 expected and 8 vs 6.5 expected, respectively), while 14 melanomas were observed compared to only 5.6 expected; most of these occurred outside the direct field of irradiation. Other hematologic malignancies and sarcomas were too rare in this population to be evaluated. This study also could not address the influence of smoking or family history due to a lack of such data.

Kleinerman *et al*. (1995) examined patterns of all incident second cancers in 86,193 primary invasive cervical cancer patients of whom 49,828 were known to have been treated with radiation between 1935 and 1990. The cervical cancers and the second cancers were reported to 13 population-based cancer registries in five countries

(Denmark, Finland, Norway, Sweden, and the U.S. [Connecticut, Iowa, and 7 SEER areas]). This report is an update of an earlier report included in the IARC monograph (Boice *et al.* 1985, Boice *et al.* 1988) (see above). According to the usual radiation treatment protocols and modalities (external beam radiotherapy or brachytherapy using an intracavitary radiation source), the radiation dose to organs of the pelvic region was estimated to have been > 30 Gy, and much less for other sites (1 to 3 Gy to the liver, pancreas, stomach, and kidney; 0.3 Gy to the esophagus, lung, and breast; 7 Gy to hematologic sites; and 0.1 Gy to the thyroid). Due to differences in treatment practices, doses may have varied widely (e.g., between 30 and 60 Gy for the bladder and the rectum).

In this new extended follow-up study, Kleinerman *et al.* (1995) concentrated on very long-term survivors (> 30 years after diagnosis of the primary cancer). Overall, 7,543 second cancers were observed versus 6,015 cancers expected based on population rates (SIR (observed/expected = 1.2; 95% CI = 1.2 to 1.3), with lung cancer accounting for nearly half of all excess cancers. Among 49,828 women treated with radiation and followed for more than 1 year, 3,750 survived 30 or more years compared to 793 out of 16,713 women not treated with radiation. Most of the long-term survivors resided in Connecticut and Denmark, because these registries have been operating the longest. Overall, a two-fold risk increase for cancers located in heavily irradiated organs was observed in the group treated with radiation. Most of the excess cancers and increasing trends with time since radiation treatment were observed for pelvic organs in close proximity to the field of irradiation, that is, after > 30 years of follow-up, SIRs for cancers of these organs in radiation-treated patients were: rectum (SIR = 4.0; [95% CI = 3.01 to 5.11]), vagina (SIR = 39.4; [95% CI = 17.2 to 78.8]), vulva (SIR = 7.9; [95% CI = 2.7 to 16.3]), ovary (SIR = 1.7; [95% CI = 1.04 to 2.64]), and bladder (SIR = 6.2; [95% CI = 4.7 to 7.9]). As mentioned above, these pelvic organs had received estimated radiation doses of ≥ 30 Gy.

Kleinerman *et al.* (1995) reported that cancers of the kidney also showed increased risks after long latency and an increasing trend with time but at much lower doses (SIR = 1.3; 95% CI = 1.0 to 1.5; SIR = 1.6; > 30 years after radiation treatment; average organ dose 2 Gy). In contrast, increases were found only in the first 10 years after radiation treatment for acute and nonlymphocytic leukemia (SIR = 1.89; 95% CI = 1.21 to 2.82, 1 to 4 years after treatment; SIR = 1.69; 95% CI = 1.05 to 2.58, 5 to 9 years after treatment; [SIR=1.08; 95% CI = 0.76 to 1.49, more than 10 years after treatment]; average dose to active bone marrow 7 Gy) and for cancers of the bone (SIR = 3.0; 95% CI = 1.7 to 4.8; average organ dose 20 Gy). In comparison to women who did not receive radiotherapy, these cancer types appeared to be linked to radiotherapy. On the other hand, increased lung cancer risks were observed in irradiated and surgically treated women, were of comparable size, and showed no increase in risk with time since treatment. Thus, the authors suggested that the lung cancers were most likely caused by cigarette smoking behavior in both patient groups. The relatively small risk increases observed for stomach, esophageal, and laryngeal cancers in irradiated patients also may have suffered from confounding by smoking, and no clear pattern with increasing time after radiation treatment was observed. Finally, breast cancers occurred less often than expected in all

irradiated survivors (SIR = 0.7); this suggests that ovarian irradiation (and subsequently lower levels of female hormones) may be protective for breast cancer.

Travis *et al.* (2000) undertook a nested case-control study of second leukemia in a cancer registry-based cohort of 18,567 males who survived one year or more after primary testicular cancer diagnosis between 1970 and 1993 as reported to 8 population-based registries. Radiation dose effects were compared for 36 men who developed leukemia and 106 control testicular cancer survivors without leukemia matched on age, registry, calendar year of, and survival time without leukemia after diagnosis (mean dose to the active bone marrow was estimated to be 12.6 Gy). Secondary leukemia developed on average 6.8 years after the diagnosis of testicular cancer. In general, survival after leukemia diagnosis was poor (median 8.4 months). Radiotherapy that resulted in a dose of ≥ 7.5 Gy without chemotherapy resulted in a threefold elevated risk of leukemia (RR = 3.1; 95% CI = 0.7 to 22 based on n = 22 cases). Risk increased with increasing dose of radiation to the active bone marrow, and patients who received radiotherapy to the chest in addition to the abdominal/pelvic fields accounted for much of the risk increase observed at higher doses. For those with only abdominal/pelvic radiotherapy (mean dose to active bone marrow 10.9 Gy), the RR was 2.9 (95% CI = 0.6 to 21), and for those with additional chest radiotherapy (mean dose to bone marrow, 19.5 Gy) the RR was 11.2 (95% CI = 1.5 to 123).

Secondary cancers after medical treatment for a primary cancer in children

Garwicz *et al.* (2000) assessed the risk of developing any second malignant neoplasm after cancer in childhood and adolescence associated with different treatment modalities including radiation treatment in Nordic countries (Sweden, Norway, Denmark, Finland, and Iceland). They performed a nested case-control study within a Nordic cohort of 25,120 patients younger than 20 years at the time of diagnosis of a first malignant neoplasm between 1960 and 1987 and diagnosed between 1960 and 1991 with a second malignant neoplasm. For each case of second malignant neoplasm, 3 controls were sampled from the registries, matched by sex, age, and calendar year of diagnosis and length of follow-up. Radiotherapeutic charts provided the information on target volume, target dose, number of days, and fractions and radiation quality employed for treatment. The high-dose local radiation group received a maximum dose of > 30 Gy at any volume, while the low-dose group received a maximum dose of ≤ 30 Gy. A third group consisted of patients radiated outside the side where the second tumor occurred. For the final analysis, there were 234 cases and 678 controls. The relative risk of developing a second malignant neoplasm in the irradiated volume was 4.3 (95% CI = 3.0 to 6.2). Specifically, risks of secondary cancers due to local irradiation were increased for cancers of the bone and connective tissue (RR = 19.8; 95% CI = 4.5 to 86.7), breast (RR = 11.5; 95% CI = 3.2 to 40.6), leukemia (RR = 2.6; 95% CI = 0.8 to 8.5), lymphoma (RR = 5.1; 95% CI = 1.0 to 25.9) and brain (RR = 2.8; 95% CI = 1.4 to 5.5). The risk was highest in children diagnosed and treated before the age of 5 years; risk also increased with the dose of radiation and with increasing follow-up time after first malignant neoplasm (i.e., risks were much greater after more than 10 years of follow-up) (RR for 0 to 9 years = 1.7; 95% CI = 0.8 to 3.9, and RR for 10 to 30 years = 4.3; 95% CI = 2.2 to 8.3). Chemotherapy alone was not associated with an increased RR but significantly potentiated the effect of

radiotherapy at low doses (interaction RR for low-dose, local radiation and chemotherapy = 7.0; 95% CI = 1.5 to 32.9).

Bhatia *et al.* (2002) followed a cohort of 8,831 children diagnosed with acute lymphocytic leukemia (ALL) before age 21 and enrolled for treatment on the Children's Cancer Group therapeutic protocols between 1983 and 1995 and followed until 1999 at 122 institutions throughout the U.S. and Canada to determine the incidence of any second neoplasms. The median age at diagnosis of ALL was 4.7 years and the cohort accrued 54,883 person-years of follow-up. The protocol required the treating institutions to record radiation doses and assigned fields that ranged from 0 to 18 Gy to the cranium (for CNS prophylaxis) and 24 Gy to the cranium and 6 to 12 Gy to the spine for treatment of CNS disease. Tumors were classified according to histology. Sixty-three patients developed second neoplasms, including solid tumors (19 brain tumors and 20 other solid tumors including 4 sarcomas and 4 thyroid cancers), myeloid leukemia or myelodysplasia (16), and lymphoma (8). The cumulative incidence of total second neoplasm (SIR = 7.2; 95% CI = 5.5 to 9.1) was compared with the general population. To calculate the risks for second cancers, expected numbers of cancers by site were based on SEER rates. Cancer risks were found to be increased for acute myeloid leukemia (SIR = 52.3; 95% CI = 28.6 to 87.7), non-Hodgkin's lymphoma (SIR = 8.3; 95% CI = 2.6 to 17.2), parotid gland tumors (SIR = 33.4; 95% CI = 9.1 to 85.6), thyroid cancer (SIR = 13.3; 95% CI = 3.6 to 34.1), brain tumors (SIR = 10.1; 95% CI = 5.9 to 16.2), and soft tissue sarcoma (SIR = 9.1; 95% CI = 2.4 to 20.2). Time to second neoplasm was on average 2 to 4 years for hematopoietic cancers and sarcomas and 7 to 8 years for solid tumors; risk for leukemia was highest in the first 5 years after radiation treatment and declined thereafter. Risk of second cancer increased with radiation dose for all cancers (1,800 cGy: RR = 1.5; 95% CI = 0.9 to 2.6; 24 Gy: RR = 3.9; 95% CI = 1.4 to 11.2) as well as in the subgroups of all solid and all hemato- and lymphopoietic cancers; 75% of all solid cancers developed within the radiation fields. Furthermore, exposure to 2,400 cGy of radiation was associated with a significantly increased risk of developing thyroid cancer (RR = 30.8; 95% CI = 1.2 to 62.9). Finally, multivariate analysis revealed that female sex, radiation to the craniospinal axis, and relapse of primary disease were independently associated with increased risk of all second neoplasms.

Ng *et al.* (2002) examined the excess risk of any second malignancy in patients treated for primary Hodgkin's disease at Brigham and Women's Hospital in Boston from 1969 to 1997. Earlier patients (665 patients) were treated predominantly with radiation and later patients (296 patients) with a combined therapy that included chemotherapy. The radiation fields included total nodal irradiation in 13% of patients, mantle and paraaortic irradiation in 66% of patients, mantle alone in 17% of patients and pelvic and paraaortic in 3% of patients. The median dose to the mantle field was 3,605 cGy, with a boost to bulk disease to a median total dose of 4,000 cGy, daily fractions ranged from 150 cGy to 200 cGy, 5 days per week. Among 1,319 patients diagnosed and treated with clinical stage I-IV Hodgkin's disease (85% suffered from disease with clinical stage I-II), 181 second malignancies and 18 third malignancies were observed. The median follow-up was 12 years, with 32% of patients having more than 15 years and 17% having more than 20 years of follow-up and 15,910 total person years of follow-up. Expected number of cases was based on rates from the SEER data, and the relative risk and absolute excess

risk of all second malignancy were estimated to be 4.6 (95% CI = 4.0 to 5.4) and 89.3/10,000 person-years, respectively. The relative risk of breast cancer by age at diagnosis of Hodgkin's disease dropped steadily and dramatically from 111.8 (95% CI = 36.2 to 261.0) at age < 15 years, to 32.0 (95% CI = 14.6 to 60.7) for ages 15 to 19, and 3.7 (95% CI = 1.0 to 9.5) for ages 30 to 35 and no increased risk after age 40. Similarly, the relative risk for all secondary malignancies dropped from 10.7 (95% CI = 7.8 to 14.4) for ages younger than 20, to 4.9 (95% CI = 4.0 to 5.9) at ages 20 to 50, to 2.4 (95% CI = 1.6 to 3.4) at ages older than 50. Women with a high risk for breast cancer had received radiation therapy to the chest prior to the age of 30 years, and the median time to development of breast cancer was 15 to 18 years. Furthermore, solid tumors showed a clear increase in risk with time since radiation treatment had occurred with the largest relative risk after 15 years for breast, lung, and gastrointestinal tract cancers. Alternatively, acute leukemias and lymphomas showed a bimodal distribution with time since treatment; the largest relative risk was observed in the first 5 to 10 years of follow-up and a second peak after 20 or more years of follow-up. The relative risk was higher with combined chemo- and radiation therapy (RR = 6.1; 95% CI = 4.7 to 8.0) than with radiation therapy alone (RR = 4.0; 95% CI = 3.4 to 4.9), and the risk increased with increasing radiation field size in patients who received combined modality therapy. Overall, after 15 and 20 years, there was, respectively, a 2.3% and 4.0% excess risk of second malignancy per person per year. The excess risk of second malignancy after Hodgkin's disease continued to be increased after 15 to 20 years and did not appear to reach a plateau.

Recent cohort studies examining medical irradiation for non-cancer diseases

Studies of diagnostic medical irradiation or medical radiation treatment for diseases other than cancers also employed cohort or nested case-control designs but in general had to rely on relatively small groups of patients compared to the studies of radiation treatment for primary cancer as discussed above. Controls are defined in a variety of ways such as patients having received a different treatment (tonsillectomy instead of radiation), having received lower levels of radiation during treatment, or radiation administered to different sites. These studies in general evaluated risks at lower radiation doses requiring larger numbers to achieve the same statistical power and, thus, rendering these studies relatively uninformative except for very common cancer sites.

Ron *et al.* (1999) followed a cohort of 968 Israeli women treated with radiotherapy for infertility at a mean age of 28 years between 1940 and 1972 (mostly treated during the 1950s). Most of these women received radiation to both the ovaries and the pituitary gland. Mean doses to the brain, colon, ovary, and bone marrow were estimated at 0.8, 0.6, 1.0, and 0.36 Gy, respectively. More than 10 years after radiation treatment, 60 incident cancers were observed compared with 74.5 expected based on national cancer incidence rates (SIR = 0.81; 95% CI = 0.6 to 1.0), the deficit being mainly due to a low breast cancer risk (SIR = 0.7; 95% CI = 0.4 to 1.1). Increased risks were suggested for cancers of the colon (SIR = 1.6; 95% CI = 0.6 to 3.3) and for the uterine corpus among women treated for infertility (SIR = 3.8; 95% CI = 1.2 to 8.8), but the small number of cases observed does not allow one to draw conclusions. Risk of colon cancer was higher among women with two or more treatments and, in addition, increased with length of follow-up. There was a slight suggestion of an increasing risk with age at exposure for all

cancers combined, but no trend was observed with attained age on subsequent cancer risk. Only 2 cases of leukemia were observed (1.61 expected). No clear excess of any cancer was observed among women at organ doses above the median compared with subjects treated with doses below the median, except for a slight increase in colon cancer.

Lichter *et al*. (2000) estimated the relative risk of developing basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) due to therapeutic ionizing radiation after identifying these skin cancers in a population-based incidence survey conducted in New Hampshire between 1993 and 1995. Cases were matched to population controls selected through driver's license and Medicare records. A total of 592 cases of BCC and 289 cases of SCC were identified and histologically confirmed, and 536 age- and sex-matched controls were selected from population lists. Information regarding radiotherapy and other factors was obtained through in-person interviews. An attempt was made to review radiation treatment records of subjects who reported a history of radiotherapy, but treatment records for nonmalignant conditions that had occurred prior to 1970 were no longer available. Increased risks of both BCC and SCC were found in relation to therapeutic ionizing radiation (mostly for benign diseases of the skin such as keloids, acne, tinea, fungus, and warts) and were confined to the site of prior radiation exposure (BCC OR = 3.30; 95% CI = 1.60 to 6.81; SCC OR = 2.94; 95% CI = 1.30 to 6.67). Effects were most pronounced for those cases previously irradiated for acne (BCC OR = 17.35; 95% CI = 2.30 to 130.8 [n = 18]; SCC OR = 9.97; 95% CI = 1.15 to 86.40 [n = 5]) and risks seemed to increase somewhat with the frequency of radiation treatments; i.e., the reported number of treatments and, thus, greater dose fractionation (10 Gy/week or less and ≤ 2 Gy per treatment). Larger risks also were observed for early age at first treatment (less than age 20) and for those treated 40 years or longer before diagnosis, suggesting an extremely long latency period for some of these skin cancers. For SCC, an association with radiotherapy was observed only among those whose skin was likely to burn with sun exposure but not among those who tan, while for BCC the risks were comparable in size in both groups.

Modan *et al*. (2000) followed 674 children through the end of 1996 who underwent diagnostic cardiac catheterization due to congenital anomalies between 1950 and 1970 in three major medical centers in Israel. 28.6% of the participants had undergone more than one procedure, the mean age at treatment was 8.9 years, and the mean follow-up time was 28.6 years. The mean dose to the active bone marrow from this procedure was estimated to have been 1.1 cGy, and the mean dose to the skin was 5 to 40 cGy. The authors conducted a review of the children's medical files in each hospital, ascertained demographic data and vital status from the Israeli National Registry and linked it with the Israeli National Cancer Registry to identify cancer cases that occurred subsequent to cardiac catheterization. While only 56.2% of the catheterized children were males, all diagnosed cancers occurred in males. The expected number of malignancies for all sites in males was 4.75, while the observed number was 11 (SIR = 2.3; 95% CI = 1.2 to 4.1). Of the 11 cancer cases, 4 were lymphomas (0.63 were expected, SIR = 6.3; 95% CI = 1.7 to 16.2), and one of these was Hodgkin's disease, but excluding this case still left an increased SIR of 6.7 (95% CI = 1.3 to 19.5). There also were three cases of melanoma as opposed to 0.62 expected (SIR = 4.9; 95% CI = 1.0 to 14.2). These findings differ from

previous studies suggesting an increased occurrence of lymphoma in the absence of an excess of leukemia. However, this study was based on a very small number of total cancers in only one gender, and the discordance of the results for males and females remains puzzling.

Yeh *et al*. (2001) followed a population of 2,925 subjects with adenoid hypertrophy. Of these, 904 subjects received radium treatment of the nasopharynx in Washington County, Maryland, between 1943 and 1960. Most controls who were not treated with radiation received a tonsillectomy or adenectomy. The authors assessed the risk of developing a subsequent neoplasm using a combination of data from the Washington County cancer registry, death certificates, and questionnaires mailed in 1978 and again in 1994 and 1995. The radium implants were estimated to emit approximately 70% gamma rays, and the dose to the thyroid ranged from less than 0.04 to 0.44 Gy, for the pituitary gland from 0.44 to 1.7 Gy, and for the salivary gland from 0.09 to 0.26 Gy. No general increase in cancer was found, with a total of 41 cancer cases identified among 808 exposed and traced patients, compared to 83 cancer cases in 1,819 traced non-exposed persons (RR = 1.0; 95% CI = 0.7 to 1.5). No salivary gland cancers were found in either group. An excess risk of thyroid cancer was suggested in the irradiated group, but this was based on only two cases in the exposed and one case in the nonexposed group (RR = 4.2; 95% CI = 0.4 to 46.6). Furthermore, seven brain tumor cases (three malignant and four benign) were identified in the irradiated group versus none in the nonirradiated group (malignant RR = 14.8; 95% CI 0.8 to 286.3; benign RR = 30.9; 95% CI = 1.9 to 541.7). The malignant brain tumors occurred within the first 25 years of follow-up while the benign cases were diagnosed 35 years or more after beginning of follow-up. Overall, the irradiated group experienced a higher risk of head and neck cancers, but a deficit was reported for all sex-hormone related cancers, i.e., decreased risks of breast cancer, female genital cancers, and prostate cancer (RR = 0.4; 95% CI = 0.2 to 1.0); the latter finding was interpreted by the authors as possibly having been due to pre-pubertal radiation damage to the pituitary, with consequent reduction in pituitary hormone output that stimulated sex-hormone production in the ovaries and testes.

3.1.2.3 Occupational exposures

Several recent cohort studies have been published for workers from the nuclear industry. All of the results presented in the following section are based on data collected in cohort studies of ionizing radiation-exposed workers, for whom cumulative workplace exposures were monitored individually, relying primarily on film badge readings and to a lesser degree on thermoluminescent or pocket dosimeters. Except for two studies that examined cancer incidence, all studies relied on mortality data and, thus, were unable to evaluate the risks of developing non-fatal cancers. Strengths of these worker cohort studies are the well-documented radiation doses, long follow-up times, internal comparisons of workers exposed at different radiation dose levels to workers exposed to little or no external radiation, and relatively complete follow-up. Limitations are mostly related to the relatively low statistical power to explore effects for cancer subtypes at these overall low levels of exposure compared to A-bomb survivors and groups that received medical treatment for cancer. These workers were overwhelmingly healthy, white, adult males and, thus, cancer risks in females and potentially more susceptible

groups in the general population at cumulative low doses of radiation could not be evaluated in most of these studies. Finally, some of these workers may have additionally been exposed to chemical carcinogens or radiation from internally absorbed radionuclides; although some studies adjusted for these concomitant exposures, others were unable to assess contributions from such additional factors.

Sont *et al*. (2001) published data for a cohort of 191,333 workers whose occupational records for ionizing radiation doses were reported to the National Dose Registry of Canada between 1951 and 1988. They used the Canadian Cancer database to identify incidence cancers among cohort members and to calculate SIRs between 1969 and 1988. Stratifying by age, sex, and calendar year, they found a healthy worker effect for nuclear workers, i.e., a deficit in the SIR for all cancers combined and specific sites except melanoma and thyroid cancers among workers compared to the national population. Internal dose-response analyses, however, showed excess relative risks with increasing radiation dose for males and females combined for cancers of the rectum (ERR per Sv = 13.8; 90% CI = 3.7 to 33.6), leukemia (ERR per Sv = 5.4; 90% CI = 0.2 to 20.0), lung (ERR per Sv = 3.0; 90% CI = 0.5 to 6.8), all cancers combined (ERR per Sv = 2.5; 90% CI = 1.2 to 4.0), all cancers except lung (ERR per Sv = 2.3; 90% CI = 0.9 to 4.1), and similarly for all cancers except leukemia (ERR per Sv = 2.3; 90% CI = 1.1 to 3.9). In addition, for males, cancers of the colon (ERR per Sv = 2.8; 90% CI = 0.0 to 8.0), pancreas (ERR per Sv = 9.2; 90% CI = 0.1 to 36.8), and testis (ERR per Sv = 38.3; 90% CI = 1.4 to 147.9) carried significantly elevated excess relative risks. This study is very important since its results are not only based on a large number of workers but is one of very few occupational radiation studies that was able to use incidence data from a National Cancer registry. Thus, it provides important information with respect to the effect of low-dose radiation on non-fatal cancers in humans exposed occupationally over extended periods of time.

Richardson and Wing (1999) reported new results of an extended follow-up of 8,307 white male workers hired at Oak Ridge National Laboratory (ORNL) from 1943 through 1972 and individually monitored for whole body exposure to ionizing radiation. In this report for ORNL workers, vital status and cause of death were ascertained for the workers through 1990. The authors focused in these analyses on the exploration of a modifying effect of age at radiation exposure. Overall, cumulative radiation dose was found to be associated with a 1.8% (SE = 0.9) increase in all-cancer mortality per 10 mSv, assuming a 10-year lag between exposure and mortality. However, radiation doses received at older ages (> 45 years of age at exposure) exhibited larger effect sizes for cancer mortality than doses received at younger ages. Specifically, doses received after age 45 were associated with a 5.9% (SE = 1.7) increase in cancer mortality per 10 mSv, and for this older age range, dose-response associations appeared consistent across periods of follow-up, periods of hire, and ages at risk. The authors interpreted these findings as suggesting an increased sensitivity to the carcinogenic effects of ionizing radiation among those exposed at older ages.

Similar effects for age at exposure were recently observed by Ritz *et al*. (1999a, 1999b) in another small nuclear worker cohort. This cohort of 4,563 nuclear workers employed at a nuclear research and production facility in Southern California was monitored for

external radiation between 1950 and 1993 and followed until December 1994 for cancer mortality. Analyses were based on 258 total cancer deaths. Internal comparisons of workers exposed at different dose levels, using risk-set analyses with adjustment for confounders, demonstrated an increased mortality rate in workers exposed to 200 mSv for hemato- and lymphopoietic cancers (RR per 100 mSv = 1.99; 95% CI = 1.17 to 3.40), and for lung cancer (RR per 100 mSv = 1.52; 95% CI = 0.90 to 2.55). Mortality rates for total cancers (ERR per 100 mSv = 1.22; 95% CI = 0.86 to 1.73) and solid cancers of "radiosensitive" sites (ERR per 100 mSv = 1.25; 95% CI = 0.80 to 1.94) increased monotonically with cumulative radiation dose, but no trends were observed for "nonradiosensitive" sites. Furthermore, analyses in which risk sets were matched for age and calendar time and cumulative radiation doses were divided according to the age intervals in which exposure occurred, suggested that workers exposed to external radiation after the age of 50 years experienced exposure-related elevations in mortality from cancer at any site (RR per 100 mSv = 1.98; 95% CI = 0.63 to 6.26), radiosensitive solid cancer (RR per 100 mSv = 3.29; 95% CI = 1.10 to 9.89), and lung cancer (RR per 100 mSv = 3.89; 95% CI = 1.23 to 12.3). These increases were substantially (1.6- to 3.5-fold) greater than were seen at earlier ages. In contrast, they found that the radiation doses contributing most importantly to mortality from cancers of the blood and lymph system were received before age 50 (for age < 50, RR per 100 mSv = 2.73; 95% CI = 1.46 to 5.10; for age ≥ 50 years, RR per 100 mSv = 0.24; 95% CI = 0.00 to 687).

In another study, Ritz (1999) made use of data from the Comprehensive Epidemiology Data Resource (CEDR) to study patterns of cancer mortality in a cohort of 4,014 uranium-processing workers. Results from this study indicated that nuclear workers employed at the Fernald Feed Materials Production Center (Ohio) exposed to ionizing radiation experienced an increase in mortality from total cancer (RR per 100 mSv external radiation = 1.92; 95% CI = 1.11 to 3.32), radiosensitive solid cancer (RR per 100 mSv = 2.00; 95% CI = 1.02 to 3.94), and lung cancer (RR per 100 mSv = 2.77; 95% CI = 1.29 to 5.95). Again, effects were strongest when exposure had occurred at older ages (> 40 years). The authors were able to adjust for internal doses from radionuclide exposures and for concomitant exposure to chemical carcinogens.

Conducting a multi-facility study of nuclear workers at U.S. Department of Energy facilities, Wing *et al.* (2000) examined the influence of radiation exposure on the occurrence of 98 multiple myeloma cases identified from the combined roster of 115,143 workers hired before 1979 at Hanford, Los Alamos National Laboratory, Oak Ridge National Laboratory, and the Savannah River site and followed for vital status through 1990 (1986 for Hanford). The authors selected 391 age-matched controls for these cases and abstracted demographic, work history, and occupational exposure data from personnel, occupational medicine, industrial hygiene, and health physics records. Cases were found to be disproportionately African American, male, and hired prior to 1948. While lifetime cumulative whole-body ionizing radiation dose was found not to be associated with multiple myeloma, at older age at exposure, a positive association between multiple myeloma was observed, and dose-response associations increased in magnitude with exposure age (from 40 to 50 years) and lag assumption (from 5 to 15 years). For cumulative doses received at ages older than 45 with a 5-year lag, the ERR

was 6.90% per 10 mSv (SE = 2.90%). This increase, however, was opposed by a similar size but non-significant deficit for multiple myeloma at younger ages of exposure (% increase per 10 mSv = -6.83% [SE = 6.11%]). The majority of workers (62 cases and 235 controls) had received less than 10 mSv of external radiation exposure and were considered unexposed in these analyses. A strength of this study was that data for exposures to radionuclides and exposures received prior to employment at the nuclear facilities, to chemicals at the facilities, and to some extent for smoking were collected and used to adjust the analyses for confounding. The authors acknowledged that the exposure age effect is at odds with interpretations of A-bomb survivor studies but saw it to be in agreement with the aforementioned studies of cancer among nuclear workers.

These four newly published studies suggest that effects of low-level radiation doses may depend on exposure age and, furthermore, that patterns of effect modification by age may differ by type of cancer.

Kreisheimer *et al.* (2000) conducted an analysis of lung cancer mortality in two subcohorts of male Mayak workers: 1) 1,669 workers who started their employment in the Mayak plutonium and reprocessing plants between 1948 and 1958 and received internal exposure from plutonium and, in addition, external gamma radiation; 2) 2,172 Mayak reactor workers who were exposed only to external gamma rays. Separate analyses for each of these two sub-cohorts allowed discrimination to some extent between the effects of external gamma-ray exposure and internal alpha-particle exposure. They reported the lung cancer mortality rate to be consistent with linear dose dependences. For the gamma-ray component, the analysis suggested an ERR for lung cancer mortality at age 60 of 0.20 per Sv (95% CI = -0.04 to 0.69) based on all workers taken together. Separate analyses for reactor workers exposed to gamma radiation only suggested that the ERR for these workers may have been larger (ERR = 0.43 [SE = 0.31]); for plutonium-exposed workers ERR = 0.24 (SE = 0.28). The authors remarked that they were unable to adjust for smoking in their analyses but that they did not expect smoking behavior to be differentially distributed according to radiation dose. They were not able to evaluate gamma radiation exposure effects in mostly non-smoking female workers since most of the female workers were employed in plutonium-related operations.

Another publication by Gilbert *et al*. (2000) reported on the incidence of liver cancers in the same cohort of Mayak workers, but this analysis included female workers. The authors found clear evidence of excess risk for workers with external doses exceeding 1 Sv to the liver and workers in the plutonium plant with detectable plutonium burdens. Especially large SMRs compared to the Russian population were found for the female workers in increasing dose categories (SMR 0 to 0.1 Sv = 0.5; 95% CI = 0.03 to 2.0; SMR 0.1 to 1 Sv = 1.3; 95% CI = 0.3 to 3.4; SMR 1 to 3 Sv = 7.9; 95% CI = 4.1 to 13; SMR > 3 Sv = 9.2; 95% CI = 2.9 to 21); the authors concluded that due to the concomitant exposure to internal and external radiation, the contributions from external gamma dose also could not be reliably evaluated.

3.1.3 Discussion

The IARC review and studies newly published and included in this review provide substantial human data for the evaluation of the carcinogenicity of X and gamma radiation. Studies chosen for review have in common that radiation exposures were directly measured or, when radiation dose estimates were reconstructed, were well-characterized. In addition, a large number of exposed subjects and site-specific cancers were evaluated and a reasonably long follow-up period was available. However, how informative even larger studies may be is limited by the rarity of certain site-specific cancers or the relatively low risk increases expected at lower radiation doses, thus limiting the sensitivity of even large studies. Furthermore, the studies included in this evaluation of the carcinogenicity of X and gamma radiation were conducted in a variety of countries and include a large number of exposure scenarios.

The IARC working group reviewed a diverse group of radiation studies, but based their conclusions solely on the results derived from high-dose radiation studies, i.e., the A-bomb studies and studies that examined medical irradiation. This document includes the most recent publications of A-bomb survivor data and medical irradiation and in addition, also considers some recent results from studies of humans exposed to radiation at relatively lower dose ranges (nuclear worker and atmospheric weapons test personnel) and addresses some important outstanding issues such as age at exposure effects (see below).

Some controversy and open questions remain concerning which organ sites ought to be considered radiosensitive and at what dose levels specific organs are affected. Discussions in the literature also revolve around the issue of the appropriateness of linear extrapolation from high to low dose levels with or without adjustment factors for fractionated low doses (such as dividing the slope by two for application in low dose settings); or alternatively using a threshold model. Addressing the latter question elegantly, Pierce and Preston (2000) examined the effects low-dose exposures exhibit on solid cancer incidence in A-bomb survivors. They concluded that for solid cancer incidence, a linear model adequately describes the dose-response curve at low dose levels. Recently, Little *et al*. (1999) addressed the issues of comparing effect sizes across populations. They compared the size of the radiation-associated relative risks of second primary cancer incidence in patients treated for first primary cancer by radiotherapy with relative risk estimates derived from the Japanese A-bomb survivor cancer incidence data. They reported the relative risks for four cancer sites, namely lung cancer, bone cancer, ovarian cancer, and leukemia, in the comparable (age at exposure, time since exposure, sex-matched) subsets of the Japanese data to be significantly greater than those reported in the majority of second cancer studies. For leukemia, they attributed this discrepancy mostly to cell-sterilization effects at very high dose levels. They cited some evidence that second cancer relative excess risks are lower among patients with cancer-prone disorders. To interpret these results correctly, however, one has to consider the higher underlying cancer risk in some of the medically exposed populations, in particular those with cancer-prone conditions. Higher background risks in populations (e.g., for breast cancer in Western populations and for stomach cancer in the Japanese population) or medical series

of patients lead to lower relative excess risks while at the same time the absolute excess risk in a population or among medically treated patients is sometimes higher.

Confounding is a common concern in epidemiologic studies, and many radiation studies lack data on lifestyle factors (such as smoking, alcohol consumption, and diet) and possible non-occupational exposures to carcinogens. However, some studies that were able to collect such data showed that the radiation effects observed were unlikely to have been biased by these factors. Furthermore, for confounding to be of major concern the factors need to be associated with both disease and exposure; in case of dose response relationships, the probability of having been exposed to the confounding factor has to increase with radiation dose, a condition that is not commonly met.

Issues that need additional future consideration include variations in cancer risk at specific sites with age and gender, the duration of radiation effects after exposure is discontinued, how to extrapolate risks from one population to another, and the possible modification (interaction) of the radiation effects by genetic or environmental risk factors and variations of the effects of radiation by histologic type. An example of a possible gene-environment interaction and the importance of histologic type is provided in the study by Lichter *et al*. (2000), which suggested that radiation effects for squamous cell carcinomas of the skin (but not basal cell carcinoma) may be limited to genetically vulnerable subjects prone to sunburn. This and several other studies reviewed here also suggested effects of age at exposure and possible effect modification by gender (Ritz 1999, Ritz *et al.* 1999b, Richardson and Wing 1999, Gilbert *et al.* 2000, Modan *et al.* 2000).

3.2 Neutrons

The IARC Working Group (2000) reviewed limited literature on human exposure to neutrons. Very few data are available for individuals exposed to neutrons, particularly since the dosimetry estimates for radiation from the atomic bombs exploded over Hiroshima and Nagasaki were revised in 1986 to such low levels (1% to 2% of the total dose at Hiroshima and less at Nagasaki) that the Working Group concluded that a useful database of human exposures is no longer available to estimate the carcinogenic risks of exposure to neutrons. The Working Group also reviewed those papers that have included reports of exposure to neutrons among workers in the nuclear industry, patients treated with neutrons, and crews of airplanes. The general conclusion about all of these populations was that the dose of neutrons received was generally too low to allow for a meaningful estimate of risk and that exposure to other ionizing radiation, particularly gamma rays, confounded the assessment of risk attributable to neutrons. IARC concluded that there was inadequate evidence in humans and sufficient evidence in experimental animals for the carcinogenicity of neutrons in humans. However, IARC classified neutrons as *carcinogenic to humans* (Group 1) because it considered genotoxic and mechanism data (See Sections 5 and 6). No new studies have been identified that provide additional information on the carcinogenicity of neutrons in humans.

3.3 Summary

3.3.1 X radiation and gamma radiation

IARC concluded in 1999 that all radiation studies taken together present a consistent body of evidence for the carcinogenicity of X radiation and gamma radiation in humans at a wide range of dose levels. This conclusion is corroborated by the newly published studies reviewed here. The recently published studies of second cancer occurrences after radiation treatment for first cancers furthermore support the A-bomb survivor results concerning differences in latency by type of cancer (higher risk of hematopoietic cancers appears in the first 10 years of follow-up compared to higher risks of solid cancers with increasing follow-up) and by age at exposure (higher risk for thyroid cancer after irradiation in childhood and for breast cancer in adolescence and during the reproductive years). Following is a description of the conclusions reached on which organ sites are to be considered radiosensitive and at what dose levels specific organs are affected.

It is largely undisputed that leukemia and cancers of the thyroid, breast, and lung are associated with radiation, and associations have been found at relatively low doses (< 0.2 Gy). The risk, however, depends to some extent on the age at exposure (exposure during childhood being mainly responsible for higher leukemia and thyroid cancer risks, exposure during reproductive age for breast cancer and, as recently suggested by some studies, lung cancer risk may be more strongly related to exposure later in life). Associations between radiation and cancers of the salivary glands, stomach, colon, bladder, ovary, central nervous system, and skin have been reported but are less well quantified. An exhaustive review by Ron (1998) noted that the relative risks for these cancer sites at 1 Gy exposure generally range from 1 to 2.5 for these sites. Some recent studies added additional evidence for cancers at these sites being caused by radiation exposures, i.e., by medical treatment with radiation (Garwicz *et al.* 2000, Bhatia *et al.* 2002, Kleinerman *et al.* 1995, Brenner *et al.* 2000, Ron *et al.* 1999, Lichter *et al.* 2000, Yeh *et al.* 2001), or by occupational low and protracted doses as reported for a large Canadian worker cohort (Sont *et al.* 2001). The first large study of sarcomas conducted by Yap *et al.* (2002) added angiosarcomas to the list of radiation-induced cancers occurring within the field of radiation at high therapeutic doses. In the IARC report, associations of ionizing radiation exposures with cancers of the liver, esophagus, and, to a lesser extent multiple myeloma and non-Hodgkin's lymphoma were considered inconsistent. Two recent studies, one conducted in a worker population (Gilbert *et al.* 2000) and another among A-bomb survivors (Cologne *et al.* 1999), suggested that liver cancers can be caused by radiation at doses above 100 mSv (in the worker population especially with concurrent exposure to radionuclides), and a linear dose-response relationship for external radiation and liver cancers was calculated for the A-bomb survivors (RR = 1.81; 95% CI = 1.32 to 2.43 per 1 Sv liver dose). A recent study by Modan *et al.* (2000) added some evidence that radiation exposure during childhood may affect the incidence of lymphomas and melanomas.

Finally, chronic lymphatic leukemia, Hodgkin's disease, cancers of the cervix, prostate, testis, and pancreas have rarely been related to radiation, although a recent large worker cohort study (Sont *et al.* 2001) suggested otherwise for the latter two cancer types (testis and pancreatic cancers).

3.3.2 Neutrons

There are no adequate epidemiological data available to evaluate the carcinogenicity of neutrons in humans.

4 Studies of Cancer in Experimental Animals

The IARC (2000) recently published a monograph on ionizing radiation that included X rays and gamma radiation and neutrons. Because of the extensive number of studies published on ionizing radiation-induced cancers in experimental animals, a comprehensive review of the literature is impractical. IARC (2000) recognized this fact in their section on experimental animal studies: *This section is not meant to be comprehensive; the studies summarized are those that provide both qualitative and quantitative information and address critical issues in radiation carcinogenesis*. Therefore, this review takes a similar approach and includes many studies that were reviewed by the IARC as well as a number of recently published studies that were not included in the IARC monograph.

It is well established that ionizing radiation induces tumors in experimental animals. IARC (2000) concluded that there is sufficient evidence in experimental animals for the carcinogenicity of X radiation, gamma radiation, and neutrons. Mice are the most extensively studied; however, there are sufficient data for rats, rabbits, dogs, and monkeys as well. The demonstration of the generality of the effect of an agent in experimental animals is considered as evidence of a high probability of a similar effect in humans. Because the evidence in humans for the carcinogenicity of X rays and gamma rays is substantial (see Section 3), consideration of the carcinogenicity of X rays and gamma rays in experimental animals might be considered superfluous. However, in the case of neutrons, experimental animal data may be considered more important because human data are lacking.

The susceptibility of different tissues is species, sex, and strain dependent. Although some experiments involved very large numbers of animals, none was large enough to establish whether certain tissues were completely resistant to the induction of cancer by radiation. Clearly, susceptibility varies considerably among tissues, and a genetic component of such susceptibility is equally clear.

One of the aims of animal experiments has been to determine the dose-response relationships of cancer induction. In many experiments, especially at low doses and despite the large numbers of animals tested in some studies, the data are insufficient to define precisely the dose-response curve. It is clear that there is not a single dose-response curve and that the curves differ depending on the tissue. However, with low-LET radiations (e.g., X rays and gamma rays) the responses are usually curvilinear in contrast to linear with high-LET radiations (e.g., neutrons). The linear-quadratic model, which was developed to describe radiation-induced chromosomal aberrations, is used to describe the dose-response curves. The simplest explanation of this model is that the linear component, alpha D, reflects single-track events and the dose-squared component, beta D^2, reflects two-track events. The values of the coefficients and dose range over which the alpha component is dominant differ among tissues. These differences likely reflect important aspects of the mechanisms but, as yet, remain unexplained.

Radiation and its effects are classified as external and internal. External radiations are emitted from sources outside the body that produce X rays, gamma rays, neutrons, and beta particles. These radiations can penetrate body tissues and, depending on their energy, deposit energy at various depths. Internal radiation comes from sources such as radionuclides that are ingested, inhaled, or injected into the body and emit alpha particles, beta particles, and/or gamma rays. Secondary radiations also occur and are produced by interactions of external radiations. Only the effects of X rays, gamma rays, and neutrons are considered in this report. See Sections 1 and 2 for further discussions of radiation types and exposure.

4.1 X radiation and gamma radiation

X rays and gamma rays are characterized by a low LET and are discussed together. Neutron radiation has a high LET and is discussed in Section 4.2. The LET accounts for the differences in biological effectiveness for a given absorbed dose in rad or Gy among various radiation sources and reflects differences in the rate of energy transfer. Low-LET radiation tends to be more effective (i.e., induces more tumors) following acute high-dose exposure than following continuous low-dose exposure or fractionated doses. At low doses, X rays are slightly more effective than gamma rays (IARC 2000).

4.1.1 Mouse

Murine animal models have been used extensively in experiments designed to study the general characteristics of low-LET radiation such as X rays and gamma rays as well as other radiation qualities. These studies concentrated on determining the physical characteristics such as dose, dose rate, fractionation and protraction. The biological aspects that have been considered include age, gender, genetic background, and relative biological effectiveness (RBE), which involves dose and the response of the radiation under investigation and a reference radiation (see Section 1.2.7). RBEs are considered in the section on neutrons.

A limited number of mouse strains have been used in the large-scale experiments, but other strains have been used to investigate the response of specific tissues and tumor types. Lymphoma and leukemia as well as various solid tumors are commonly reported. Results are discussed in the following sections according to the life stage at exposure (i.e., postnatal, prenatal, and parental).

4.1.1.1 Postnatal exposure

The following discussion is organized according to general tumor types. Many studies focused on neoplasms of the bone marrow and lymphoreticular system (i.e., leukemias and lymphomas). Some studies included data for various solid tumors as well as leukemia and lymphoma and are discussed under the “mixed tumor” subheading while other studies presented data only for solid tumors.

Although ovarian tumors are reported in many of the studies involving female mice in this section and in Section 4.2.1.1, it is important to note that the murine ovary is exquisitely sensitive. When sufficient oocytes are killed, the production of hormones is changed altering the pituitary-ovary axis, and the resulting increase in gonadotrophins

plays an important part in the production of ovarian tumors. Thus, the effects of dose, dose rate, and radiation quality on tumor induction may reflect cell killing rather than an actual influence of these factors on some molecular process involved in neoplastic transformation. Unfortunately, the altered hormonal balance after irradiation of the ovary may result in greater risks in some organs than would occur independent of the ovarian damage (Fry *et al.* 1978).

Lymphoma or leukemia

The complexity of time-dose relationships became clear in the extensive studies on the induction of thymic lymphoma, which was one of the first murine tumors to be examined in detail (Kaplan *et al.* 1953, Kaplan 1964). This tumor occurs in a number of mouse strains and has been studied extensively in C57Bl and RFM mice, with female mice markedly more susceptible than the male. Most of the tumors arise from T cells, which is not common in humans. The thymus microenvironment appears to determine the type of malignancy, and a heterogeneity of cell type has been noted (Boulton *et al.* 2002).

The time-dose relationships for thymic lymphoma differ from those of most tumor types. While single doses will induce lymphoma, fractionation is more effective. The number of fractions, the interval between fractions, the dose per fraction, and the total dose all influence the effectiveness. The induction of this type of lymphoma can be classified as abscopal, i.e., the effect on nonirradiated tissue results from irradiation of other tissue of the organism. It has been shown that the thymus does not need to be present at the time of the irradiation. It can be removed prior to irradiation and replaced after exposure. Obviously the target cell is not in the gland but is in the bone marrow. A certain amount of cell killing seems to be involved, and shielding of a portion of the bone marrow can reduce the induction rate. Some of these characteristics were part of the argument in support of the role of activation of an endogenous virus (Kaplan 1964). Age dependency must be added to all the variables in the induction of thymic lymphoma. Very early in life the susceptibility for induction is low but rises rapidly to a peak at about 150 days of age and then decreases to a very low level.

Studies by Sloan *et al.* (1990) detected recurrent *Ras* gene activation in radiation-induced thymic lymphomas and deletions in chromosome 4 and T-cell receptor (Tcr). Gene rearrangements have been described (Meijne *et al.* 2001).

In studies on the loss of heterozygosity, an important step in tumorigenesis, 12 tumor suppressor loci were mapped on eight chromosomes (Cleary *et al.* 2001). In acute myeloid leukemia, allelic loss on chromosome 2 predominates. Other types of lymphoma include B-cell lymphoma, which increases with age and exposure to radiation, and the reticulum cell type (often called reticulum cell sarcoma). The latter is like chronic lymphocytic leukemia (CLL) in humans in that it does not appear to be induced by radiation and decreases at higher doses.

Myeloid leukemia occurs naturally in a number of strains and is now classified as acute myeloid leukemia (AML). This type of leukemia is distinguished by an aberration on chromosome 2 in both the naturally occurring and radiation-induced disease and has been found in all of the strains that are susceptible to this type of leukemia (Hayata *et al.*

1983). The chromosomal sensitivity appears to be related to hot spots that result in a specific deletion that involves telomere-like repeat sequences in the chromosome breakage and rearrangement (Bouffler *et al.* 1996, 1997). There is a selection of the chromosome 2 aberrant clones, and the cells have a proliferative advantage. While these events are required for the development of AML, their occurrence does not ensure that overt leukemia will develop (Bouffler *et al.* 1997). Cleary *et al.* (2001) reported that there is also a preferential loss of the maternally transmitted allele at the TLSR 5 locus on chromosome 4 in CBA/A mice.

Considering the central role of the chromosome aberration in leukemogenesis, it is not surprising that the data for the dose-response relationship are consistent with a linear-quadratic model. However, Mole *et al.* (1983) fitted their data to a quadratic response with an added killing term, and Ullrich and Preston (1987) chose a linear model. A primary reason for the different model choices is that the delineation of the initial part of the curve requires an inordinate number of mice.

Upton (1961) published a dose-response curve for the incidence of myeloid leukemia in RF male mice exposed to 250 kVp X rays. The response was curvilinear, rising to a peak of about 35% at about 250 rads (about 2.5 Gy). The curve started to bend over at about 150 rads, becoming almost bell-shaped. The decrease in incidence at higher doses has been interpreted to be due to cell killing. However, the dose-response curve was for incidence uncorrected for competing risks; when the correction was made (Upton *et al.* 1958) the upward curve decreased in slope but did not become a negative slope. This is a good example of the necessity for appropriate analysis in the interpretation of dose-response curves.

Seki *et al.* (1991) fitted their data for myeloid leukemia in C3H/He mice exposed to 0.47 to 4.75 Gy to a linear-quadratic response with a peak incidence of 24% at a dose of 2.84 Gy. These results are consistent with those of Mole (1983) for CBA/H mice. Seki *et al.* (1991) also found that the incidence of myeloid leukemia was significantly increased (peak incidence of about 39%) when a single dose of prednisolone was injected after the exposure to radiation. This is one example of the modifying factors that can affect the production of myeloid leukemia. Yoshida *et al.* (1992) reported an enhancement of the radiation effect when they introduced cellulose acetate membrane into the peritoneal cavity to stimulate an inflammatory reaction. Walburg *et al.* (1968) showed that the susceptibility for radiation-induced myeloid leukemia was markedly reduced in germ-free mice. The microenvironment influences the level of cytokines and proliferation of the bone marrow cells, and this influences the probability of leukemia developing.

Mixed tumors

Di Majo *et al.* (1996), examined the influence of sex on tumor induction and life shortening in CBA/Cne mice exposed to doses of 1, 3, 5, or 7 Gy from a 250-kVp X-ray source. Mice were observed for their entire lifespan, and mean survival time decreased with increasing radiation exposure. In general, male mice were more susceptible than female mice. Both myeloid leukemia and malignant lymphoma were increased in males, while Harderian gland tumors were increased in a dose-dependent manner in both sexes. There was a significant positive dose-related trend (data corrected for differences in

longevity) for all tumor types evaluated except for lung and liver tumors in male mice (Table 4-1). However, incidences of total solid tumors were not significantly different from controls.

Table 4-1. Tumor occurrence in CBA/Cne mice following acute exposure to X rays

Sex and dose (Gy)	No. of mice	Number of neoplastic lesions[a]					
		Acute myeloid leukemia	Malignant lymphoma	Harderian gland	Lung	Liver	Ovary
Female							
0	50	0	7	5	3	8	6
1	50	0	15	7	0	11	11
3	49	1	12	9	3	9	8
5	61	0	11	18	5	23	4
7	49	0	8	12	1	8	3
Trend[b]		–	$P = 0.02$	$P < 0.001$	$P = 0.001$	$P < 0.001$	$P = 0.03$
Male							
0	60	0	0	10	7	40	nap
1	60	6	2	17	13	42	nap
3	55	8	2	24	7	33	nap
5	58	2	8	16	3	35	nap
7	56	1	1	21	6	22	nap
Trend[b]		$P = 0.05$[c]	$P = 0.03$	$P < 0.001$	ns	ns	nap

Source: Di Majo *et al.* 1996.

[a]*P* values provided only for trend analysis.

[b]The data were adjusted to account for decreased survival with increasing dose.

[c]In the dose range 0–5 Gy.

– Symbol was undefined.

nap = not applicable.

ns = not significant.

Upton *et al.* (1970) investigated the induction of tumors in RF/Un male and female mice after whole-body exposure to either acute doses of X rays ranging from 0.25 to 4.5 Gy or chronic ^{60}Co gamma rays ranging from about 1 Gy to about 58 Gy. The incidences of a broad spectrum of tumors were increased, even with the lowest X-ray dose, and included myeloid leukemia in males (discussed above) and thymic lymphoma and ovarian tumors in females. The low-dose-rate gamma irradiation, which was continuous for 23 hours per day, was less effective than the acute doses by a factor significantly greater than 2. Since X rays are considered to be more effective than gamma rays, at low doses the reduction in effectiveness caused by the reduction in dose rate may be even greater.

In a series of studies (Ullrich and Storer 1979a, 1979b, 1979c) the induction of cancer by single doses and protracted exposures to ^{137}Cs gamma rays in male and female RFM/Un

and female BALB/c mice was investigated. Results are summarized in Table 4-2 and Figure 4-1. A significant increase in thymic lymphoma in female RFM/Un mice was found at a dose of 0.25 Gy and above. The incidence of this type of tumor was increased in males but less than in females; the corrected peak incidence in females was about 44% at 2.0 Gy compared to about 26% in males at 3.0 Gy. In the case of myeloid leukemia, the corrected incidence at 3.0 Gy reached about 20% in males and about 5% in females. In contrast, female BALB/c mice were not susceptible to the induction of either thymic lymphoma or myeloid leukemia. Increased incidences of several solid tumors also were noted (Table 4-2). In RFM/Un male mice, the incidence of Harderian gland tumors increased as a function of dose. In females, increases in the incidence of pituitary, Harderian gland, and ovarian tumors were noted with a three-fold increase in ovarian tumors with a dose of 0.25 Gy.

Table 4-2. Age-adjusted tumor incidences in RFMf/Un mice following acute exposure to gamma radiation

Sex and dose (Gy)	No. of mice	Reticular tissue tumors (% ± SE)		Solid tumors (% ± SE)		
		Thymic lymphoma	Myeloid leukemia	Harderian gland	Pituitary	Ovarian
Female						
0	4,014	13.4 ± 0.60	0.77 ± 0.14	1.2 ± 0.38	6.6 ± 0.87	2.4 ± 0.55
0.1	2,827	14.2 ± 0.63	0.72 ± 0.15	1.3 ± 0.45	5.8 ± 1.0	2.0 ± 0.61
0.25	965	20.8 ±1.3	0.84 ± 0.30	1.6 ± 0.88	5.5 ± 1.5	6.4 ± 1.7
0.5	1,143	27.6 ± 1.2	1.1 ± 0.32	2.3 ± 1.0	9.1 ± 1.8	35.5 ± 2.8
1.0	1,100	30.3 ± 1.3	1.6 ± 0.41	6.6 ± 1.6	9.5 ± 1.9	35.1 ± 1.9
1.5	1,043	38.3 ± 1.2	3.6 ± 0.76	5.3 ± 1.7	9.4 ± 2.1	42.4 ± 3.0
2.0	333	44.4 ± 3.1	3.5 ± 0.78	15.4 ± 2.4	10.2 ± 4.1	43.9 ± 6.8
3.0	4,133	52.4 ± 1.3	5.2 ± 0.56	16.2 ± 1.6	20.9 ± 1.8	47.8 ± 1.9
Male						
0	430	6.6 ± 1.3	1.3 ± 0.59	1.2 ± 0.92	nr	nap
0.1	256	6.5 ± 1.7	0.8 ± 0.56	1.6 ± 0.96	nr	nap
0.25	94	9.6 ± 3.4	1.2 ± 0.92	2.1 ± 1.4	nr	nap
0.5	247	9.1 ± 2.8	4.5 ± 1.5	2.5 ± 1.2	nr	nap
1.0	230	15.9 ± 2.2	9.1 ± 2.2	3.0 ± 1.5	nr	nap
1.5	204	20.3 ± 3.6	10.2 ± 2.7	4.5 ± 1.7	nr	nap
3.0	571	25.9 ± 2.6	19.9 ± 2.4	14.8 ± 2.3	nr	nap

Source: Ullrich and Storer 1979a, 1979b.

Note: *P* values were not provided (see text for description).
nap = not applicable; nr = not reported.

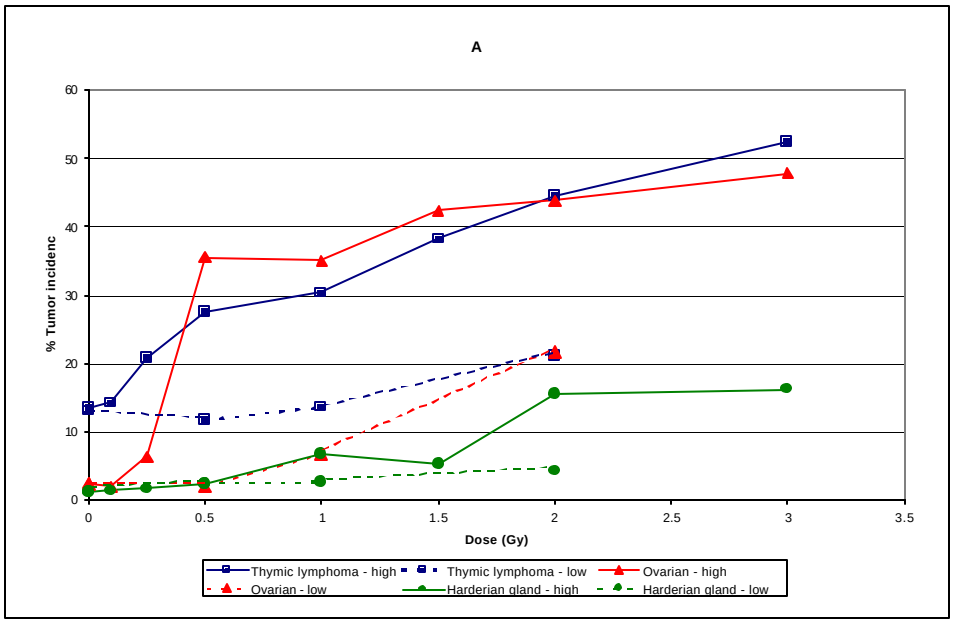
Ullrich and Storer (1979c) examined the effects of dose rate on the development of neoplasms in female RFMf/Un and BALB/c mice. BALB/c mice were included to provide more data on solid tumors that occur later in life than reticular tissue tumors. Mice were irradiated with ^{137}Cs gamma rays at dose rates of 40 or 45 cGy/min or 8.3 cGy/day. The high-dose-rate groups contained 749 to 865 mice each, and the low-dose-rate groups contained 1,239 to 1,531 mice each. The tumorigenic effectiveness was reduced at the lower dose rate for both reticular and solid tumors (Figure 4-1). These included thymic lymphoma, myeloid leukemia, ovarian tumors, pituitary tumors, and Harderian gland tumors in RFM mice and ovarian, mammary, and lung tumors in BALB/c mice. The dose-response relationships observed in various tissues at various dose rates could not be described by a single model and suggested that different mechanisms were involved.

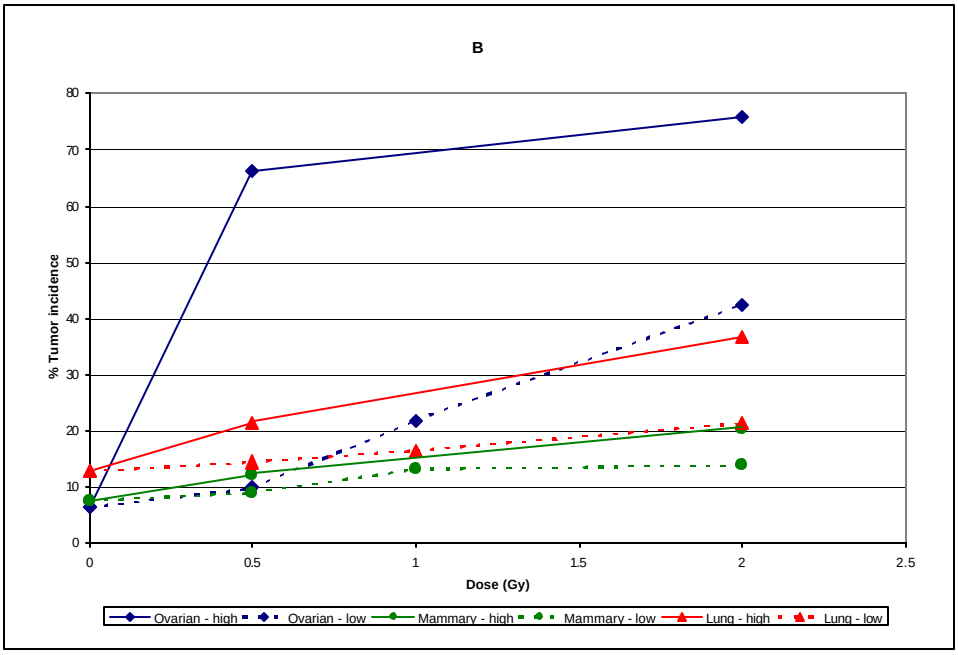
Grahn *et al.* (1992) reported an increase in the incidence of lymphoreticular tumors and tumors of the lung and Harderian gland in both sexes of B6CF$_1$ mice, and increases in ovarian tumors in female B6CF$_1$ mice exposed to ^{60}Co gamma rays. Both protraction and fractionation decreased the effect.

Solid tumors

In a series of experiments (Ullrich 1983, Ullrich *et al.* 1987), female BALB/c mice were exposed to graded single doses (0.1 to 2 Gy) and fractionation and low-dose-rate regimens of ^{137}Cs gamma rays. The data for mammary and pulmonary adenocarcinomas could be fitted with a linear-quadratic dose-response relationship. The curve for mammary carcinomas tended to flatten from about 0.5 Gy. Reduction of dose rate and fractionation reduced the tumorigenic effect. An important finding, as yet not understood, was that the full reduction of effectiveness in the induction of lung tumors occurred with fractions of below about 0.2 Gy; whereas, for mammary cancer, the dose per fraction had to be about 0.02 Gy or lower to obtain the full reduction in effectiveness.

Sasaki and Fukuda (1999) investigated the response of neonatal female B6C3F$_1$ mice exposed to 0.48 to 5.7 Gy of ^{137}Cs gamma rays. The dose-response curves for the liver, lung, pituitary, and ovary were convex upward with a rapid increase at doses below 1 Gy. The dose at which the peak incidence occurred was tissue dependent. When the data were analyzed taking into account competing risks and cell killing, dose response for these tissues was proportional to dose but for bone was proportional to the square of the dose. Sasaki (1992) also studied the influence of age at exposure on the risk of cancer in B6C3F$_1$ mice. Mice exposed at 17 days gestational age were not as sensitive as in infancy. After 20 days of age there was a steady decline in sensitivity. This is important for two reasons. First, any model of mechanisms of radiation carcinogenesis must take into account this age dependency and second, most of the studies of radiation carcinogenesis in mice have been carried out when the sensitivity was declining or had nearly reached a minimum.





Source: Ullrich and Storer 1979c .

Figure 4-1. (A) Incidences of thymic lymphoma, ovarian tumors, and Harderian gland tumors in female RFM mice following gamma irradiation at 45 cGy/min (high rate) or 8.3 cGy/day (low rate). (B) Incidences of ovarian, mammary, and lung tumors in female BALB/c mice following gamma irradiation at 45 cGy/min (high rate) or 8.3 cGy/day (low rate).

The natural incidence and susceptibility of tumors of the gastrointestinal tract for radiation induction have been reviewed (Boice and Fry 1995). Such tumors are rare in mice, less than 1.0 percent in five strains studied and 2.0 percent in C57BL/ mice. Nowell and Cole (1959) found it necessary to use high doses 250 kVp X rays to induce adenocarcinomas of the gut in C57L x LAF1 mice. They reported a 3% incidence after 800 roentgen (about 8 Gy) and 22% after 1,000 to 1,100 roentgen (about 10 to 11 Gy) based on a small number of animals. The contribution of gastric cancer to the total incidence was very small. The failure of Saxén (1952) to induce gastric carcinomas after exposure to local irradiation of 1,000 roentgen (about 10 Gy) substantiates the conclusion that the murine stomach is very resistant to the induction of cancer.

Hirose *et al.* (1977) exposed ICR and CF1 mice to local X irradiation at weekly intervals. In ICR mice, the incidence of rectal carcinomas rose from zero after a single dose of 20 Gy to 42% after two doses of 20 Gy and to 95% after three such doses. After a single dose of 30 Gy, the incidence was 31% but if split into 2 doses of 15 Gy the incidence was only 6%. There is a very high susceptibility for intestinal tumors in mice carrying a germline mutation in the *mApc* gene, which is responsible for the phenotype of the so-called Min mouse (Moser *et al.* 1990). Mice carrying this dominant mutation, which is the homologue of APC in humans, develop multiple adenomas, mainly in the small intestine. Exposure of Min mice to radiation increases the number of adenomas. The mutant forms of APC found in colorectal tumors cannot down regulate the trans-activation function of b-catenin, and thus, the expression of MYC and Cyclin D1, regulators of cell proliferation.

4.1.1.2 Prenatal exposure

Mixed tumors

Sasaki (1978a, 1978b) reported on two studies of tumor incidence in mice whose dams had been irradiated during pregnancy. In the first study (Sasaki *et al.* 1978a), pregnant C57BL/6 mice, which had been mated with WHT/Ht males, were exposed to 2 Gy of 180 kVp X rays on days 12 or 16-18 post coitum. Litter size was not affected by irradiation, but the number of stillborn pups and perinatal deaths (13.9% to 40.5%) was significantly increased in irradiated groups compared to controls (1.4%). Body weights and life spans also were significantly depressed in both irradiated groups. Mice irradiated during the late prenatal stage had significantly increased incidences of lung, pituitary, and ovarian tumors with slight increases in liver and skin tumors. Tumor incidences were not increased in mice in the middle prenatal-irradiated group, and in some cases, tumor incidences were less than in controls (Table 4-3). In the second study (Sasaki *et al.* 1978b), liver tumors increased in a dose-dependent manner in both the male and female progeny of $B6WF_1$ female mice irradiated with 1.5 or 3.0 Gy of 200 kVp X rays on day 17 post coitum.

Table 4-3. Tumor incidence in B6WF$_1$ mice following prenatal exposure to X rays

Treatment group	No. of mice	Tumor incidence (%)					
		Lympho-reticular	Lung	Liver	Pituitary	Ovary	Total
Female							
Controls	77	30	17	7	1	1	65
2 Gy, gd 12	53	6**	4	0	0	0	15**
2 Gy, gd 16-18	140	24	39**	10	9*	14*	77
Male							
Controls	55	16	24	7	0	nap	46
2 Gy, gd 12	44	2*	5*	0	0	nap	11**
2 Gy, gd 16-18	126	10	56**	17	1	nap	73**

Source: Sasaki *et al*. 1978a.
*$P < 0.05$, **$P < 0.01$ vs. controls
gd = gestation day; nap = not applicable.

Lumniczky *et al*. (1998) mated female C57Bl/6 mice with male DBA/2 mice and irradiated the F$_1$ hybrids (B6D2F$_1$) *in utero* on day 13 or 18 of gestation. Mice received single doses of 0.2, 0.5, 1.0, or 2.0 Gy gamma rays from a ^{60}Co source and were sacrificed at 2 years of age or when they became moribund. There was no difference in tumor latency among controls and irradiated mice. Tumors appeared within 18 to 24 months. Liver, lung, and lymphoid tumor incidences increased in mice irradiated on gestation day 18 (Table 4-4). In contrast, uterine tumor incidence decreased in irradiated mice. Statistical comparisons were reported only for total tumors, which showed a dose-related increased incidence. Data for mice irradiated on gestation day 13 were not provided, but tumor incidences were reportedly lower in these animals.

Table 4-4. Tumor incidence in B6D2F$_1$ hybrid mice following prenatal exposure to gamma radiation on day 18 of gestation

Dose (Gy)	No. of mice	Tumor incidence (%)					
		Liver[a]	Lung	Uterus[b]	Lymphoid	Other	Total
0	1,009	36 (3.6)	10 (1.0)	27 (2.7)	29 (2.9)	51 (5.1)	153 (15.2)
0.2	72	5 (6.9)	2 (2.8)	2 (2.8)	1 (1.4)	3 (4.2)	13 (18.1)
0.5	79	6 (7.6)	3 (3.8)	2 (2.5)	4 (5.1)	3 (3.8)	18 (22.7)*
1.0	145	9 (6.2)	4 (2.8)	3 (2.1)	17 (11.7)	7 (4.8)	40 (27.6)**
2.0	114	12 (10.5)	3 (2.6)	2 (1.8)	9 (7.9)	14 (12.3)	40 (35.1)***

Source: Lumniczky *et al*. 1998.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$
[a]Includes adenomas and adenocarcinomas.
[b]Includes fibromyomas and fibromyosarcomas.

Interaction with other carcinogens

The possibility that prenatal exposure to ionizing radiation predisposes the progeny to an enhanced risk if exposed postnatally to carcinogenic agents, mutagens, and promoting agents has been investigated (Nomura 1984, Nomura *et al.* 1990, Schmahl 1988).

Nomura (1984) irradiated ICR mice on days 0, 2, 4, 6, 8, 10, 12, 14 or 16 of gestation with 0.36 Gy of 180 kVp X rays. The offspring were administered urethane at 21 days of age, and after five months the number of lung tumors was counted. No increase in the incidence of tumors was found in mice that were exposed to radiation alone, but a significant increase was noted in the mice receiving the combination of prenatal irradiation on days 0 to 14 and postnatal urethane. For some unexplained reason, the mice that had been irradiated on day 6 of gestation did not show an enhanced risk. In a later study, Nomura *et al*. (1990) exposed fetuses of coat-colored mutants of the PT and HT strains to 0, 0.3 or 1.03 Gy of X rays on day 10 of gestation at a high dose rate (0.543 Gy/min) or a low dose rate (4.3 mGy/min). Mutations were assessed at six weeks of age and a group of the mice was treated with 12-O-tetradecanoylphorbol 13-acetate (TPA). At twelve months of age the mice were killed. A significant linear dose-dependent increase in mutations was found in mice that received radiation alone, but no increase in tumors was found. However, the incidences of liver and skin tumors were increased in mice that received postnatal treatment with TPA. Lowering the dose rate to 4.3 mGy per min reduced the tumor incidence by about a factor of five.

Schmahl (1988) reported a study of the interaction of prenatal irradiation and postnatal treatment with N-ethyl-N-nitrosourea (ENU), a potent mutagen. NMR1 fetuses were exposed to 0.2, 0.4, 0.8 or 1.6 Gy of 180 kVp X rays on day 15 of gestation and treated with ENU at 21 days of age. At 22 months there was no increase in the incidence of tumors in mice exposed to 0.2 or 0.8 Gy alone (the incidences in the other two groups were not determined). There were significant increases in the incidences of tumors of the liver, intestine, uterus, and ovary in mice exposed prenatally to 0.2 or 0.8 Gy and treated with ENU postnatally compared to mice that received ENU alone. However, prenatal exposure to 1.6 Gy combined with ENU exposure resulted in lower incidences of liver and ovarian tumors. These data are summarized in Table 4-5 and Figure 4-2.

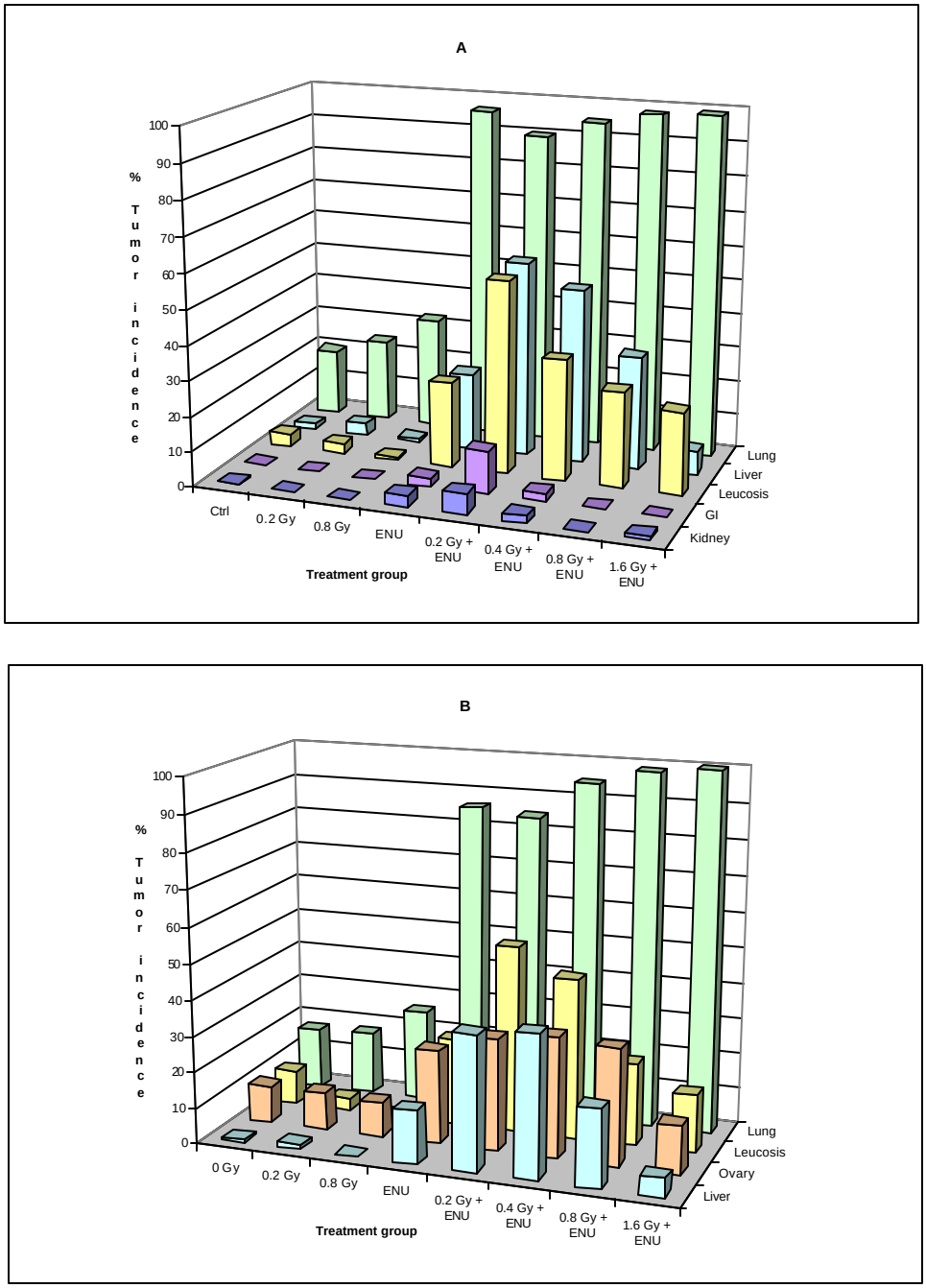
4.1.1.3 Parental exposure

Nomura (1982) exposed male and female ICR mice to 0.36 to 5.04 Gy of 180 kVp X rays. Mice then were mated with nonirradiated mice at various intervals after irradiation. The sensitivity of germ cells at different stages was determined by examining fetuses on day 18 of gestation and offspring of the irradiated parents. Significant dose-dependent increases in dominant lethal mutations and congenital abnormalities were noted when the exposure had been at the spermatozoa and spermatid stages. At eight months of age there was an increase in the total tumors, mainly due to an increase in lung tumors. In a follow-up study, Nomura (1983) demonstrated that F1 of parents that were treated with 2.16 Gy of X rays and mated to unexposed mice were highly susceptible to additional exposure to carcinogens. One group of F1 offspring was sacrificed at eight months of age without further treatment. A second group received a single s.c. injection of 5 µmoles/g b.w. urethane at 21 days of age and were sacrificed five months later. Offspring of non-irradiated parents were similarly treated. Large clusters of lung tumors developed in 18%

Table 4-5. Tumor incidence in NMRI mice following prenatal exposure to X rays alone or combined with postnatal exposure to ENU

Treatment group	No. of mice	Tumor incidence (%)					
		Leucosis[a]	Lung	Liver	GI	Kidney	Ovary
Females							
Controls							
Exp. 1	192	8.3	15.1	0.5	0	0	9.9
Exp. 2	152	11.2	19.7	0.6	0	0	11.2
ENU							
Exp. 1	92	18.5†	92.4***[b]	14.1***[b]	1.1	0	28.3***[b]
Exp. 2	115	28.7†	80.9***[b]	15.7***[b]	0.9	3.5	24.3***[b]
0.2 Gy	143	3.5**[b]	17.5	1.4	0	0	10.5
0.8 Gy	116	0**[b]	25.9	0	0	0	9.5
0.2 Gy + ENU	128	52.3***[c]	84.4	37.5***[d]	7.8†	2.3	31.3*[c]
0.4 Gy + ENU	116	44.8***[c]	94.8	39.6***[d]	7.8†	1.7*[c]	33.6*[c]
0.8 Gy + ENU	74	23	98.6	21.6	0	1.3	32.4
1.6 Gy + ENU	74	16.2	100	5.4***[c]	0	1.3	13.5**[c]
Males							
Controls							
Exp . 1	148	4.1	12.2	1.3	0	0.7	
Exp. 2	159	3.8	26.4	1.9	0	0	nap
ENU							
Exp. 1	129	22.5†	97.7***[b]	23.3***[b]	2.3	0.8	
Exp. 2	127	26.8†	94.5***[b]	21.3***[b]	2.4	6.3	nap
0.2 Gy	171	2.9	24.0	3.5	0	0	nap
0.8 Gy	139	0.7**[b]	32.4	0.7	0	0	nap
0.2 Gy + ENU	180	55.6**[e]	90.0	56.7**[d]	12.2**[e]	6.1	nap
0.4 Gy + ENU	152	34.9***[c]	94.7	50.6**[d]	5.3†	2.0*[c]	nap
0.8 Gy + ENU	98	27.6	98.0	32.6*[c]	0	0	nap
1.6 Gy + ENU	97	23.7	98.9	7.2***[c]	0	1.0	nap

Source: Schmahl 1988.
*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
† Significance level relative to the control or ENU groups were not stated.
[a]Primarily lymphatic leukemia with some myeloid leukemia and reticulosarcomas.
[b]Compared to the respective control group.
[c]Compared to the respective ENU group.
[d]Compared to the 0.8 Gy + ENU group.
[e]Compared to the 0.4 Gy + ENU group.
ENU = ethylnitrosourea; Exp = experiment; nap = not applicable.



Source: Schmahl 1988.

Figure 4-2. Tumor incidences in male (A) and female (B) NMRI mice following prenatal exposure to X rays alone or combined with postnatal exposure to ENU

of the urethane-treated offspring of irradiated parents (either male or female) compared to about 11% in offspring of irradiated parents without postnatal urethane and 2.8% in non-irradiated controls treated with urethane. The authors concluded that urethane treatment increased the penetrance of tumor mutations.

In subsequent studies (Nomura 1986, 1989), male N5 and LT mice were exposed to 5.04 Gy of X rays at the spermatogonial or postmeiotic stages, and the progeny were killed at twelve months. There were significant increases in the incidences of lymphocytic leukemia in both strains. Cattanach *et al.* (1995, 1998) repeated the work of Nomura (1982, 1983) with different mouse strains but did not get the same results. In the first experiment, male inbred BALB/cJ mice were exposed to single X-ray doses of 0, 2.5, or 5 Gy and were mated with nonirradiated females. Twenty-one replicate experiments were conducted over a one-year period. The BALB/cJ strain was selected because the spontaneous tumor incidence was similar to the strains used by Nomura (1982, 1983). Offspring were examined after 8 and 12 months for lung tumors. Radiation exposure reduced litter size, showing a dose-dependent dominant-lethal response. Total tumor incidences were 179/1,832 (9.8%) and 67/190 (35.3%) at 8 and 12 months, respectively. Although there was a significant difference in tumor incidences among the replicates, there were no statistically significant differences in the incidences of lung adenomas or total tumors between male and female offspring, or between the control and treatment groups. The majority of tumors were benign adenomas. Eleven and 13 adenocarcinomas were observed at 8 and 12 months, respectively. Cattanach *et al.* (1998) repeated this study using C3H/HeH mice. The same protocol was used with the exception that half of the offspring from each treatment group were given 5 µmoles/g b.w. urethane at three to four weeks of age. Offspring were examined at 6 or 12 months for lung tumors. Results were similar to the previous experiment. Litter size was reduced in the irradiated groups, but tumor incidence was not increased in any of the treatment groups compared to controls.

In a similar experiment, Daher *et al.* (1998) reported that offspring of male N5 mice exposed to 5.0 Gy of 160 kVp X rays had higher mortality from leukemia at one year of age than did controls.

Mohr *et al.* (1999) conducted a transgenerational study of the carcinogenic effects of X rays and urethane in CBA/J mice. The study included a control group, a urethane group (1 mg/g b.w.) by subcutaneous injection, and two X-ray-exposed groups (1 Gy or 2 Gy, testicular exposure). Male mice were treated and mated to virgin females at one, three, or nine weeks after treatment. Offspring from each treatment group received s.c. injections of urethane (0.1 mg/kg b.w.) or saline at 6 weeks of age. The numbers of litters and litter sizes were decreased in the X-ray treatment groups. Paternal males treated with urethane had statistically significant increased incidences of lung ($P < 0.001$) and liver tumors ($P \leq 0.05$). There were no statistically significant increased incidences of lung, liver, or hematopoietic tumors in offspring from the X-ray-exposed groups compared to controls. Nevertheless, higher incidences of hematopoietic system tumors and lung adenocarcinomas were observed in female and male offspring, respectively, in the 2 Gy X-ray group mated one week after exposure (Table 4-6). No increases were observed in offspring of males treated with X rays and mated three or nine weeks after treatment.

Table 4-6. Tumor incidence in CBA/J mice following paternal exposure to X rays or urethane

F1 treatment	Paternal treatment group[a]	No. of mice (M/F)	Tumor incidence					
			Males			Females		
			Lung	Liver	HP	Lung	Liver	HP
Saline	control	119/111	20 (17%)	64 (54%)	6 (5%)	21 (19%)	6 (5%)	13 (12%)
	urethane	78/63	19 (24%)	45 (58%)	5 (6%)	7 (11%)	2 (3%)	7 (11%)
	1 Gy	60/45	10 (17%)	41 (68%)	7 (12%)	2 (4%)*	0	5 (11%)
	2 Gy	76/69	20 (26%)	47 (62%)	3 (4%)	13 (19%)	5 (7%)	12 (17%)
Urethane	control	83/84	41 (49%)	55 (66%)	7 (8%)	28 (33%)	2 (2%)	12 (14%)
	urethane	105/83	53 (50%)	67 (64%)	6 (6%)	33 (40%)	10 (12%)*	8 (10%)
	1 Gy	88/62	44 (50%)	55 (63%)	12 (14%)	23 (37%)	3 (5%)	8 (13%)
	2 Gy	100/93	52 (52%)	68 (68%)	11 (11%)	28 (30%)	4 (4%)	22 (24%)

Source: Mohr *et al.* 1999.

*$P < 0.05$

HP = Hematopoietic system tumors included lymphomas and histiocytic sarcomas.

[a]One week prior to mating.

Male DBA-2 mice were irradiated with 0 or 3 Gy of ^{60}Co gamma rays 12 weeks before mating with C57BL/6 mice (Lord and Hoyes 1999). Eighty-three female offspring from the control group and 63 female offspring from the treated group were injected with 50 mg/kg b.w. methylnitrosourea (MNU) at 10 weeks of age. The incidence of myeloid leukemia showed a significant increase ($P < 0.01$) in the preconception paternal irradiation group. The incidence of lymphoid leukemia was unchanged. The authors concluded that preconception paternal irradiation increased the susceptibility of offspring to a subsequent exposure to a carcinogen. Although the mechanism is unknown, damage to the spermatagonial stem cells was likely an important factor.

Reaching a consensus on transgenerational effects of parental exposure is confounded by strain differences in susceptibility to specific types of tumors. However, most of the studies have demonstrated that paternal exposures appear to increase the susceptibility to other carcinogenic agents and promoters administered to the offspring. This effect is often expressed as a shortening of the latent period.

4.1.1.4 Genetically engineered mice

Much insight into the genetic targets and molecular mechanisms of radiation-induced carcinogenesis has been gained thorough the use of genetically engineered mice. This section presents a brief review of studies using *p53*-deficient or null mice, *Atm*-disrupted mice, or Eμ-*pim*-1 transgenic mice.

_p53_-deficient mice

Kemp *et al.* (1994) exposed 33 heterozygous *p53*-deficient (+/–), 28 wild type (+/+), and 27 *p53* null (–/–) adult mice (7 to 12 weeks old) to a single dose (4 Gy) of whole-body gamma radiation from a ^{60}Co source. Mice deficient in *p53* normally develop lymphomas

and sarcomas; therefore, untreated controls including 18 null and 14 heterozygous mice were used to determine the spontaneous rate of tumor development. Mice were observed daily and sacrificed and necropsied after they became moribund. Wild-type mice did not develop tumors after irradiation. Irradiated heterozygous mice developed tumors much earlier (median age of 40 weeks) than nonirradiated heterozygous controls (median age of 70 weeks). The spectra of tumors in irradiated and nonirradiated mice were similar; however, loss of heterozygosity (LOH) was more prevalent in tumors from irradiated mice compared to nonirradiated mice. Tumor latency was not significantly reduced in irradiated null mice. Therefore, additional experiments were conducted using younger mice.

Twelve two-day-old null mice received 1 Gy, and 14 null and 15 wild-type mice received 4 Gy at six days of age. One irradiated wild-type mouse developed a lymphoma at 40 weeks. The median tumor latency was reduced in irradiated newborn null mice from 21 weeks in controls to 14 weeks (4 Gy) or 15 weeks (1 Gy) (Kemp *et al.* 1994). These fragmentary data do not give a full account of the influence of *Trp 53* on radiation carcinogenesis.

Atm-disrupted mice

Gene targeting has been used to disrupt the *Atm* gene and in *Atm*-/- mice, rapidly growing thymic lymphomas appear by three months of age (Barlow *et al.* 1996). As yet it is not known whether the heterozygote, *Atm*-/+, is more susceptible to radiogenic cancers than the wild type.

Eμ-*pim*-1-transgenic mice

Heterozygous Eμ-*pim*-1 transgenic mice overexpress the *pim*-1 oncogene and have a low incidence of spontaneous T-cell lymphomas but are susceptible to genotoxic carcinogens. Van der Houven van Oordt *et al.* (1998) exposed Eμ-*pim*-1 transgenic mice and non-transgenic littermates to four weekly doses of whole body X irradiation at 0, 0.5, 1.0, or 1.5 Gy. Mice were four to seven weeks old at the beginning of the experiment and were monitored for up to 250 days after the last exposure. Radiation exposure resulted in a dose-related increase in lymphomas in transgenic but not wild type mice (Table 4-7). There was a higher incidence of lymphomas and reduced latency in treated transgenic mice compared to treated wild type or untreated transgenic mice at all doses tested.

Table 4-7. Incidences of lymphoma in transgenic and wild-type mice following X irradiation

Mouse type	Incidence[a]			
	Controls	4 ´ 0.5 Gy/wk	4 ´ 1.0 Gy/wk	4 ´ 1.5 Gy/wk
Eμ-pim-1	3/25 (12%)	17/61 (27.9%)	20/22 (91%)	26/26 (100%)
Wild type	0/24	0/62	6/31 (19.4%)	0/12[b]

Source: van der Houven van Oordt *et al*. 1998.

[a]Data for male and female mice are combined, *P* values were not provided.

[b]Only 12 of 31 mice survived the 250-day study period.

4.1.2 Rat

Rats have been used to study a selected number of tissues, such as the mammary gland, skin, and thyroid, but not in large-scale experiments to determine the general characteristics of radiation carcinogenesis. Studies have investigated the effects of whole-body irradiation and localized exposure.

The induction of mammary tumors has been studied in Sprague-Dawley rats, in particular, at Brookhaven National Laboratory (BNL) (Shellabarger *et al.* 1966, Shellabarger *et al.* 1980, Bond *et al.* 1960) and the Organization for Applied Scientific Research, Netherlands (Broerse *et al.* 1986, 1987). The latter group also studied other strains of rats. Both fibroadenomas and carcinomas occur naturally, and the incidence can be influenced by radiation. The incidence of fibroadenomas in the strain used at BNL reaches about 100%. Therefore, the effect of radiation must be an advancement in the time of appearance of this neoplasm. This may be true of a number of tumors but not in such a clear-cut manner. The analysis of the dose-response of such tumors must take this into account.

Female Sprague-Dawley rats exposed to single or fractionated doses of gamma rays or single doses of X rays developed increased incidences of mammary tumors (Table 4-8). The earlier study examined the effects of fractionation and age at exposure in rats exposed to a total whole-body dose of 0.5 Gy from a ^{60}Co source (Shellabarger *et al.* 1966). Groups of animals were exposed to a single dose of 0.5 Gy at 40 or 160 days of age. Other groups were exposed to a total dose of 0.5 Gy in 4, 8, 16, or 32 equal doses administered twice per week beginning at 40 days of age. The mean life span was reduced by 15% to 17% in rats given a single dose of 0.5 Gy; however, the life-shortening effect diminished with dose fractionation and protraction (Table 4-8). The cumulative risk of cancer in all irradiated groups was greater than in the control group at all time intervals; however, differences among the irradiated groups were not significant. Adenocarcinomas and adenofibroma-fibroadenomas were the predominant neoplasms and occurred in all groups. In the control group, adenocarcinomas did not occur in significant numbers until late in life, while adenofibroma-fibroadenomas occurred early and continued to occur throughout the experiment. In contrast, adenocarcinomas began to appear before adenofibroma-fibroadenomas in irradiated animals and were more abundant than in the control group. The incidences of adenocarcinoma also increased with dose fractionation (Table 4-8). The authors concluded that radiation exposure accelerated the appearance of these naturally occurring neoplasms; however, the normal sequence was disrupted in that adenocarcinomas appeared before adenofibroma-fibroadenomas.

Shellabarger *et al.* (1980) investigated the induction of mammary tumors in Sprague-Dawley rats following single doses of X rays or neutrons (see Section 4.2.1.1 for neutron radiation results). Animals were exposed to 0, 0.28, 0.56, or 0.85 Gy at 61 to 63 days of age and examined frequently. When breast tumors were discovered they were surgically removed, and the animal was returned to the experiment. Animals were only sacrificed when death appeared imminent. The experiment was terminated after 1,053 days. The mean life span decreased and the incidences of mammary tumors increased in all

irradiated groups (Table 4-8). Incidences of adenocarcinomas were less than in the earlier (Shellabarger *et al.* 1966) study but did show a slight increase in the irradiated groups. However, statistical analyses for adenocarcinomas were uncertain because of the low numbers of tumors. Nevertheless, radiation exposure reduced the latency period for both adenocarcinomas and fibroadenomas.

Table 4-8. Mammary tumor incidences in female rats exposed to X rays or gamma rays

Strain	Dose (Gy)	No. of rats	Life-span (days ± S.E.)	Incidence[a] (%)	Total tumors[b] (% AC)	Reference
Sprague-Dawley	0	75	793 ± 25	48 (64)	189 (19)	Shellabarger *et al.* 1966
	0.5 at 40 days	54	657 ± 21	50 (93)	394 (23)	
	0.125 × 4	54	636 ± 25	52 (96)	350 (37)	
	0.062 × 8	54	705 ± 21	48 (89)	415 (45)	
	0.031 × 16	54	705 ± 30	46 (85)	382 (56)	
	0.015 × 32	54	786 ± 12	47 (87)	420 (66)	
	0.5 at 160 days	53	676 ± 22	48 (91)	282 (44)	
Sprague-Dawley	0	167	748 ± 13	112 (67)	311 (10)	Shellabarger *et al.* 1980
	0.28	95	708 ± 18	68 (72)	193 (14)	
	0.56	48	729 ± 21	37 (77)	138 (12)	
	0.85	48	667 ± 26	38 (79)	165 (16)	

[a]Incidences in all irradiated groups reported as higher than in controls, but *P* values were not provided.
[b]Includes adenocarcinomas and adenofibroma-fibroadenomas.
AC = Adenocarcinomas.

Broerse *et al.* (1986, 1987) found that the dose-dependent increase in mammary tumors in rats exposed to 300 kVp of X rays was strain dependent. The order of sensitivity was Sprague-Dawley, WAG/Rij, and BNBi rats. The substrain of Sprague-Dawley that they used appeared less sensitive than the rats used at BNL. Bartstra *et al.* (1998) exposed female WAG/Rij rats to either 1 or 2 Gy of ^{137}Cs gamma rays at ages ranging from 8 to 64 weeks of age. There was a reduction in the risk in the 64-day-old rats.

Barstra *et al.* (1998) also investigated the effects of age on mammary tumors in female WAG/Rij rats exposed to ^{137}Cs gamma-rays. Groups of 40 animals were exposed to single doses of 1 or 2 Gy whole-body irradiation at 8, 12, 16, 22, 36, or 64 weeks of age and observed for life. Irradiation of young rats with doses up to 2 Gy resulted in a dose-related excess relative risk of mammary cancer. The normalized excess risk for carcinoma was 0.9 and 2.2 for the age groups 8 to 36 weeks exposed to 1 Gy and 2 Gy, respectively. There was no difference in tumor incidences in groups exposed at 8 to 36 weeks of age. The incidence of carcinoma in the group exposed at 64 weeks was less than in the control group.

Inano *et al.* (1999) reported that pregnant or lactating rats were more susceptible to radiation-induced mammary tumorigenesis than virgin rats. Consequently, these authors

investigated the role of prolactin in mammary adenocarcinoma. Ovariectomized Wistar-MS rats were used to control for ovarian hormones. Two pituitary glands, removed from mature rats, were transplanted underneath the kidney capsule of each of 23 rats at 2.5 months of age. The transplanted pituitaries increased serum prolactin levels in the test animals. Two weeks after the transplants, rats were exposed to 2.6 Gy of whole-body irradiation from a ^{60}Co source. Animals then were treated with diethylstilbestrol (DES) as a tumor promoter. The control group (42 animals) included ovariectomized rats treated in the same manner but without pituitary transplants (sham operation only). Five animals from each group were sacrificed after two weeks to determine hormone concentrations. Pituitary-transplanted rats had a significantly increased incidence of adenocarcinoma and fibroadenoma (77.8% compared to 21.6%). The authors concluded that hypersecretion of prolactin accelerated mammary gland tumorigenesis induced by radiation in the absence of ovarian hormones.

In another study, Inano *et al.* (2000) reported that 19 of 27 (70.3%) Wistar-MS rats irradiated with 1.5 Gy gamma rays on day 20 of pregnancy and implanted with DES developed mammary tumors. Rats similarly treated but fed a diet containing 1% curcumin (a plant extract with anti-inflammatory and anti-oxidant properties) from day 11 of pregnancy to parturition (day 23) had a significantly lower tumor incidence (18.5%).

Female Long-Evans rats were used to study the carcinogenic effects of localized X irradiation to the thyroid and pituitary glands (Lee *et al.* 1982). Groups of 300 rats received doses of 0, 0.94, 4.1, or 10.6 Gy delivered to the thyroid at 2.8 Gy/min and were maintained for two years. Two additional groups received 4.1 Gy to the pituitary or 4.1 Gy to both the pituitary and thyroid at 2.5 Gy/min. Animals that died within the first six months were not included in the results. Animals becoming moribund after the first six months were sacrificed and necropsied. The incidences of thyroid tumors increased with dose ($P < 0.001$), and irradiation of the pituitary did not affect tumor incidence (Table 4-9). Because tumor rates were approximately proportional to the square root of dose, the risk for total thyroid tumors per cGy X rays decreased with increasing dose and ranged from 2.6×10^{-4} at 10 Gy to 4.2×10^{-4} at 0.8 Gy.

Table 4-9. Thyroid tumor incidences in female Long-Evans rats exposed to X rays

Dose (Gy)	No. of rats	Tumor incidence[a] (%)		
		Carcinomas	Adenomas	Total tumors
0	281	2 (0.7)	7 (2.5)	9 (3.2)
0.94 (T)	275	4 (1.5)	7 (2.5)	11 (4.0)
4.1 (T)	282	15 (5.3)	20 (7.1)	35 (12.4)
10.6 (T)	267	21 (7.9)	55 (20.6)	76 (28.5)
4.1 (P)	267	0	3 (1.1)	3 (1.1)
4.1 (T + P)	269	14 (5.2)	17 (6.3)	31 (11.5)

Source: Lee *et al.* 1982.

[a] *P* values not provided.

T = Dose delivered to the thyroid gland.
P = Dose delivered to the pituitary gland.
T + P = Dose delivered to both the thyroid and pituitary gland.

Tinkey *et al.* (1998) studied the effects of localized radiation treatment on nerve engraftment. A single hind leg of male Sprague-Dawley rats was irradiated before or after nerve isograft surgery was performed on the right posterior tibial nerve. A ^{60}Co source was used to deliver radiation in 2-Gy fractions at a dose rate of 73 cGy/min. The first phase included a nonirradiated control group and two treatment groups that received a cumulative dose of 66 or 106 Gy beginning three days after surgery. In the second phase, four other treatment groups received cumulative doses of 46, 66, 86, or 106 Gy, and surgery was performed six weeks later. All groups were observed for eight months after completing treatment. Results were not reported separately from the two phases of the experiment. Combined tumor incidences were 0/7 (controls), 0/20, 2/27, 2/20, and 8/41 in the respective treatment groups. Tumor types included osteosarcoma, malignant fibrous histiocytoma, and fibrosarcoma and occurred within four to eight months following irradiation treatment.

4.1.3 Rabbit

Male and female adult Dutch rabbits were exposed to gamma rays (4.4 to 14.1 Gy) and fission neutrons (see Section 4.2.3) and maintained throughout their lifespan (six to nine years) (Hulse 1980). Six tumors were detected in four rabbits in the control group. Forty-two tumors were detected in 15 of 21 rabbits (71%) exposed to gamma rays. Twenty-six of these tumors occurred in the mid-dose group. Although a wide variety of tumors occurred in the irradiated groups, only basal-cell tumors of the skin ($P = 0.009$) and fibrosarcomas ($P = 0.04$) were significant when the number of animals with tumors in all irradiated groups were compared to the nonirradiated group. When the comparison was based on the total number of tumors per rabbit, osteosarcomas were statistically significant ($P = 0.016$). About half of the rabbits with tumors had more than one tumor. Data for the more prevalent tumors are shown in Table 4-10.

Table 4-10. Selected tumor incidences in rabbits exposed to gamma radiation

Dose (Gy)	No. of rabbits (M/F)	Number of rabbits with malignant tumors (%)				Number of rabbits with benign tumors (%)	
		OS	FS	Sertoli-cell	Uterine carcinoma	Skin basal-cell	Skin fibroma
Controls	14/3	0	0	0	2 (67)	0	0
4.4	4/0	0	0	2 (50)	–	2 (50)	1 (25)
8.8–10.6	4/4	1 (13)	4 (50)	1 (13)	1 (25)	4 (50)	1 (13)
11.5–14.1	7/2	3 (33)	1 (11)	0	1 (50)	1 (11)	0
Total[a]	15/6	4 (19)	5 (24)*	3 (14)	4 (67)	7 (33)**	2 (10)

Source: Hulse 1980.

*$P < 0.05$, **$P < 0.01$ compared to controls

[a]Total for all irradiated groups (statistical comparisons were only provided for the totals).

– No female rabbits in this group.

OS = osteosarcomas; FS = fibrosarcomas.

4.1.4 Dog

Beagle dogs were used as the test animal in a few radiation studies. These included continuous lifetime whole-body exposures to various doses of gamma rays or single whole-body exposures to gamma rays at various life stages, including prenatal exposure.

Gamma irradiation delivered in four fractions per week to the spinal cord for five weeks, lung for six weeks, or brain for seven weeks of male beagle dogs did not result in neoplasms (Bradley *et al.* 1981). Total doses ranged from 30 to 78.75 Gy.

Carnes and Fritz (1993) investigated mortality patterns in beagle dogs exposed 22 h/day, 7 days/week to whole-body gamma radiation from a ^{60}Co source. Doses were administered at 3, 7.5, 18.8, 37.5, 75, 127.5, 262.5, 375, or 540 mGy/day beginning at approximately one year of age and continuing until death. The risk of acute death and late-occurring death from causes other than cancer were determined by dose rate and accumulating dose. The risk of death from neoplastic disease rose rapidly with accumulating dose but was not significantly affected by dose rate.

Benjamin *et al.* (1991) investigated carcinogenesis in beagle dogs exposed to ^{60}Co gamma rays. The study included 10 treatment groups with 120 to 240 dogs per group and a sham-irradiated control group containing 360 dogs. Each group contained an equal number of males and females. Treatments were administered during prenatal development on gestation days 8, 28, or 55 or during postnatal development on days 2, 70, or 365. All the prenatal groups and the youngest postnatal group included both low-dose (0.16 to 0.18 Gy) and high-dose (0.81 to 0.88 Gy) groups. Groups treated at 70 or 365 days of age received mean doses of 0.83 and 0.81 Gy, respectively. There was a statistically significant increase in both fatal and total neoplasms in the perinatally-exposed groups (gestation day 55 and day 2 postpartum). The risk was greater in females

than in males. There were significantly increased risks of lymphoid cancers in dogs exposed on gestation day 55 and hemangiosarcomas in dogs exposed on gestation days 8 or 55. Other tumors reported included malignant lymphoma, leukemia, thyroid carcinoma, mammary carcinoma, and other nonspecified malignant tumors. However, the risk of mammary carcinoma was not significantly increased compared to controls.

Non-neoplastic and neoplastic thyroid disease occurring in these beagles was reported in a subsequent publication (Benjamin *et al.* 1997). Higher incidences of malignant and multiple neoplasms were observed in dogs with hypothyroidism; however, there was no evidence that this effect was related to radiation exposure. Irradiated dogs had a lower incidence of hypothyroidism, but the authors did not have an explanation for this finding. Dogs exposed at 70 days of age had an increased risk of thyroid follicular cell adenoma and carcinoma. When the analysis was limited to dogs with normal thyroid function, groups exposed at 2 days of age (high-dose only) and 70 days of age had an increased risk of thyroid follicular cell neoplasia. There were no differences in response between males and females.

4.1.5 Monkey

Three-year-old rhesus monkeys were exposed to supralethal whole-body X rays or neutron radiation prior to receiving an autologous bone marrow transplant (Broerse *et al.* 1981, Broerse *et al.* 1991, Broerse *et al.* 2000). Monkeys that survived more than three years after the bone marrow transplant were observed for tumor incidence and longevity. These included nine monkeys exposed to 2.3 to 4.4 Gy (mean of 3.4 Gy) neutron radiation (see Section 4.2.5), 20 monkeys exposed to 3 to 8.6 Gy (average 6.8 Gy) X rays, and 21 untreated controls. All animals entered the study between 1960 and 1973. By 1981, 8 of 12 X-irradiated monkeys, but none in the control group, had developed malignant tumors. Several animals in the irradiated groups also had benign tumors. The number of monkeys with a malignancy increased to 10 in the X-irradiated groups and 7 in the control group by 1995. In terms of the total observation period for the entire group (monkey-years), malignant tumor incidences were 10/257 and 7/482 for X rays and controls, respectively. Malignant tumors of the kidney, bone, vascular system, nervous system, thyroid gland, ileum, colon, and multiple myeloma were reported. The average latency period for tumor development was 12 years following X irradiation (range 7 to 16 years) (Broerse *et al.* 2000).

4.2 Neutrons

The available data for the determination of the risks to humans from exposure to neutron radiation or other high-LET radiations are inadequate (with the exception of alpha particles from exposure to radon and perhaps plutonium). Thus, experimental data have to be used to estimate the effect of high-LET radiations. To obtain the effect of high-LET radiations for radiation protection purposes, the effect per unit dose is determined for a low-LET reference radiation and is multiplied by the radiation weighting factor appropriate for that radiation quality (see Section 1.2.4.2).

In the IARC (2000) report, the data for the induction of tumors by low-energy neutrons, mainly fission-spectrum neutrons, and one report on 5.0-MeV and one on 7.5-MeV

neutrons were discussed. In addition to the IARC (2000) report, Neutron Carcinogenesis, a report of the Commission of European Communities (Broerse and Gerber 1982) provides comprehensive coverage of the neoplastic effects of neutrons.

4.2.1 Mouse

Mouse studies have shown that the dose-response curves for low-LET radiation and neutron radiation are different and that RBE values vary with total dose, dose rate, and sex. The carcinogenic effects of neutron radiation on mice are presented in this section. Although many of the sources of neutron radiation used in these experiments contained some gamma radiation, in most cases, the gamma component was assumed to be negligible.

4.2.1.1 Postnatal exposure

Most of the studies reviewed for neutron radiation reported on mixed tumors (lymphoma, leukemia, and solid tumors) or solid tumors only. One study reported only on incidences of myeloid leukemia in male mice. Findings from these studies are summarized below.

Lymphoma or leukemia

Ullrich and Preston (1987) investigated the relative effectiveness of acute doses of fission neutrons (0.005 to 0.8 Gy) and ^{137}Cs gamma rays in the induction of myeloid leukemia in RFM/Un male mice. Based on fitting the data for the responses to both radiation qualities to a linear model, the RBE was estimated to be 2.8. If the dose response for the gamma rays were in fact linear quadratic, the value of the RBE would have been higher.

Mixed tumors

Upton *et al.* (1970) exposed groups of 8- to 10-week-old RF/Un mice of both sexes to varying acute and chronic doses and dose rates of whole-body irradiation with 1-MeV or 5-MeV neutrons. The study included 301 females and 115 males in the control groups; 2,537 females in 28 treatment groups; and 538 males in 8 treatment groups. Dose rates ranged from 0.004 to 11.4 cGy/day, and total doses ranged from 0.016 to 9.3 Gy for females and 0.17 to 3.32 Gy for males. Animals were sacrificed after they became moribund or died a natural death. Neoplasms occurred in 47% to 64% of nonirradiated male and female mice, respectively. Radiation exposure had variable effects depending on dose rate, radiation type, duration, sex, and tumor type. Neutron irradiation was associated with increased incidences of myeloid leukemia and thymic lymphoma at intermediate doses; however, results for thymic lymphomas were somewhat inconsistent. Ovarian tumors were only increased at the lowest dose (0.02 Gy) and dose rate (0.004 cGy/day) tested. Relatively few ovarian tumors were induced at higher doses and dose rates. The incidences of nonthymic lymphomas and pulmonary adenomas generally decreased with increasing dose rate of neutron radiation. In contrast to the response observed in X- or gamma-irradiated mice (see Section 4.1.1.1), chronic exposure to low dose rates of neutrons was as effective or more effective than an equivalent acute exposure. Therefore, the RBE of neutrons was higher when given at low dose rates than when given at high dose rates.

Storer *et al.* (1979) investigated the induction of tumors in female RFM/Un mice exposed to high-dose-rate fission neutrons and californium-252 (^{252}Cf) neutrons at a low dose rate

in comparison to ^{137}Cs gamma rays. In general, neutrons were more effective than gamma rays, and there was less of a dose-rate effect. In the case of thymic lymphoma and ovarian tumors, effects were not completely independent of dose rate. The reason for this is not clear, but the results should not necessarily be considered as an exception to the usual finding of an effect of neutrons that is independent of the dose rate.

Extensive studies on the effects of neutrons have been carried out using the JANUS reactor. Neutrons produced by this reactor are fission spectrum with a mean energy of 0.85 MeV with a gamma-ray component of only 2.5%. Initial studies were designed to test the assumption of an additivity of the effects of neutrons (i.e., that the responses are independent of dose rate and fractionation). It was established early in the studies that at higher total doses, fractionated exposures were more effective than single doses (Ainsworth *et al.* 1975). Subsequent experiments, also using male and female B6CF1 (C57BL/6 x BALB/c) mice which were exposed at about 110 days of age, were designed to determine primarily the relative life-shortening effect of fission neutrons and ^{60}Co gamma rays under different regimens of exposure. Mice were exposed to single, 24 once-weekly, or 60 once-weekly doses of either gamma rays or neutrons and in a group of males five times per week for 59 weeks. RBE values derived from the data for the different regimens ranged from 6 to 43 (Carnes *et al.* 1989). At low doses, an excess of malignancies accounts for life shortening. Thus, life shortening is an integrated index of the impact of induced cancers. The large variation in RBEs reflects the influence of the type of exposure on the effects of low-LET radiations. Dose-dependent increases in the incidence of lymphoreticular, lung, liver, Harderian gland, and ovarian tumors were reported. Based on the histopathology of about 19,000 mice, the data for the induction of tumors were fitted by linear and linear-quadratic equations for two intervals, 600 to 799 days and 800 to 999 days. RBE values were derived from the ratios of the linear coefficients of the responses to gamma radiation and neutrons, and they increased as the doses were protracted, ranging from 2 to over 50 (Grahn *et al.* 1992). Epithelial tissues were more sensitive, and RBE values were higher than for other types of tissue.

Covelli *et al*. (1989, 1991) determined the dose-response curves for tumors in male $BC3F_1$ mice (C5BL/Cne × C3H/HeCne) induced by doses ranging from 0.17 to 2.14 Gy of 1.5-MeV fission neutrons with 12.5% gamma-ray contamination. A significant decrease in life span was detected at 0.36 Gy. The data for myeloid leukemia were fitted to a curvilinear model, and a significant increase was observed at 0.71 Gy and up to 1.79 Gy. The RBE, using acute 250-kVp X rays as the reference radiation, was about 4. Incidences of solid tumors were increased significantly at doses at 0.36 Gy and above. However, the incidence of malignant lymphoma was decreased at 1.43 Gy and above.

Seyama (1991) exposed seven- to eight-week-old B6CF1 mice to a dose of 0.27 Gy at 0.059 mGy/min or 2.7 Gy at 0.53 mGy/min of ^{252}Cf neutrons with a mean energy of 2.13 MeV and 35% gamma-ray contamination. There were significant increases in the incidences of tumors of the liver, mammary, pituitary, and Harderian glands, ovary in both dose-rate groups and reticulum cell sarcoma at the 2.7 Gy dose level. Surprisingly, an increase in lipoma was found at only the 0.27-Gy dose level. No increases in the incidences of tumors in other major organs examined nor in leukemia were found. These

findings indicate that there was no dose-rate effect with these neutrons. The authors noted that the neutrons were more effective than gamma rays but did not give any RBE values.

Di Majo *et al.* (1994) exposed three-month-old male BC3F1 mice to cumulative doses of 0.025, 0.05, 0.1, 0.17, 0.25, 0.36, 0.535 and 0.71 Gy at a 4 mGy/min. Incidences of liver tumors and lung tumors were increased significantly at a dose of 0.025 Gy and above and skin tumors at 0.36 Gy. The incidence of soft-tissue tumors was increased at 0.71 Gy, and myeloid leukemia showed a positive trend in the dose range of 0 to 0.36 Gy.

Three-month-old CBA/Cne mice of both sexes were whole-body irradiated with fission neutron doses of 0, 0.1, 0.2, 0.4, 0.8, 1.2, and 1.8 Gy (Di Majo *et al.* 1996). Animals were observed for their entire life span. Mean survival time decreased with increasing radiation exposure, dropping from an average of 852 days (controls) to 523 days (0.8 Gy) in males and 849 days (controls) to 528 days (1.8 Gy) in females. Male mice were more susceptible to tumorigenesis than female mice. The incidences of AML and malignant lymphoma were significantly increased in irradiated males and in irradiated males and females combined. However, AML did not occur in controls or in irradiated female mice. Although slightly more malignant lymphomas were observed in female mice, the incidence in female controls was about 10% compared to 4% in males. Incidences of Harderian gland tumors were significantly increased in treated mice of both sexes. Lung, liver, ovarian, and several other tumors were observed, but incidences were not significantly increased by radiation exposure; nevertheless, there was a significant dose-related trend (after adjusting for differences in longevity) for all tumor types examined (Table 4-11). Neutron RBE values compared to X irradiation ranged from 2.3 for AML to 20.2 for Harderian gland tumors in male mice.

Storer and Fry (1995) investigated whether the initial part of the dose-response relationship for life shortening and tumor induction was linear and if the slope could be determined using multiple small doses suitably spaced. Male and female BC3F1 mice were exposed to a total dose of 0.06 to 0.48 Gy of fission neutrons in fractionation regimens. The regimens were: 24 exposures weekly, 12 exposures every 2 weeks, 6 exposures every 4 weeks, and 3 exposures every 8 weeks. No change in age dependency was found over the period of the experiment. The dose-response relationships for life shortening and most radiogenic tumors were linear. The effect per unit dose was independent of the fractionation regimen. It was concluded that the initial slope of the dose-response curve for fission neutrons was linear and could be economically and accurately determined using a regimen of multiple small doses.

Table 4-11. Tumor occurrence in CBA/Cne mice following acute exposure to fission neutrons

Sex and dose (Gy)	No. of mice	Number of neoplastic lesions[a]					
		Acute myeloid leukemia	Malignant lymphoma	Harderian gland	Lung	Liver	Ovary
Male							
0	50	0	2	1	3	28	
0.1	79	3	8	7	8	52	
0.2	71	6	4	21	6	40	
0.4	56	4	8	12	2	36	
0.8	57	13	12	13	2	24	
1.2	65	3	12	6	2	32	
1.8	63	1	8	8	4	25	
Trend[b]		$P = 0.003$[c]	$P < 0.001$	$P = 0.005$	$P = 0.02$	$P < 0.001$	
Female							
0	58	0	6	7	4	14	12
0.1	70	0	14	12	3	24	7
0.2	68	0	6	20	3	20	17
0.4	59	0	9	12	3	19	9
0.8	57	0	10	19	5	17	5
1.2	69	0	7	21	3	14	13
1.8	70	0	12	19	3	11	11
Trend[b]		NAP	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Source: Di Majo *et al.* 1996.

[a]*P* values provided only for trend analysis.

[b]The data were adjusted to account for decreased survival with increasing dose.

[c]In the dose range of 0–1.2 Gy.

Solid tumors

Ullrich *et al.* (1977) reported that in female BALB/c AnNBd mice exposed to whole-body radiation with fission neutrons, the increase in incidences of pulmonary, mammary, and ovarian tumors occurred a doses as low as of 0.005 to 0.01 Gy delivered at a high-dose rate (0.25 Gy/min).

Ullrich *et al.* (1979) and Ullrich (1980) used the same strain and gender of mice as Storer *et al.* (1979). With localized thoracic irradiation they found a linear increase up to about 0.25 Gy in the number of lung tumors per mouse at nine months and an RBE that increased to about 40 with reduction in dose to about 0.01 Gy. The increase in RBE is because of the reduction in effect of the X rays, used as the reference radiation, at lower doses. Dose fractionation did not alter the effectiveness of the neutrons.

Female BALB/c mice exposed to neutron radiation developed increased incidences of lung, mammary, and ovarian tumors (Ullrich 1983, 1984). These studies were designed to examine responses in a low dose range (0 to 0.5 Gy). The first study included a control group (263 animals) and six treatment groups (0.025, 0.05, 0.1, 0.2, 0.5, and 2 Gy) with 140 to 182 animals each. Mice received whole-body exposures at dose rates of 5 to 25 cGy/min (Ullrich 1983). The second study examined the effects of a fractionated or protracted dosing protocol (Ullrich 1984). Doses of 0.025 to 0.5 Gy were delivered in two equal doses separated by 24 hours or 30 days. Other groups received doses of 0.025 to 0.4 Gy from a ^{252}Cf source delivered at 1 or 10 cGy/day for 20 h/day. The treatment groups contained 140 to 193 animals.

The data showed that fractionation and dose-rate effects varied according to tumor type (Ullrich 1984). Incidences of lung and mammary tumors were the same in groups exposed to a single dose or fractionated doses separated by 24 hours. When animals were dosed 30 days apart, incidences of lung and mammary tumors were similar to the first two groups at the lower doses but were higher at 0.5 Gy. Incidences of ovarian tumors were the same for the single-dose groups and both fractionated-dose groups. Lung and mammary tumor responses were slightly enhanced by continuous low-dose-rate neutron irradiation while the opposite was true for ovarian tumors. The dose-response relationships for both mammary and lung tumors appeared to bend over at between 0.1 and 0.2 Gy, resulting in convex upward dose-response curves. This illustrates the difficulty in delineating the slope of the initial curve, which is a prerequisite for the calculation of the RBE. The incidence of mammary tumors rose from 8.0% in controls to 25% at 0.5 Gy, and the percentage of lung tumors rose from about 15% to about 37% (Table 4-12).

Covelli *et al.* (1988) compared the life shortening and tumor induction in BC3F1 female mice exposed to 0.5 to 16 cGy of 1.5 MeV neutrons and to 4 to 256 cGy of X rays. Significant life shortening was found at doses of 8 cGy and above of neutrons. On the assumption of linear responses for both neutrons and X rays, the reference radiation, an RBE of 12.3 was calculated. An increase in the incidences of solid tumors also was found at doses of neutrons of 8 cGy and 1 Gy and above of X rays. The results were considered to be consistent with an advancement in time of the appearance of tumors.

Coggle (1988) exposed thoraxes of three-month-old male and female SAS/4 albino outbred mice to neutrons with a mean energy of 7.5 MeV and 3% gamma-ray contamination. The single doses were 0, 0.1, 0.25, 0.5, 0.75, 1.0, 2, 3 or 4 Gy, and at 12 months the incidence of lung tumors was determined. Data for benign and malignant tumors were pooled. The incidence in females increased from 9% in controls to a peak value of 34% at 2 Gy; at higher doses the incidence decreased. In males, the incidence increased from 16.5% in controls to 46.5% at 1 Gy (Table 4-13). RBE values using 250-kVp X rays as the reference radiation were about 5 for females and 7 in males.

Table 4-12. Tumor incidences in female BALB/c mice following single, fractionated, or continuous exposure to neutron radiation

Total dose (Gy)	Tumor incidence[a] (% ± SE)			
	Single dose	24-hr interval	30-day interval	Continuous[b]
Lung adenocarcinomas				
0	14.6 ± 2.4	14.6 ± 2.4	14.6 ± 2.4	15.8 ± 2.5
0.025	16.6 ± 3.7	15.4 ± 3.3	18.0 ± 3.8	18.7 ± 2.4
0.05	20.9 ± 4.3	18.3 ± 3.8	19.1 ± 3.4	21.7 ± 2.7
0.1	18.0 ± 4.0	23.8 ± 4.7	21.7 ± 3.2	28.0 ± 2.7
0.2	30.5 ± 6.1	28.3 ± 4.6	29.8 ± 4.7	32.9 ± 2.7
0.4	na	na	na	42.3 ± 4.3
0.5	37.4 ± 6.9	39.9 ± 7.4	50.2 ± 7.9	na
2.0	26.8 ± 6.1	na	na	na
Mammary adenocarcinomas				
0	7.9 ± 1.7	7.9 ± 1.7	7.9 ± 1.7	8.5 ± 2.3
0.025	10.5 ± 2.9	12.6 ± 3.4	12.2 ± 3.0	19.2 ± 2.5
0.05	16.8 ± 3.8	14.0 ± 3.1	15.1 ± 3.5	23.6 ± 2.9
0.1	17.9 ± 4.2	18.5 ± 4.1	20.0 ± 4.4	25.9 ± 2.2
0.2	20.0 ± 4.7	20.3 ± 4.5	22.6 ± 4.8	27.4 ± 2.6
0.4	na	na	na	29.2 ± 4.0
0.5	25.4 ± 5.5	26.4 ± 4.9	35.6 ± 6.9	na
2.0	8.4 ± 3.2	na	na	na
Ovarian tumors				
0	2.4 ± 1.0	2.4 ± 1.0	2.4 ± 1.0	4.9 ± 1.8
0.025	2.5 ± 1.4	2.5 ± 1.3	2.1 ± 1.5	3.3 ± 1.2
0.05	6.5 ± 2.1	5.5 ± 1.5	4.2 ± 1.4	3.8 ± 1.1
0.1	9.5 ± 2.6	8.0 ± 1.5	6.0 ± 1.5	6.9 ± 1.8
0.2	16.4 ± 3.7	17.5 ± 2.3	16.2 ± 1.6	8.5 ± 2.1
0.4	na	na	na	27.2 ± 3.6
0.5	76.2 ± 3.0	77.0 ± 5.2	72.0 ± 4.9	na
2.0	56.3 ± 3.8	na	na	na

Source: Ullrich 1983, 1984.

[a]*P* values not provided.
[b]Data were pooled for the two dose rates (1 and 10 cGy/day).
na = not available.

Table 4-13. Primary lung tumor incidences in SAS/4 mice following acute exposure to neutron radiation

Dose (Gy)	Males			Females		
	No. of mice	No. of mice with tumors	Incidence[a] (% ± SE)	No. of mice	No. of mice with tumors	Incidence[a] (% ± SE)
0	291	48	16.5 ± 2.2	210	19	9.0 ± 2.0
0.1	60	17	28.3 ± 5.8	57	10	17.5 ± 5.0
0.25	52	17	32.7 ± 6.5	54	13	24.1 ± 5.8
0.5	58	16	27.6 ± 5.9	55	14	25.5 ± 5.9
0.75	55	16	29.1 ± 6.1	61	17	27.9 ± 5.7
1.0	71	33	46.5 ± 5.9	59	18	30.5 ± 6.0
2.0	64	27	42.2 ± 6.2	59	20	33.9 ± 6.2
3.0	69	31	44.9 ± 6.0	61	18	29.5 ± 5.8
4.0	45	9	20.0 ± 6.0	58	9	15.5 ± 4.8

Source: Coggle 1988.
[a]*P* values not provided

Ito *et al.* (1992) examined the strain and gender dependence of induction of tumors. Six-week old mice were exposed to 0.125, 0.5 or 2 Gy of the same type of neutrons described by Seyama *et al.* (1991) above, but at 6 to 8 mGy/min. The order of susceptibility, as assessed by the increase in total tumor incidence, was C3H/HeN, B6CF1 and C3B6F1 and C57Bl/6N. There was a dose-dependent increase of the incidence in liver tumors of all strains with a greater increase in males.

Male and female B6C3F$_1$ mice were exposed to whole-body irradiation from a ^{252}Cf (2.13 MeV) source at doses ranging from 0 to 2 Gy and observed for 13 months (Takahashi *et al.* 1992). Thirteen months was selected because this mouse strain has a high spontaneous incidence of liver tumors after 14 months of age. Survival was not adversely affected by radiation exposure. The incidence of liver tumors, number of liver tumors, and tumor size increased in a dose-dependent fashion in both sexes (Table 4-14). Liver tumors occurred in 3.8% and 3.2% of male and female controls, respectively, and rose to 62.1% (males) and 27.6% (females) in mice exposed to 2 Gy. There also was a dose-dependent increase in total tumor incidence. Overall, the relative RBE, compared to ^{60}Co gamma-radiation, was 15.2 in males and 2.5 in females.

Table 4-14. Survival and tumor incidences in B6C3F$_1$ mice following acute exposure to neutron radiation

Dose (Gy)	Males				Females			
	No. of mice	Survival rate (%)	Tumor incidence (%)		No. of mice	Survival rate (%)	Tumor incidence (%)	
			Liver	Total			Liver	Total
0	53	96	3.8	5.7	63	95	3.2	12.7
0.03	24	100	12.5*	20.8	0	nap	nap	nap
0.06	24	100	20.8**	25.0	0	nap	nap	nap
0.125	30	94	36.7**	43.3	29	91	3.4	13.8
0.5	30	94	43.3**	46.7	30	94	6.7	23.3
2.0	29	91	62.1**	79.3	29	91	27.6**	69.0

Source: Takahashi *et al*. 1992.

*$P < 0.05$, **$P < 0.01$ (P values not provided for total tumors).

nap = not applicable.

4.2.1.2 Prenatal exposure

Di Majo *et al*. (1990) and Covelli *et al*. (1991) investigated the effect of exposing pregnant BC3F$_1$ mice on the 17th day of gestation to 0.09, 0.27, 0.45, or 0.62 Gy of fission neutrons with a mean energy of 0.4 MeV and 12% gamma-ray contamination. Progeny were observed for their lifetime. Trend analysis over the dose ranges of 0 to 0.27 Gy, 0 to 0.45 Gy, and 0 to 0.62 Gy were all highly significant ($P < 0.001$). The incidence of liver tumors increased from 11% in the controls to 52% in the 0.45-Gy dose group and decreased to 18% after the 0.62-Gy dose (Table 4-15). An RBE of 28 was estimated at the neutron dose of 0.09 Gy compared to 250-kVp X rays. The authors concluded that these data indicate that the fetal liver is highly susceptible to neoplastic transformation by neutron irradiation.

Table 4-15. Tumor incidences in BC3F$_1$ mice following prenatal exposure to neutron radiation

Total dose (Gy)	No. of mice	Hepatocellular adenoma	Hepatocellular carcinoma	Total[a] (%)	Age-adjusted incidence[a] (%)
0	230	24	2	26 (11)	11
0.09	49	15	0	15 (31)	25
0.27	42	9	3	12 (29)	35
0.45	25	10	3	13 (52)	81
0.62	33	5	1	6 (18)	21

Source: Di Majo *et al*. 1990.

[a]P values not provided.

4.2.1.3 Parental exposure

Several studies have shown a relationship between parental exposure to fission neutrons and liver tumorigenesis in offspring (Takahashi *et al.* 1992, Watanabe *et al.* 1996, Shoji *et al.* 1998). Each of these studies used a similar protocol. Male C3H mice received whole-body irradiation at seven weeks of age from a ^{252}Cf source. The ^{252}Cf source was reported to consist of 67% fission neutrons and 33% gamma rays. At two weeks or three months after irradiation, irradiated male mice were mated to nonirradiated female C57BL mice. Control mice had nonirradiated fathers. Male mice mated two weeks after irradiation had a significantly increased incidence of abnormal sperm that led to an increased incidence of embryo lethality. This was not the case when male mice were mated three months after irradiation (Watanabe *et al.* 1996). A statistically significant increased incidence of liver tumors was observed in male but not female offsprings when the male parent was exposed to radiation two weeks prior to mating. When males were mated three months after radiation treatment, a statistically nonsignificant increase in the incidence of liver tumors was seen in male offsprings of fathers exposed to 0.5 or 1 Gy. Results are summarized in Table 4-16.

Table 4-16. Paternal radiation exposure to fission neutrons and liver tumorigenesis in offspring

Dose (Gy)[a]	Mating time[b]	Male offspring			Female offspring			Reference
		No. of mice	Incidence (%)	Tumors per mouse	No. of mice	Incidence (%)	Tumors per mouse	
0	2 wk	31	3.2	0.03	30	3.3	0.03	Takahashi *et al.* 1992
0.5		44	43.2**	0.91	58	1.7	0.02	
2.0		0[c]	nap	nap	0[c]	nap	nap	
0	2 wk	31	3.2	1.08	30	3.3	nr	Watanabe *et al.* 1996
0.5		44	43.2**	6.49*	58	1.7	nr	
1.0		39	15.4	2.28*	35	0	0	
2.0		0[c]	nap	nap	0[c]	nap	nap	
0	3 mo	33	9.1	1.23	nr	nr	nr	Watanabe *et al.* 1996
0.5		20	30	3.16*	18	5.6	nr	
1.0		22	22.7	5.93*	24	0	0	
2.0		19	5.3	1.13	14	0	0	
0	2 wk	42	11.9	0.17	43	0	0	Shoji *et al.* 1998
0.125		50	24[d]	0.3	70	0	0	

*$P < 0.05$, **$P < 0.01$.
[a]Values represent the reported total dose. The pure neutron dose is 67% of the value listed.
[b]Time after irradiation.
[c]All offspring died within two weeks after birth.
[d]The *P* value was not reported.
nap = not applicable; nr = not reported.

4.2.2 Rat

Many of the studies on the effect of neutrons had companion studies of the effect of some low-LET radiation, which were described in Section 4.1.2.

Vogel (1969) reported the earliest study of the effect of fission neutrons on mammary tumors in rats. In a subsequent study, Vogel and Zaldivar (1972) exposed two- to three-month-old Sprague-Dawley rats to single doses of 0.05, 0.1 to 0.12, 0.18 to 0.22, 0.35, 1.5 or 2.5 Gy of fission neutrons with 10% to 15% gamma-ray contamination. The animals were followed for their life span. The incidence of tumors rose from 48% in the controls to 78% at 0.05 Gy and varied between 73% and 87% at the higher doses. Tumors were predominantly benign, and the RBE based on comparison with the effect of 250-kVp X rays was 20 to 60. There was little difference in the induction of tumors between whole-body and partial-body irradiation.

Vogel (1978) investigated the influence of splitting the neutron dose on the incidence of mammary tumors determined twelve months after exposure to single doses of 0.1, 0.2, and 0.7 Gy or the same total doses split into two and delivered with a 24-hour interval. No significant difference in the incidence of tumors was found.

Vogel and Turner (1982) determined the induction of mammary tumors in five strains of rats: Long-Evans/Simonsen, Sprague-Dawley/Harlan, Buffalo/Simonsen, Fischer 344/Simonsen, and Wistar-Lewis/Simonsen. The incidences of tumors were determined at one year post irradiation in controls and in rats exposed to 0.5 Gy of fission neutrons. The incidences in the irradiated rats of the five strains were 56%, 56%, 29%, 26%, and 5.5% respectively. These results were interpreted as an indication of the role of a genetic component in the susceptibility for radiation-induced mammary cancer.

Vogel and Dickson (1982) reported on the comparative effectiveness of protracted exposures and single doses of fission neutrons and the influence of exposure pattern on RBE using gamma rays as the reference radiation. Protraction of exposures to gamma rays reduced the effectiveness of mammary tumor induction in Sprague-Dawley rats and increased the RBEs, which were 58 to 82 for protracted exposures and 8 to 16 for the acute doses. In contrast, protraction of the neutron exposures tended to increase the effectiveness.

Shellabarger (1976) and Shellabarger *et al.* (1978, 1980, 1982, 1983) conducted a number of studies on the induction of mammary tumors in Sprague-Dawley rats by low-energy neutrons. In the comparison of the effect of 0.43-MeV neutrons and X rays (Shellabarger *et al.* 1980), the following conclusions were made: 1) single doses as low as 1 mGy of neutrons increased total mammary tumor rate significantly, 2) the dose-response relationship for neutrons was consistent with a slope of 0.5 on a log-log plot and of 1.0 for X rays. However, it is possible that the initial slope for the neutron response is initially linear and bends over at very low doses, which has been noted in other experiments, 3) the results suggested that the RBE at the lowest doses may be as high as 100, and 4) the relationship of RBE to dose suggested that RBE is inversely proportional to the square root of the neutron dose, a relationship proposed by Rossi and Kellerer (1972). This suggestion of a dose exponent of 0.5 was interpreted to indicate that more

than one cell was involved in tumorigenesis in the rat mammary gland. As noted in the section on low-LET radiations, any interpretation of the increase in tumor rates in Sprague-Dawley rats must be cognizant of the fact that radiation appears to advance the time of appearance of tumors, in particular of benign fibroadenomas, rather than induce them.

The incidences of mammary tumors in WAG/Rij, Bn/BiRij and Sprague-Dawley rats were determined by Broerse *et al.* (1986, 1987) after exposure to 0.5, 4, or 15 MeV neutrons, respectively. The frequency of specific types of mammary tumors varied among the different strains. Based on the comparative effect of 300-kVp X rays, the RBE for 0.5-MeV neutrons was 15 for adenocarcinomas and 13 for fibroadenomas in WAG/Rij rats and 7 for fibroadenomas in Sprague-Dawley rats. In an earlier publication (Broerse and Gerber 1982), it was noted that the effectiveness of 15-MeV neutrons was between that of 0.5-MeV neutrons and X rays.

Montour *et al*. (1977) studied the induction of mammary tumors in Sprague-Dawley rats exposed to 14.5-MeV neutrons. Neutron doses ranged from 0.025 to 0.4 Gy, and RBEs were calculated from the data for mammary tumor incidences at 11 months after irradiation using gamma rays as the reference radiation. The RBE increased from 5 at 0.4 Gy to 13.8 at 0.025 Gy.

Chmelevsky *et al.* (1984) reported the incidence of lung tumors in Sprague-Dawley rats of both sexes exposed to 0.012, 0.02, 0.06, 0.1, 0.3, or 0.5 Gy over a one-day period; 1.5 or 2.3 Gy over a 14-day period; 3.0 Gy over a 23-day period; and 5.3 or 8 Gy over a 42-day period. The radiation was 1.6-MeV neutrons with a high contamination of gamma rays, a ratio of neutrons to gamma rays of 3:1. There was a dose-dependent increase in lung tumors up to a dose of 2.3 Gy.

Lafuma *et al.* (1989) investigated the relative effectiveness of exposure to alpha particles from radon, 2.1-MeV neutrons (25% gamma-ray contamination), and gamma rays. At doses of 10 to 20 mGy, the RBE for neutron induction of lung tumors in Sprague-Dawley rats was about 50; the RBE was 30 to 40 at 0.1 Gy. Wolf *et al.* (2000) reanalyzed the data and, based on a retrospective classification of the lung tumors into lethal and nonlethal, calculated an RBE of 50 using different models. The choice of model did not affect the estimated RBE value.

Spiethoff *et al.* (1992) irradiated the liver of female Wistar rats locally with 0.2 Gy of neutrons at 14-day intervals for two years, for a total dose of 10 Gy. Eighty-three of the 114 rats irradiated developed liver tumors, which varied in the cell of origin.

A number of reports on the influence of modifying factors, apart from time-dose regimens, have been made. Yokoro *et al.* (1980, 1987) demonstrated that grafts of prolactin-secreting tumor cells made 25 days after exposure of Wistar/Furth rats to 0.048, 0.089, or 0.195 Gy of neutrons, with a mean energy of 2.0 MeV, promoted the cells initiated by neutrons to develop into overt mammary tumors. Promotion could be demonstrated even when the grafts were made 12 months after irradiation.

4.2.3 Rabbit

Hulse (1980) exposed a small number of 7- to 18-month-old male and female Dutch rabbits to 1.8 to 5.5 Gy of 2.5-MeV neutrons with about 12% gamma-ray contamination. There was an increase in the incidence of subcutaneous fibrosarcomas in the 1.8- to 5.5-Gy dose groups, and some osteosarcomas and basal cell carcinomas were found among the various groups. In the irradiated groups, 36 of 38 rabbits (95%) had a total of 124 tumors with 86 tumors detected in the low-dose group. Based on gamma rays as the reference radiation (see Section 4.1.3), an RBE of about 2.5 was estimated for osteosarcomas and 3.0 to 3.5 for pooled data for other tumors.

4.2.4 Dog

Bradley *et al.* (1981) examined the effects of partial-body irradiation. The brain, lung, and spinal cord were exposed separately to 15-MeV neutrons or gamma rays. The brain received four fractions per week for seven weeks, the lung for six weeks, and the spinal cord for five weeks. Seven dogs out of 46 irradiated developed nine tumors in 1 to 4.5 years. The lowest total dose to induce a cancer, a hemangiosarcoma of the heart, was 10 Gy. An osteosarcoma and a subcutaneous myxofibrosarcoma of the spinal cord occurred after 26.25 Gy. Other tumors occurred in the brain (oligodendroglioma and glioblastoma), hemithorax (subcutaneous osteosarcoma), lung adenocarcinoma, and neurofibroma (cervical nerve). The data were too sparse for meaningful estimates of RBEs.

4.2.5 Monkey

Broerse *et al.* (1981, 1991, 2000) exposed nine rhesus monkeys to supralethal whole-body radiation. Doses were 2.3, 3.5, 3.8, 4.1, or 4.4 Gy of 1 MeV neutrons. This resulted in a very small number of animals per dose group; there were 21 control monkeys. After irradiation, autologous bone marrow cells were injected intravenously to obviate acute radiation effects. Monkeys that survived more than three years after the bone marrow transplant were observed for tumor incidence and longevity. All animals entered the study between 1960 and 1973. By 1981, six of nine neutron-irradiated monkeys and none in the control group had developed malignant tumors. Several animals in the irradiated groups also had benign tumors. By 1995, eight animals in the neutron-exposed groups and seven animals in the control group had developed malignant tumors. In terms of the total observation period for the entire group (monkey-years), malignant tumor incidences were 8/101 and 7/482 for neutron-exposed and controls respectively (Broerse *et al.* 2000). Malignant tumors of the bone (including a synovial sarcoma of the humerus), kidney, brain, vascular system, liver, colon, and thyroid were observed. The average latency period for tumor development was 11.9 years following neutron irradiation (range 4 to 21 years). An RBE value of 3.6 was calculated based on results from gamma-irradiated monkeys (see Section 4.1.5) (Broerse *et al.* 2000).

4.3 Summary

The carcinogenic effects of all types of ionizing radiation are well established in experimental animals. This section reviewed the effects of X rays, gamma radiation, and neutrons given as whole-body or localized exposures in single, fractionated, or continuous doses at different life stages to various species and strains of experimental animals. The general findings are summarized below.

4.3.1 X radiation and gamma radiation

X rays and gamma rays are clearly carcinogenic in all the species tested (Table 4-17), although tissues differ in their susceptibility to both radiation qualities. The degree of susceptibility for the induction of benign and malignant tumors is species-, strain-, age- and gender-dependent. While genetic factors play a major role in the probability of initiation, host factors, which also are determined by the genetic background, are paramount in the expression of the essential initial events and the progression to an overt tumor. The effects of radiation on the initial events and the mutations to which they lead have been studied in much more detail than the effects on host factors. The incidences of certain leukemias and many solid tumors increase in a dose-dependent manner. Fractionation and lowering the dose rate reduce carcinogenic and other effects of low-LET radiations. Even in experiments involving a large number of animals, it often is difficult to delineate the dose-response with precision. However, in many cases the data can be fitted using the linear-quadratic model. The finding of a reduction in effect with a lowering of the dose rate is consistent with either elimination or diminution of the quadratic component of the dose-response curve.

Exposures in the early prenatal stages do not appear to increase cancer rates, but exposures in the later stages may do so. The question of whether parental irradiation increases the susceptibility for radiogenic cancer is controversial, and conflicting results have been obtained in different experiments.

Table 4-17. Tumor sites in experimental animals following exposure to X or gamma radiation

Test animal	Radiation type	Dose range (Gy)	Tumor sites
Mouse	X ray	0.2-30	Lymphoma Leukemia GI tract Harderian gland Lung Liver Ovary
	Gamma ray	0.1-58	Lymphoma Leukemia Harderian gland Pituitary Liver Lung Mammary Ovary
Rat	X ray	0.28-10.6	Mammary Thyroid
	Gamma ray	0.5-2.6 46-106	Mammary Bone[a]
Rabbit	Gamma ray	4.4-14.1	Skin Bone
Dog	Gamma ray	0.003-0.83	Lymphoma Leukemia Vascular system Thyroid gland
Monkey	X ray	3-8.6	Bone Colon Ileum Kidney Multiple myeloma Nervous system Thyroid gland Vascular system

[a]Localized high-dose exposure.

4.3.2 Neutrons

Low-energy neutrons, such as fission neutrons, are significantly more carcinogenic than low-LET radiations, such as X or gamma rays. The dependence on neutron energy for carcinogenesis has not been adequately defined, but it is clear that at increasing energies, above about 2 to 4 MeV, the effectiveness declines. How much greater the relative effectiveness of low-energy neutrons is at very low doses compared to low-LET radiation is poorly understood. RBEs are tissue dependent and very dependent on the response to the reference radiation. The maximum RBE is what is required in the adjustment of doses of radiations of different qualities to equivalent doses, and this entails determination of the initial slopes of the relevant dose responses. The best approach for determining the slopes of the dose-response curves is to use low-dose-rate exposures or multiple fractions that are very small in dose.

There are some differences in the effects among radiations of different quality, but none of the differences have been reason to reject the assumption made in risk estimation for

radiation protection purposes, namely, that the effects of radiations of different LET differ quantitatively but not qualitatively. Unfortunately, the term inverse dose rate has been introduced to describe an apparent enhancement of effect when doses of high-LET radiations are protracted or fractionated. A rigorous examination of whether dose rate is the reason for an increased effect with lower dose rates, especially in the case of protraction, is required. Time-dose relationships are complex and require more meticulous investigation than they have had.

There is, as yet, no evidence of a signature alteration that might distinguish tumors induced by high-LET radiations from those induced by low-LET radiations. Tumors induced in experimental animals following exposure to neutron radiation are summarized in Table 4-18.

Table 4-18. Tumor sites in experimental animals following exposure to neutron radiation

Test animal	Dose range (Gy)	Tumor sites
Mouse	0.001-9.3	Lymphoma Leukemia Bone Epithelial tissues Harderian gland Liver Lung Mammary Ovary Pituitary Skin Soft tissues Vascular system
Rat	0.001-2.5	Lung Liver Mammary
Rabbit	1.8-5.5	Bone Skin
Dog	10-26.25	Bone Brain Heart Hemithorax Lung Spinal cord
Monkey	2.3-4.4	Bone Brain Colon Kidney Liver Thyroid gland Vascular system

5 Genetic and Related Effects

There is a considerable literature on the genetic effects of ionizing radiations of a broad range of qualities. These effects can be subdivided into somatic cell and germ cell effects. In risk-assessment terms, somatic cell effects are primarily used for cancer risk assessment and germ cell effects for genetic (or heritable) risk assessments. Both classes will be discussed in this section. Extensive reviews have been published on both somatic and genetic effects of low-LET (X and gamma rays) and high-LET (including neutrons) radiation (Sankaranarayanan 1991a, 1991b, IARC 2000, UNSCEAR 2000, 2001).

Since there have been recent, comprehensive reviews of the mutagenic effects of ionizing radiation, this section will concentrate on studies of exposed human populations, experimental studies with laboratory animals, and studies of human and other mammalian cells in culture. A very large amount of data have been published on prokaryotes and lower eukaryotes that provide support for the conclusions developed here; however, since a very large literature also exists for human and rodent data, this review focuses on these publications.

The major characteristics of X rays, gamma rays, and neutrons have been described previously (see Section 1). For the purposes of the present discussion, X rays and gamma rays are examples of low-LET radiations, and neutrons are an example of a high-LET radiation. This distinction becomes of particular significance when discussing mechanisms of induction of genetic effects. Given this difference in radiation quality, and the separate nominations, this section will consider X rays and gamma rays separately from neutrons, which are discussed in Section 5.2.

5.1 X radiation and gamma radiation

The IARC Working Group (2000), in its review of the genetic and related effects of X rays and gamma rays, concluded that ionizing radiation, including X rays and gamma rays, can induce gene mutations across a wide variety of cellular systems. Chromosomal aberrations have been found in all eukaryotic systems examined, with the predominant mutations identified being deletions that result in gene inactivation. In addition, persistent genomic instability may be induced, including chromosomal aberrations, gene mutations, and reduced plating efficiency, that are detected many cell generations after exposure in a variety of systems. While most of the effects of ionizing radiation on induction of chromosomal aberrations have been established through *in vitro* and *in vivo* assays, a great deal has been learned from large-scale human exposures to radiation after atomic bombings and accidental exposures that occurred at Chernobyl, Ukraine, and Goiânia, Brazil. Studies of these exposed populations published since the IARC review and some papers reviewed by IARC are discussed below.

5.1.1 Human studies

The major applications for ionizing radiation-induced genetic alterations are for biological dosimetry in exposed humans and as supporting data in cancer and genetic risk assessments. Thus, the thrust is towards obtaining human data. Hence this review will

first discuss the available human data and then data for laboratory animals and cellular systems.

5.1.1.1 Atomic bomb survivors

As discussed in Section 3, the cancer risk assessment process relies very heavily on tumor data obtained from the atomic bomb survivor group. Similarly, genetic risk assessments have used a combination of data from the offspring from A-bomb survivors and mouse data (UNSCEAR 2001). Extensive data also have been collected on the frequencies of chromosomal alterations and mutations in peripheral lymphocytes and on a number of effects in offspring of exposed parents for identifying possible inherited effects.

In brief, the frequencies of both unstable chromosome alterations (dicentrics, rings, and deletions) and reciprocal translocations assessed by conventional Giemsa staining and chromosome fluorescence *in situ* hybridization (FISH) showed an increase with estimated dose (Awa 1997, Nakano *et al.* 2001). These data have in turn been used to support some of the dose estimates. For Giemsa-stained preparations, the proportion of cells containing at least one reciprocal translocation or inversion was highly non-linear with dose. The shape of the dose response was concave upwards for doses below 1.5 Sv, with some leveling off at higher doses (Kodama *et al.* 2001). Results with FISH are quite similar to those obtained with Giemsa-staining, except that the latter method detected only about 70% of the value for the genome-equivalent translocation frequency obtained using FISH (Nakano *et al.* 2001).

Somatic cell mutations have been assessed at the glycophorin A (GPA) locus (Kyoizumi *et al.* 1996) and the hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus (Hirai *et al.* 1995) in A-bomb survivors. For GPA, the frequency of hemizygous mutant erythrocytes was measured by flow cytometry in 1,226 heterozygous A-bomb survivors in Hiroshima and Nagasaki. The mutant frequency was higher in males than in females and higher in Hiroshima than Nagasaki survivors. An analysis of the dose-response relationship for GPA frequency using a descriptive model showed that the doubling dose obtained was similar to that for solid cancer incidence in A-bomb survivors. This has led to the suggestion that radiation-induced somatic mutations are the major cause of excess cancer risk after radiation exposure (Mendelsohn 1996).

The mutation frequency at the *HPRT* locus in peripheral blood T-lymphocytes was measured in 171 exposed A-bomb survivors and 83 control survivors (Hirai *et al.* 1995). There was an increase in *HPRT* mutant frequency in exposed individuals, but the dose-response curve was quite shallow and far less steep than the curve for chromosome aberrations, suggesting that the *HPRT* assay is not sufficiently sensitive to be used for biodosimetry. The weak response may have been due to the time lapse of 50 years since the time of exposure. There was no correlation between mutant frequency and chromosomal aberrations.

In parallel to the assessment of genetic effects in somatic cells in A-bomb survivors, considerable effort has been expended to determine if there are any heritable effects of radiation exposure to the A-bombs in Hiroshima and Nagasaki. The general conclusion is

that no increase above background has been observed using a variety of endpoints and a range of techniques (Neel 1998, 1991, UNSCEAR 1993). Adverse effects considered included: untoward pregnancy outcomes (congenital malformations, stillbirths, and neonatal deaths); deaths among children before reproductive age (excluding malignant tumors); cancer before the age of 20; increases in certain classes of chromosomal alterations (balanced rearrangements and sex chromosome abnormalities); increased frequencies of mutations affecting protein characteristics; altered sex ratios and impaired physical development of children. The average combined dose to the gonads of the parents was 0.4 Sv. A recent reconsideration of the heritable risk from radiation exposure by UNSCEAR (2001), using human data as the basis for the estimates, provides evidence that the nonsignificant increase in heritable effects in A-bomb survivors is predicted from the doubling dose.

A more recently developed technique for assessing human germ cell alterations measures changes in a set of minisatellite loci in exposed and control groups. Ideally, the approach is to measure minisatellite length in parents and offspring and search for differences. Including offspring born before and after an exposure would add strength to any conclusion concerning mutations resulting from exposure. This technique was applied to A-bomb survivors and their offspring (Kodaira *et al.* 1995). These investigators screened 64 children from 50 exposed families and 60 children from 50 control families for mutations at six minisatellite loci. The average parental gonadal dose in exposed families was l.9 Sv. The mutation frequency per locus was 1.5% in the exposed parents and 2.0% in the unexposed parents. There was no significant difference in the mutation rates in the children of exposed and unexposed parents ($P = 0.37$ using a Fisher's exact probability test). This observation is not in agreement with some data from persons exposed as a consequence of the Chernobyl accident. These data are discussed in Section 5.1.1.2 below.

5.1.1.2 Chernobyl accident

A considerable volume of data has been collected from individuals exposed as a result of the Chernobyl accident. A comprehensive review of the exposure scenarios and potential health outcomes has been provided by UNSCEAR (2000). The accident led to acute irradiation exposures, both external and internal, from ^{131}I (half-life of 8 days) and then chronic exposures from more stable isotopes, mainly ^{137}Cs. A significant difficulty with the interpretation of these studies is a lack of a clear assessment of exposure for individuals and groups. This has led to some discrepancy among the reports.

Peripheral blood samples taken between 1986 and 1992 from 102 workers on-site at the time of the accident or who served as clean-up workers shortly after the accident were analyzed for GPA mutants using flow cytometry. The frequency of hemizygous deletion (N/Ø) variant red cells increased in proportion to the estimated exposure for each individual (Jensen *et al.* 1995). The dose-response curve was similar quantitatively to that obtained for the A-bomb survivors in a previous study (Langlois *et al.* 1993). Jones *et al.* (2002) conducted a similar study using blood samples collected between 1992 and 1999 from 625 Russian Chernobyl clean-up workers and 182 Russian controls. They performed the GPA assay for both deletion (N/Ø) and recombination (N/N) events

detected by flow cytometry in lymphocytes. Some 30 exposure and lifestyle covariates were available and incorporated into the analysis. No increase in variant frequency was observed for either endpoint in the GPA assay for the exposed group compared to the control. Of interest was the finding that in the same group, an increase in chromosomal translocations (assessed by FISH) and an increase in *HPRT* mutant frequency were observed. The average dose estimated from the translocation data was 9.5 cGy. These data indicate that the GPA assay is insensitive for biodosimetry.

Analysis of chromosome aberrations in persons exposed as a result of the Chernobyl accident has been conducted in a number of studies. Padovani *et al*. (1993, 1997) conducted cytogenetic studies on groups of children exposed as a consequence of the Chernobyl accident as a more acute exposure and as a longer term chronic exposure. The first study (Padovani *et al.* 1993) was a cytogenetic analysis using conventional staining on children from the Belarussian, Ukrainian, and Russian Republics. These children had varying amounts of ^{137}Cs contamination based on whole-body counts. Chromosome aberrations were observed at a low frequency; since the types analyzed are the unstable ones, their frequency is reduced as a function of time after exposure. It was not possible to determine the dose-response relationship.

It is apparent from biodosimetric studies that low-LET radiation induces chromosome damage that persists in peripheral lymphocytes for many years after exposure. Littlefield *et al*. (1998) conducted an extensive cytogenetic monitoring study to define the magnitude of exposures as a result of the Chernobyl accident. Cytogenetic analysis using FISH was conducted on 118 Estonian clean-up workers, selected from a defined cohort of 4,833 cleanup workers. The mean estimated doses were 10.3 cGy with a maximum of 25 cGy. A group of 29 Estonian population controls and a group of 21 American controls also were analyzed. There was no correlation between aberration frequency and recorded measurements of physical dose or any category of high-dose, or high-dose rate exposure related to cleanup function. Using simulated exposure, the investigators determined that the mean dose estimated from physical parameters would have led to an increase in mean translocation frequency of more than 40% to 65% compared to nonirradiated controls. They concluded that the recorded doses for this group of cleanup workers overestimated their average bone marrow dose, perhaps substantially. Thus, studies on possible adverse health outcomes in groups such as this have to be very carefully evaluated as regards to actual exposure.

Published studies of the possible effects of exposure as a result of the Chernobyl accident on mutation induction in germ cells and recovery in offspring are quite discordant and remain difficult to interpret. Studies by Dubrova *et al*. (1996, 1997) reported an increase in minisatellite mutations in children born in heavily polluted areas of the Mogilev district of Belarus after the Chernobyl accident compared to a control group. The problem with interpretation is that the control group was from the United Kingdom and could not be accurately used to account for possible confounders of response (i.e., genetics, lifestyle, and environment). In a subsequent study, Dubrova *et al.* (2002) assessed germline mutation frequency at eight minisatellite loci in families from rural areas of Kiev and Zhitomir regions of Ukraine that were heavily contaminated as a result of the Chernobyl accident. The control group was matched to the exposed group for a number

of possible confounders of response (e.g., ethnicity, maternal age, parental occupation, and smoking habits). The authors reported a 1.6-fold increase in mutation rate in the germline of exposed fathers but not in exposed mothers. These data suggest a germline induction of mutations by radiation exposure.

In contrast, Livshits *et al*. (2001) did not show an increase in inherited mutation alleles at seven hypermutable minisatellite loci in 183 children born to Chernobyl cleanup workers (liquidators) compared to the frequency in 163 children born to control families living in a non-irradiated area of Ukraine. If the subjects were divided into subgroups based on time of conception, the mutation frequency was higher, although not statistically so, in children conceived within 2 months of paternal exposure. This potential difference in sensitivity during spermatogenesis is predicted based on rodent studies and might be a part of the explanation for differences in minisatellite mutation induction between post-Chernobyl individuals and A-bomb survivors; the latter were conceived over a much longer post-exposure period.

Weinberg *et al*. (2001) reported a much higher (7-fold) increase in new bands using multi-site DNA fingerprinting in individuals born to liquidator (cleanup workers) families. Controls were unexposed families and the siblings in the test group who were conceived before parental exposure. A strong criticism by Jeffreys and Dubrova (2001) describes the fact that the method used by Weinberg *et al*. (2001) is unreliable, "the mutants were not validated and had no obvious molecular basis." They may have arisen as PCR artifacts through non-paternity or sample mix-up. Thus, this study is considered to be very equivocal, pending further assessment.

In short, a small increase in paternal germline mutations is possible, but further study and validation is needed. In particular, dose-response relationships are needed for which the dose estimates are accurate.

5.1.1.3 Other radiation accidents

The development and use of cytogenetic and gene mutation assays for conducting biological dosimetry in cases of accidental radiation exposures has covered some 40 years. A comprehensive review of many of these accidents can be found in Bender *et al*. (1988). From this review it is apparent that for acute exposures to X rays or gamma rays, when samples are taken a few days to weeks after exposure, the frequency of unstable chromosome aberrations is a good predictor of dose. It is less well documented how effective the analysis of stable aberrations using FISH is at predicting dose. Initial reports suggest that this method can be used with reasonable predictive power when known confounders of outcome are taken into account. The analysis of stable aberrations can be more predictive of dose at long time intervals after exposure than that of unstable aberrations.

The relatively recent radiation accident at Goiânia (Brazil) serves to illustrate the use of different methods to predict dose. In this case a ^{137}Cs radiotherapy source (51×10^{12} Bq) was abandoned at a hospital and recovered by a scrap metal dealer in Goiânia (IARC 2000). The source was destroyed, thereby releasing the radioactive material. The highest individual initial dose rate was 0.25 Gy per hour. The mostly highly exposed group

received doses of 4 to 7 Sv. Four people died within six weeks of exposure. Two hundred forty-nine persons showed detectable contamination, with 129 of those having internal contamination. To estimate the absorbed radiation dose, the frequencies of unstable chromosomal aberrations were measured in 110 exposed persons. The frequencies of dicentrics were consistent with the estimated doses (Natarajan *et al.* 1991a). The frequencies of dicentrics declined as predicted after one year (Natarajan *et al.* 1991b). Translocations were assessed by FISH eight years after the accident, and it was reported that their frequencies were about one-third to one-half the initial dicentric frequencies, with the biggest differences being at the higher doses (> 1 Gy) (Natarajan *et al.* 1998). These studies suggest that the FISH assay for reciprocal translocations in peripheral lymphocytes can only provide an approximate estimate of dose at longer times after exposure. Clearly there is a loss of translocation-carrying lymphocytes over time. A small but significant increase in *HPRT* mutants was observed in 11 subjects who had received doses of 1 to 7 Gy, between 2.3 and 4.5 years prior to analysis (da Cruz *et al.* 1996). In a subsequent experiment, the HPRT mutant frequency was about 10-fold higher among 17 exposed individuals compared to nine unexposed controls (Skandalis *et al.* 1997).

The analysis of genetic alterations in individuals exposed to low-LET radiation as a result of accidents shows that radiation induces somatic genetic alterations in humans and that the frequencies of some of these classes of genetic alterations can be used to estimate dose received.

5.1.1.4 Occupational and environmental exposures

There have been many studies of the genetic effects of occupational or environmental exposures to low-LET radiation. The significance of these for the present discussion is that the exposures are chronic. Examples are presented here to demonstrate how the chromosome aberration frequencies observed in peripheral lymphocytes can generally be predicted from the linear component of a linear quadratic dose-response curve that is obtained for acute exposures for the same endpoint.

Evans *et al*. (1979) reported on chromosome aberration frequencies in 197 nuclear dockyard workers who were followed over a 10-year period. These workers were exposed to mixed neutron-gamma radiation during the refueling of nuclear reactors, with most exposures being below the internationally accepted maximum permissible exposure level of 50 mSv/year (5 rem/year). Aberration frequency was linear as a function of dose and was influenced by age and time of blood sampling after exposure. Susceptibility to chromosome damage increased with age. The aberration frequency for this chronic occupational exposure for dicentrics (incidence per cell per Gy) was $2.32 \pm 1.01 \times 10^{-6}$. The aberration frequency for acentric elements, dicentrics, and rings combined was $4.38 \pm 1.4 \times 10^{-6}$. These results were similar to the mean value of the alpha coefficient of $2.5 \pm 1.1 \times 10^{-6}$ in the linear terms of the dose-response curve that was derived from four other large studies summarized in Bender *et al*. (1988).

Lloyd *et al*. (1980) studied aberration frequencies in peripheral lymphocytes of 146 radiation workers from United Kingdom nuclear establishments. The authors employed a half-life of three years to weigh individual increments of dose, and they obtained a linear

dose-response curve for dicentrics, with a coefficient of $2.22 \pm 0.94 \times 10^{-6}$ per Gy, which was similar to that observed by Evans *et al*. (1979).

A number of investigators have observed chromosome aberration rates to be elevated in persons residing in areas of high natural background radiation (e.g., Wang *et al.* 1990), and a number of additional studies have been conducted on populations exposed to high background radiation (Pohl-Ruling and Fischer (1983). The major problem with these studies, with the exception perhaps of the one conducted on individuals from "the Radon Spa" in Bad-Gastein, Austria, is that it is very difficult to estimate dose when exposure occurs from both external and internal sources. In general, dose-response curves for chromosome aberrations were linear. At low dose levels and low dose rates the dose-response curve for total chromosome aberrations was linear, up to annual doses of 3 mGy X rays, with a plateau for additional dose increments (Pohl-Ruling *et al.* 1983).

5.1.1.5 Summary of human studies

Genetic damage has been detected in various human populations (e.g., atomic bomb survivors, radiation accidents, radiation workers, and people living in areas with high background radiation) exposed to ionizing radiation levels exceeding normal background. Chromosomal aberrations and mutations are commonly detected in peripheral lymphocytes collected from these individuals; however, there is little evidence for heritable effects. The data reviewed in this section are summarized in Table 5-1.

Table 5-1. Genetic effects in human populations exposed to ionizing radiations

Population	Endpoint	Results[a]	References
Atomic bomb suvivors	Chromosomal aberrations	+	Awa 1997 Nakano *et al*. 2001
	Somatic mutations (GPA, HPRT locus)	+	Kyoizumi *et al*. 1996 Hirai *et al*. 1995
	Germ cell mutations	–	Neel 1991, 1998 UNSCEAR 1993 Kodaira *et al*. 1995
Chernobyl accident	Chromosomal aberrations	+	Padovani *et al*. 1993, 1997 Jones *et al*. 2002
		–	Littlefield *et al*. 1998
	Somatic mutations (GPA, HPRT locus)	+	Jensen *et al*. 1995 Jones *et al*. 2002
		–[b]	Jones *et al*. 2002
	Germ cell mutations (minisatellite loci)	+	Dubrova *et al*. 1996, 1997, 2002
		–	Livshits *et al*. 2001
		±	Weinberg *et al*. 2001
Goiânia, Brazil	Chromosomal aberrations	+	Natarajan *et al*. 1991a, 1991b, 1998
	Somatic mutations (HPRT)	+	da Cruz *et al*. 1996 Skandalis *et al*. 1997
Nuclear workers or high background radiation	Chromosomal aberrations	+	Evans *et al*. 1979 Lloyd *et al*. 1980 Pohl-Ruling *et al*. 1983 Bender *et al*. 1988

[a] + = positive, – = negative, ± = equivocal

[b]GPA locus

5.1.2 Human cells

5.1.2.1 Gene mutations

The evidence for radiation-induced germline mutations in humans remains inconclusive. Studies investigating the heritable effects following the Hiroshima and Nagasaki, Japan, bombings and the Chernobyl nuclear accidents reported contradictory results (see Section 5.1.1). Part of the difficulty resides in the inability to determine an accurate dose-response relationship *in vivo*. Dubrova *et al*. (1997) reported the existence of germline mutations in a Belarussian population. However, the results were controversial. The vast majority of data on the subject of germline mutations as a direct result of radiation exposure were conducted *in vitro*. Human spermatozoa are thought to be more susceptible to mutagens such as ionizing radiation than oocytes and somatic cells because spermatozoa are devoid of cytoplasm (Kamiguchi and Tateno 2002). Consequently, the majority of inherited chromosome structural changes are paternal in origin. The potential implications of ionizing radiation on germ cells are multifactorial. Kamiguchi and Tateno

(2002) have emphasized the following points: (1) there is a difference in susceptibility to mutations between somatic and germ cells which should not be ignored, (2) mature sperm lack a cytoplasm and consequently have an impaired DNA repair ability and a higher contribution of inheritable effect to the subsequent generation than the oocyte, and (3) sperm have been shown to retain their fertilizing ability following a high dose of gamma-irradiation of 4.23 Gy.

Sankaranarayanan (1991b) reviewed the effects of radiation on mutation induction. In human T lymphocytes, up to 80% to 97% of the spontaneous *HPRT* mutations resulted in a normal Southern blot pattern while the rest are the result of gross intragenic deletions. The complete deletion of the *HPRT* gene is rarely observed except in the TK6 cell line. The percentage of mutations with normal Southern patterns was in the 50% to 60% range at the HLA-A locus. Further, mitotic recombination constitutes a substantial portion of the mutation spectrum at the HLA-A locus. The vast majority of the radiation-induced mutations showed Southern patterns indicating deletions and possible gross changes. Spontaneous mutation frequencies range from $0.04/10^6$ cells for the sickle cell mutations at the human HBB locus to $30.8/10^6$ cells for HLA-A mutations in T lymphocytes. The locus of interest and system of investigation also affect the mutation frequency. In studies of the nature of radiation-induced mutations using the Southern blot technique, experiments are usually conducted with minimum doses of 1.0 Gy. The results indicate a 25% to 50% relative frequency of HPRT mutants with normal Southern blots in human lymphoblast cell lines (up to 80% frequency has been reported in human T cells *in vivo*), a high proportion of which, 60%, constitutes deletions (vs. 20% *in vivo*) (Sankaranarayanan 1991b).

5.1.2.2 Chromosomal aberrations

Reports of X ray-induced chromosomal aberrations first surfaced in the late 1930s. Many aspects of chromosomal aberrations were extensively and accurately described at a time when notions of the genetic material were still nebulous. Progressively, links have been documented between radiation exposure and genetic abnormalities. It is known that ionizing radiation not only produces clastogenic events, but that the effects are dependent on the cell cycle phase at the time of exposure. Irradiation of cells during G_1 or G_0 phase results in aberrations due to rejoining, misrejoining, deletions, broken ends, and exchanges following post-irradiation mitosis. All these types of aberrations occur because the cell is affected at the same locus on both chromatids. A subset of defects yields sister chromatid exchanges (SCE). Cells irradiated during the S or G_2 phase of the cell cycle lead to single chromatid breaks following the first post-irradiation mitosis. This pattern of aberration is generally seen only for ionizing radiation exposure and varies from defects emanating from exposure to clastogenic chemical agents, which often result in chromatid-type and not chromosomal-type aberrations (reviewed by Bedford and Dewey 2002). Exposure to X radiation of 1 Gy is accompanied instantaneously by approximately 20 to 40 double-strand breaks, 1,000 DNA single-strand breaks, 1,000 defective bases, and 150 DNA-protein crosslinks per diploid mammalian cell (Bedford and Dewey 2002). X rays and ionizing radiation are among the very few agents capable of an immediate production of DNA double-strand breaks.

The vast majority of DNA single-strand breaks are repaired efficiently in normal cells during the G_1 and G_0 phases of the cell cycle and present little clastogenic risk to the cell. However, DNA repair-deficient cells are highly likely to display DNA double-strand breaks. It is important to note that both DNA double-strand breaks and single-strand breaks are seen following cell exposure to ionizing radiation probably because of the cumulative effects of DNA misrepair, base damage, and other additive chemical and molecular effects. Moreover, the chromatin structure at the time of radiation has a significant effect on the DNA double-strand breaks. There seems to be a higher susceptibility to DNA double-strand breaks in response to ionizing radiation in regions of DNA that are active transcriptionally. This evidence was supplied by studies indicating that supernumerary human X-chromosomes that are inactive transcriptionally are resistant to ionizing radiation-induced aberrations.

The development of FISH with multicolor chromosome painting expanded the level of understanding of ionizing radiation-induced chromosomal aberrations. These aberrations are much more complex in nature, involving three or more breaks in two or more chromosomes (Bedford and Dewey 2002). For X rays and gamma rays, the level of complexity is relatively low up to a dose of 1 Gy. The level of complex aberrations can reach 26% at 4 Gy in human lymphocytes (Loucas and Cornforth 2001) and 50% at 6 Gy in human fibroblasts (Brown and Kovacs 1993).

Various studies of contact-inhibited non-cycling normal human fibroblasts have reported five to six breaks per cell per Gy as a result of ionizing radiation exposure. These cells are in G_0 arrest, and the same population of cells can be studied for chromosomal breaks chronologically hours post-exposure to agents. G_0 human lymphocytes were used in other studies with similar results. There are a few reports of lower sensitivity in normal cells. Cells from *ataxia telangiectasia* (AT) patients exhibited similar radiosensitivity; however, rejoining of breaks differed from that in normal cells. Evidence of the disparity in the studies seems to point to post-irradiation misrepair and misrejoining of the strand breaks. A comparative study conducted at a 6-Gy dose of X rays indicated that the initial breaks occur at the same level; however, the residual number of breaks following repair differs for normal and AT cells at 1.7 and 9.6 breaks, respectively. In normal human cells, rejoining of breaks post-irradiation occurs with a half-life of 1.5 hours (Bedford and Dewey 2002). AT cells are characterized by a radioresistant DNA synthesis inhibition (Painter and Young 1980).

A method of study worth mentioning, due to its contribution to radiation genetics, is premature chromosomal condensation (PCC). This technique was developed following the observation that the fusion of interphase cells with mitotic cells causes PCC. Further, PCC induction after cell irradiation permits the chronological observation of chromosome breakage and rejoining. The method revealed 10-fold more breaks with immediate fusion following irradiation than would be assessed by allowing the cells to reach mitosis (Bedford and Dewey 2002). Far fewer breaks were noted two hours post-irradiation as a result of rejoining. These observations highlight the importance of time–dependent repairability in the DNA breaks.

Kamiguchi and Tateno (2002) reviewed the effects of various types of radiation on chromosome structural changes. The results of various studies of X rays and gamma rays and the generated dose-effects equations are summarized in Table 5-2a. Structural chromosomal aberrations increased exponentially as a result of increased gamma-radiation dosage, beginning with a linear dose-dependent increase at low dose. A similarity in dose-dependent increase of chromosomal aberrations in spermatozoa was reported between acute (1.36 Gy/min) and chronic (1.7 cGy/min) exposure for gamma irradiation. These results in spermatozoa differ from somatic cell data, which show a lower effectiveness of chromosomal aberration induction following chronic exposure. The difference is attributed to the absence of a cytoplasm in spermatozoa which consequently lack DNA repair capacity. Chromosomal breaks occur with a higher frequency than exchange-type aberrations. Further, spermatozoa have a tendency to remain fertile following gamma irradiation at a dose reaching 4.23 Gy. This observation leads to the speculation that radiation-induced DNA damage in spermatozoa may be transmitted to subsequent generations.

Table 5-2a. Dose-response relationship for radiation-induced structural chromosomal aberrations in human spermatozoa

Radiation	Dose (Gy)	Dose rate (Gy/min)	Dose-effect equation[a]
^{137}Cs gamma rays	0.0–4.0	5.0	Y = 0.96 + 15.14D
^{137}Cs gamma rays	0.0–4.23	1.36	$Y = 100\ (1-e^{-0.514D})$
^{137}Cs gamma rays	0.0–2.11	1.36	Y = 3.70 + 32.52D
^{60}Co gamma rays	0.0–2.0	0.017	Y = 1.68 + 33.85D
^{60}Co gamma rays	0.0–4.0	1.07-1.17	Y = 1.05 + 17.98D
X rays	0.0–1.82	0.44	Y = 00.8 + 34.52D

Source: Kamiguchi and Tateno 2002.

[a] Y indicates the percentile yield of spermatozoa with chromosome aberrations and D the dose in Gy.

The micronucleus test is less complicated and is a more rapid method for measuring chromosomal damage than sperm chromosome analysis. In two studies reviewed by Kamiguchi and Tateno (2002), hamster oocytes were fertilized with gamma-irradiated human spermatozoa and examined for micronuclei at the two-cell stage. Incidences of micronuclei were consistent with the incidences of spermatozoa with chromosomal breaks and fragments (Table 5-2b); however, the radiosensitivity measured by either test differed between the two studies reviewed. Although these differences were puzzling, the authors thought they might be due to differences in the protocols used (e.g., chromosome preparation technique, radiation source, and dose rate) in the studies.

Table 5-2b. Clastogenic effects of gamma rays on human spermatozoa chromosomes: comparison of micronuclei and chromosome aberrations

Index	Radiation			Dose-effect equation[a]
	Source	Dose (Gy)	Dose rate (Gy/min)	
Two-cell embryo with MN	^{137}Cs	0.0–2.13	1.36	Y = 1.38 + 38.00D
Spermatozoa with SCA	^{137}Cs	0.0–2.13	1.36	Y = 1.70 + 36.39D
Two-cell embryo with MN	^{60}Co	0.0–4.0	1.07–1.17	Y = 0.63 + 12.00D
Spermatozoa with SCA	^{60}Co	0.0–4.0	1.07–1.17	Y = 0.29 + 14.76D

Source: Kamiguchi and Tateno 2002.

[a] Y indicates the percentile yield of spermatozoa with micronuclei (MN) or structural chromosome aberrations (SCA) and D the dose in Gy.

Boei *et al*. (2000) used telomeric and centromeric peptide nucleic acid probes to localize both telomeres and centromeres to accurately detect asymmetrical chromosomal aberrations in X ray-exposed human lymphocytes. Analysis was restricted to cells containing all 92 pairs of telomeric signals and all 46 centromeric signals; therefore, incomplete elements were always observed in pairs. Three different pairs of incomplete elements were observed. These included an incomplete chromosome with a terminal fragment (ic+tf), two incomplete chromosomes (ic+ic), or two terminal fragments (tf+tf). Exposure to 1, 2, 3, 4, or 6 Gy of X rays resulted in increased frequency of all pairs, but the greatest increase was in the number of cells containing an incomplete chromosome with a terminal fragment (ic+tf). The dose-response curves followed a linear-quadratic function. Unexposed cells contained no aberrations in 373 cells observed; whereas, about 68%, 26%, 6%, and 36% of the irradiated cells contained ic+tf, ic+ic, tf+tf, and terminal deletions, respectively.

Immortalized human foreskin keratinocytes (HPV-G cells) were exposed to 0.5, 1.0, or 3.0 Gy of gamma rays or 0.25 or 0.5 Gy of alpha particles (Mothersill *et al.* 2000). Cytogenetic analysis revealed that radiation exposure induced a significant increase in the number of mean aberrations per cell (Fisher's exact test, $P < 0.01$). Chromosomal instability persisted in the 1 Gy group up to 72 population doublings, but declined in the 0.5 and 3 Gy groups between 30 and 72 population doublings.

Human peripheral blood lymphocytes exposed to 0, 0.5, 1, 2, 3, or 4 Gy of gamma rays (^{137}Cs) exhibited a clear dose-response increase in the number of translocations (Matsumoto *et al.* 1998). Translocations were more persistent than dicentrics, acentric fragments, or ring chromosomes. The reduction in the frequency of translocations over seven days was significant. Using FISH to analyze human lymphocytes exposed to 0 to 4 Gy of ^{60}Co gamma-radiation, Finnon *et al*. (1999) showed that complex rearrangements occurred more frequently as the dose increased. The frequency of dicentrics, acentrics, centric rings, two-way translocations, and one-way translocations increased, in the whole genome as well as in the painted chromosomes, in a dose-dependent manner.

Deininger *et al*. (1998) assessed the ability of gamma radiation to induce leukemia-associated fusion genes in human cells. These included the BCR-ABL hybrid gene (a product of the t(9;22)(q34;q11) translocation of chronic myeloid leukemia), the AML1-ETO fusion gene (a product of t(8;21) translocation of acute myeloblastic leukemia), and the DEK-CAN fusion gene (a product of t(6;9)(p23;q34) translocation of acute myeloblastic leukemia). The HL60 and KG1 cell lines did not contain any of these translocations. Cells were exposed to 50 or 100 Gy of gamma radiation, and mRNA transcripts were detected by RT-PCR. Fusion genes were induced in both cell lines but at a higher frequency in KG1 cells. For KG1 cells, exposure to 50 or 100 Gy of gamma rays resulted in a significant ($P < 0.0001$) increase in the formation of AML1-ETO fusion genes. A significant increase was not observed for the other fusion genes. The authors noted that the data support the idea that ionizing radiation can directly generate leukemia-specific fusion genes and demonstrate that there are differences in susceptibility among different cell populations.

5.1.2.3 Summary of human cell studies

Human T lymphocytes, the TK6 lymphoid cell line, fibroblasts, sperm, keratinocytes, and other cell lines have been studied in a number of *in vitro* assays for genetic effects induced by exposure to ionizing radiation. Ionizing radiation is capable of immediate production of DNA strand breaks, DNA-protein crosslinks, and defective bases in exposed cells. The data reviewed in this section are summarized in Table 5-3.

Table 5-3. Genetic effects of ionizing radiation in cultured human cells

Cell type	Endpoint	Results[a]	References
T lymphocytes TK6 lymphoid cells	Mutations (HPRT, TK, HLA -A locus)	+	Sankaranarayanan *et al*. 1991b
Lymphocytes Fibroblasts	Chromosomal aberrations DNA strand breaks	+	Brown and Kovacs 1993 Matsumoto *et al*. 1998 Boei *et al*. 2000 Loucas and Cornforth 2001 Bedford and Dewey 2002
Keratinocytes (HPV-G cells)	Chromosomal aberrations	+	Mothersill *et al*. 2000.
Spermatozoa	Chromosomal aberrations Micronucleus[b]	+	Kamiguchi and Tateno 2002
HL60 and KG1 hematopoietic cell lines	Fusion genes	+	Deininger *et al*. 1998

[a] + = positive

[b] Hybrid two-cell embryos generated from irradiated human sperm and hamster oocytes

5.1.3 Experimental animals

5.1.3.1 Germ cell studies

The majority of the data on the induction of mutations and chromosomal alterations by X rays or gamma rays in germ cells is for the mouse. There are more limited data that can be used for comparative purposes in rats, guinea pigs, Chinese hamsters, marmosets,

rhesus monkeys, and humans. Comprehensive reviews have been provided by Sankaranarayanan (1991a) and IARC (2000).

The available germ-cell studies in experimental animals have considered acute versus chronic exposures, male versus female, and germ cell stage at the time of exposure because all these factors influence the overall sensitivity. A summary of germ-cell mutation rates is provided in Table 5-4 for various endpoints. Further discussion is provided in Appendix D.

Table 5-4 Estimated induced mutation rates per cGy for low-LET radiation (mouse unless otherwise noted)

Genetic end-point	Cell stage and sex	Relative dose rate	
		High	Low
Dominant lethal	postgonial, male	1×10^{-3}/gamete	5×10^{-4}/gamete
	gonial, male	1×10^{-4}/gamete	2×10^{-5}/gamete
Recessive lethal	postgonial, male	1×10^{-4}/gamete	nr
	gonial, male	1×10^{-4}/gamete	nr
Dominant visible	gonial, male	2×10^{-5}/gamete	nr
	skeletal	5×10^{-7}/gamete	nr
	cataract	$5–10 \times 10^{-7}$/gamete	nr
	other	$5–10 \times 10^{-7}$/gamete	1×10^{-7}/gamete
	postgonial, female	$5–10 \times 10^{-7}$/gamete	nr
Recessive visible (specific locus test)	postgonial, male	6.5×10^{-7}/locus	nr
	postgonial, female	4.0×10^{-7}/locus	$1–3 \times 10^{-8}$/locus
	gonial, male	2.2×10^{-7}/locus	7×10^{-8}/locus
Reciprocal translocations	gonial, male		
	mouse	$1–2 \times 10^{-4}$/cell	$1–2 \times 10^{-5}$/cell
	rhesus monkey	2×10^{-4}/cell	nr
	marmoset	7×10^{-4}/cell	nr
	human	3×10^{-4}/cell	nr
	postgonial, female	$2–6 \times 10^{-4}$/cell	nr
Heritable translocations	gonial, male	4×10^{-5}/gamete	nr
	postgonial, female	2×10^{-5}/gamete	nr
Congenital malformations	postgonial, female	2×10^{-4}/gamete	nr
	postgonial, male	4×10^{-5}/gamete	nr
	gonial, male	$2–6 \times 10^{-5}$/gamete	nr
Aneuploidy (trisomy)	postgonial, female		
	preovulatory oocyte	6×10^{-4}/cell	nr
	less mature oocyte	6×10^{-5}/cell	nr

Source: BEIR V 1990, IARC 2000., nr = not reported.

5.1.3.2 Somatic cell mutations

Although germ cell mutations have been more extensively studied in mice exposed to X or gamma radiation, there are sufficient data to establish somatic cell mutations. IARC (2000) reviewed several studies in mice and reported mutations in the *Hprt* and *Aprt* genes of T lymphocytes isolated from the spleen and in transgenic mice carrying a marker gene. However, ionizing radiation did not induce loss of heterozygosity at the *Aprt* locus in mice.

Hoyes *et al*. (1998) examined the effects of administering 1 Gy of ^{60}Co gamma rays on the mutation frequency in somatic and germ cells. Big Blue *lacI* transgenic mice were treated with 1 Gy of gamma rays whole-body irradiation. At 35 days post-irradiation, mean mutant frequencies increased 1.5 fold in the testes and spleen (not statistically significant), but increased 4.5 fold in the liver ($P = 0.022$).

Transgenic Muta mice were used to determine the molecular characteristics of mutants induced by a high dose of radiation (Ono *et al.* 1999). At two months of age, mice received 200 Gy of whole body X rays and were sacrificed 3.5 days after exposure. There were 4.8-, 10.8-, and 4.8-fold increases in *lacZ* mutant frequency in the spleen, liver, and brain, respectively. The most prevalent mutations in irradiated tissues were deletions (approximately 55% in each of the three tissues) compared to ≥ 80% base substitutions observed in spontaneous mutations. Radiation-induced deletions were small and not different in size from spontaneous deletions. Deletions in irradiated tissues were primarily simple deletions without direct repeats at the termini and complex deletions (deletions that cannot be explained by one deletion event), neither of which was found in control tissues. These data indicate that radiation-induced mutations are different from spontaneous mutations.

5.1.3.3 Chromosomal aberrations

Swiss mice were exposed to 1 or 3 Gy of whole-body X rays and sacrificed at 1, 7, 21, or 100 days after irradiation (Xiao *et al.* 1999). Approximately 38% of the mouse genome was painted and examined by FISH. Animals in the unexposed control group had no dicentrics, translocations, or trisomies in bone marrow cells. In exposed mice, the frequencies of dicentrics, fragments, and translocations in the bone marrow cells were high on the day after exposure and decreased with time after exposure. By day 21, the frequencies of dicentrics and fragments decreased to control levels. The frequencies of translocations following exposure to 1 Gy were not significantly different from day 1 to day 100. However, mice exposed to 3 Gy showed significant declines in translocations by day 7 but leveled off through day 100. The frequencies of trisomy increased with time. The authors suggested that the increase in trisomy indicated the aneugenic properties of X rays.

Spruill *et al*. (2000) examined the decline of stable aberrations, i.e., chromosomal translocations, over time. Female C57BL/6 mice were exposed to a single dose of 0, 1, 2, 3, or 4 Gy of ^{137}Cs gamma rays. At various intervals between day 1 and 21 months after exposure, peripheral blood was obtained from the mice and analyzed by FISH. The results showed that translocations decreased dramatically the first three months after

irradiation. From three months to one year, the frequencies of translocations were relatively constant; however, from one year to 21 months frequencies of translocations were highly variable due to the significant effects of aging and clonal expansion. Dicentrics decreased rapidly after exposure and reached baseline levels within three months.

Rhesus monkeys (*Macaca mulatta*) were exposed to 5 Gy of X rays, either total-body or partial-body irradiation, to test the ability of cytogenetic methods to assess the persistence of radiation damage and determine the unirradiated fraction (Darroudi *et al.* 1998). At various times after exposure, blood lymphocytes were assessed for chromosomal aberrations, micronuclei, and premature chromosome condensation. One day after either total- or partial-body irradiation, the frequency of dicentrics was 66% and 62.5%, respectively; control cells had no dicentrics. The values decreased to 39.5% and 36% for total- and partial-body irradiation, respectively, after seven days. The total number of micronuclei per 1,000 binucleated cells was 6.7 in control cells, but total- and partial-body irradiation resulted in 748 and 705 total micronuclei, respectively, one day after exposure. Seven days after exposure, the total number of micronuclei fell to 436 and 403 in cells isolated from monkeys exposed to total-body and partial-body irradiation, respectively. Premature chromosome condensation analysis showed that total-body exposure resulted in all cells carrying excess breaks (no cells without breaks); whereas 94% of the cells recovered after partial-body exposure contained excess breaks. None of the unexposed cells contained excess breaks.

5.1.3.4 Summary of experimental animal studies

Mutations in germ cells and somatic cells and chromosomal aberrations in somatic cells occur in experimental animals exposed to X or gamma radiation. Most of these studies were conducted in mice. Results are summarized in Table 5-5.

Table 5-5. Genetic effects of ionizing radiation in experimental animals

Test animal	Endpoint	Results[a]	References
Mice	Germ cell mutations	+	Sankaranarayanan *et al.* 1991a IARC 2000
Transgenic mice	Somatic cell mutations (*lacI*, *lacZ*)	+	Hoyes *et al.* 1998 Ono *et al.* 1999
Mice	Chromosomal aberrations Trisomy	+	Xiao *et al.* 1999 Spruill *et al.* 2000
Rhesus monkeys	Chromosomal aberrations Micronucleus DNA strand breaks	+	Darroudi *et al.* 1998

[a] + = positive

5.1.4 Experimental animal cells

5.1.4.1 Mutations

The development of mammalian single cell plating generated new opportunities in the field of somatic genetic studies. One of the first series of studies conducted involved the detection of resistance to the toxic purine analogs 8-azaguanine (8-AG) and 6-

thioguanine (6-TG) resulting from mutation in the X-linked gene coding for a purine salvage pathway enzyme (Bedford and Dewey 2002). A series of studies of the X-linked HPRT assay indicated yields of approximately 20×10^{-6} mutants per locus per Gy for high-dose-rate X rays or gamma rays. These results are in close agreement with those of the mouse spermatogonial stem cell studies. Some studies have reported high ratios of intragenic to intergenic *hprt* mutations in CHO cells induced by ionizing radiation at low dose of 0.5 cGy. Interestingly, only 30% of the *hprt* mutations were intragenic, with the remaining 70% involving intergenic mutations including deletions of the entire *hprt* gene and rearrangements.

The CD59 assay is used to investigate the deactivation of the gene *CD59* located on the human chromosome 11, coding for the cell surface antigen of the same name. The human hybrid cell A_L/CHO contains a single copy of chromosome 11, which allows the isolation and mapping of the latter chromosome for the human genome project. A_L/CHO cells are still viable following the loss of the large section of this chromosome, thus permitting the study of large mutations. Using this cell assay system, Waldren and colleagues (reviewed by Bedford and Dewey 2002), produced *CD59* mutants at a level of 10^{-3}/Gy of X rays, and was two orders of magnitude higher than that demonstrated with the HPRT method. Closer molecular scrutiny indicated that the vast majority of the deleterious effects were non-point mutations, which were only detected following additional exposure to UVC light or alkylating agents.

An additional radiation assay can be conducted with the use of the enzyme thymidine kinase (*TK*). The enzyme intervenes in the phosphorylation catalysis of thymidine incorporation into DNA. *TK* deactivation through mutation can prevent the incorporation of the toxic analog trifluorothymidine (TFT). Thus, colony growth would be an indication of the *TK* gene deactivation via mutation. The *TK* gene is autosomally linked and located on the human chromosome 17 and synthenically located on mouse chromosome 11. For purposes of radiation mutation research, *TK*+/- cells were produced by exposure to X rays. Results of studies using mouse L5178Y cells indicated a sensitivity range of 350 to $2{,}300 \times 10^{-6}$/Gy, respectively (reviewed by Bedford and Dewey 2002). These values are in agreement with those generated with the CD59 hybrid cell system and higher than the figures generated through the HPRT assay on a unit dose basis.

5.1.4.2 Chromosomal aberrations

The frequency and distribution of X ray-induced chromosomal aberrations were examined in female Chinese hamster embryonic cells (CHE) (Xiao and Natarajan 1999a). Using FISH, these authors determined the frequency of aberrations on specific arms of the X chromosome. The most frequently observed exchanges were dicentrics and translocations and were proportionally distributed. However, breaks involved the long arms of the X chromosome more frequently than the short arms, resulting in a nonproportional distribution of breaks between the arms ($P < 0.001$). This bias was significant before radiation exposure but achieved greater significance after exposure to 1 or 4 Gy of X rays. The authors noted that the difference in the response of the two X chromosome arms might be explained by their chromatin constitution; the long arm is heterochromatic while the short arm is euchromatic. The sensitivity of heterochromatin

also was noted by Puerto *et al*. (2000). The induction, processing, and persistence of radiation-induced CAs were studied in the euchromatin of the short arm of the X chromosome and in the heterochromatin of the long arm of the X chromosome in hamster splenocytes. Irradiation with 4 Gy of X rays resulted in a greater frequency of breaks in the heterochromatin of the long arm of X ($P < 0.001$) when compared to the short (euchromatin) arm of the X chromosome. The heterochromatic Y chromosome of male animals also was over-involved in radiation-induced chromosomal aberrations. The authors noted that the data confirmed earlier studies showing that radiation damage is non-randomly distributed and that there is a prevalence of damage in heterochromatin and telomeres. The authors concluded that the level of DNA repair is similar in Xp and Xq and that differences in the chromosome aberrations frequency is caused by the greater radiosensitivity of heterochromatin and not by differential repair of heterochromatin and euchromatin.

Xiao and Natarajan (1999b) utilized CHE cells to examine X ray-induced aberrations on chromosomes 3, 4, 8, and 9. The short arms of chromosome 3 and the long arms of chromosome 4 were involved more than expected in breaks and dicentrics, respectively. Chromosome 8 was preferentially involved in chromosomal exchanges and was highly sensitive to radiation. The authors suggested that the sensitivity of chromosome 8 might be due to the enrichment of telomeric sequences on this chromosome.

Suzuki *et al*. (2000) used Syrian hamster embryonic cells (SHE) to investigate the formation of chromosomal aberrations following gamma irradiation (1, 2, or 4 Gy) with a ^{137}Cs source. The frequency of aberrations (gaps, deletions, and exchanges) increased in a dose-dependent manner. Bingham *et al*. (2000) also saw dose-related increases in the frequency of chromatid type aberrations in rat prostate adenocarcinoma cells irradiated with 1, 2, 3, or 5 Gy of gamma rays. Chromosome-type aberrations were less common; the number of dicentrics and ring structures was slightly higher than control levels.

CHO cells exposed to 2.5 Gy of gamma radiation had a significant increase ($P < 0.05$) in clastogenic damage when compared to unirradiated controls (Araújo *et al.* 1999). Cells irradiated in G_1/S phase contained mainly chromosome-type aberrations, while cells irradiated during S phase showed chromatid- and chromosome-type aberrations. Cells irradiated during G_2/S phase exhibited large increases in chromatid-type aberrations. The number of abnormal metaphases was increased in all irradiated cultures.

Slijepcevic *et al*. (1998) used four immortalized rodent cell lines (CHE, SCID ST, CHO K1, and CB17) with variable telomere lengths. Irradiation with 1.0 Gy of gamma rays resulted in chromosome breaks, some of which exhibited telomeric signals. Chromosome breaks exhibiting telomeric signals occurred only in cell lines with FISH-detectable telomeres. The authors suggested that interstitial telomeric sites might be involved in radiation-induced chromosome instability.

5.1.4.3 Genomic instability

The notion of genomic instability intervenes when the rate of introduction of genomic changes, including point mutations, chromosomal aberrations, aneuploidies, and gene amplifications, become grossly elevated in comparison with the normal condition.

Generally, a system of surveillance and damage processing maintains genomic integrity in all living organisms (reviewed by Bedford and Dewey 2002). Genomic instability is often associated with neoplastic cells that display additional defects, such as DNA repair deficiencies. A number of studies have reported the acquisition of genomic instability in cells and their progeny in response to ionizing radiation treatment. The observed aberrations are delayed and are seen in cells only at mitosis. Once acquired, genomic instability is permanent with reports of evidence in 15 to 20 subsequent generations of cells. However, there seems to be a susceptibility difference with respect to genomic instability. DNA repair proficiency can be a mitigating factor in irradiation susceptibility. In addition, there are some inherent differences between genomic events detected in cell culture systems and their actual occurrence *in vivo.*

Irradiation with 6 Gy gamma rays resulted in a G_2/M delay during which normal rat embryo fibroblasts, transformed with E1Aad5 and cHa-*ras* oncogenes, replicated the cellular DNA resulting in the formation of polyploid cells with micronuclei or enlarged lobular nuclei (Bulavin *et al.* 1999). At 48 hours post-exposure, the number of polyploid cells decreased and the number of hypodiploid cells increased, indicating that a population of the exposed polyploid cells underwent apoptosis. The authors concluded that gamma irradiation results in a temporary G_2/M delay during which replication continues, resulting in the formation of polyploid cells that die by apoptosis.

Limoli *et al*. (1999) showed (in the absence of BrdU) there was a 3% probability of observing chromosomal instability per Gy of X rays in GM10115 human-hamster hybrid cells (CHO cells carrying a single copy of human chromosome 4). In cells substituted with BrdU, a dose-response relationship was observed at doses <1 Gy but leveled off at doses >1 Gy. Gamma radiation (^{137}Cs) of unsubstituted cells resulted in frequencies of chromosomal instability similar to those observed with X rays. There were no significant differences in the induction of chromosomal instability when exposure to gamma radiation occurred at different dose rates. The BrdU-enhancement of chromosomal instability suggests that DNA is at least one of the critical targets in the induction of genomic instability.

5.1.4.4 Cell transformation

Cell lines including the mouse 3T3 cells, C3H 10T1/2 cells, and primary cultures of hamster embryo cells have been shown to yield to malignant transformation as a direct result of X radiation (Han and Elkind 1979, Kennedy *et al.* 1984). These cells, following transformation in monolayer culture, display characteristic non-contact inhibition with clonal foci and when transplanted into animals will form tumors. However, transformation of normal cells has proven to be difficult, especially in studies involving normal human cells, though normal hamster embryo cells have been transformed via radiation treatment with great success (Han and Elkind 1979, Kennedy *et al.* 1980, Borek *et al.* 1978). Multiple factors are generally required for easy transformation of cells including combinatory treatment with other agents, telomere maintenance, and functional endogenous cellular repair mechanisms.

5.1.4.5 DNA damage

DNA damage after exposure to ionizing radiation has been demonstrated by a number of techniques, including the comet assay. Based on comet moment and comet length as measurements of DNA damage, rat lymphocytes (0.6 to 5 cGy) or mouse C3H 10T1/2 cells (1 to 200 cGy) irradiated with ^{137}Cs gamma radiation contained significant damage to DNA (Malyapa *et al.* 1998).

DNA unwinding assays also have been used to detect DNA damage in CHO cells and in human and rodent cell lines. Dahm-Daphi *et al.* (2000) found dose-dependent DNA damage (double-strand breaks and single strand breaks) over a range of 0 to 60 Gy X rays. At a given dose, radioresistant (repair proficient) cell lines showed more intact DNA than did radiosensitive (repair deficient) cells lines (Roos *et al.* 2000).

DNA repair processes also may be reflected in assays of DNA damage. In a study of the ability of gamma radiation to induce gene conversion between the endogenous major histocompatibility complex class II genes, Hogstrand and Bohme (1999) irradiated F1(Balb/c $\times$ C3H/HeJ) testis cells (FBCTCL) with ^{137}Cs gamma rays (0.1 to 10 Gy). The frequency of gene conversion was increased by one order of magnitude at the lowest doses (0.1, 0.5, and 1 Gy), but at 10 Gy, the frequency of gene conversion was similar to control levels. The authors proposed that the lack of obvious gene conversion events at the higher doses might reflect differences in the repair processes, which depend on the extent and nature of DNA damage.

5.1.4.6 Summary of animal cell studies

Numerous *in vitro* assay systems have utilized rodent cell lines to assess the genetic effects of ionizing radiations. Ionizing radiations induce mutations, chromosomal aberrations, polyploidy, DNA strand breaks, chromosomal instability, and cell transformations. Data reviewed in this section are summarized in Table 5-6.

Table 5-6. Genetic effects of ionizing radiation in cultured animal cells

Cell type	Endpoint	Results[a]	References
CHO A_L/CHO hybrid L5178Y	Mutations (*hprt*, CD59, TK)	+	Bedford and Dewey 2002
CHE	Chromosomal aberrations	+	Xiao and Natarajan 1997a, 1997b
CHO	Chromosomal aberrations	+	Araújo *et al*. 1999
Hamster splenocytes	Chromosomal aberrations	+	Puerto *et al*. 2000
SHE	Chromosomal aberrations	+	Suzuki *et al*. 2000
Rat prostate adeno-carcinoma cells	Chromosomal aberrations	+	Bingham *et al*. 2000
CHE SCID ST CB17 CHO K1,	Chromosomal aberrations	+	Slijepcevic *et al*. 1998
Rat embryo fibroblasts	Polyploidy	+	Bulavin *et al*. 1999
Human-hamster hybrid CHO cells	Chromosome instability	+	Limoli *et al*. 1999
3TC C3H10T½ CHE	Cell transformation	+	Borek *et al*. 1978 Han and Elkind 1979 Kennedy *et al*. 1980, 1984
Rat lymphocytes C3H10T½	DNA damage	+	Malyapa *et al*. 1998
CHO	DNA damage	+	Dahm-Daphi *et al*. 2000
Mouse testis (FBCTCL)	DNA damage	+	Hogstrand and Bohme 1999

[a] + = positive

5.1.5 Mechanistic considerations

In order to predict the adverse health outcomes of exposure to ionizing radiations for humans, it is frequently necessary to utilize data obtained from other animals and, by using a series of correlation factors, extrapolate to the anticipated response in humans. This extrapolation of genetic and somatic risk to humans from animal data can be markedly improved by incorporating an understanding of the mechanism of formation of end-point (tumor or birth defect) into the approach. Since this can only be partially feasible because of the current incomplete nature of our knowledge, it is sensible to consider the use of surrogate end-points for cancer, namely chromosomal alterations and mutations. This is reasonable since specific genetic alterations are clearly involved in the production of tumors and birth defects. The following sections address the mechanism of induction of chromosome alterations and point mutations by X and gamma rays and discusses factors that might influence the dose-response for these genetic end-points at low exposures.

5.1.5.1 DNA damage

Despite many years of study, the specific nature of damage to DNA induced by ionizing radiations of different qualities is only now beginning to be determined. In somewhat simplistic fashion, the most common types of DNA damage induced by ionizing radiations include single-strand breaks, double-strand breaks, base damages, DNA-DNA and DNA-protein crosslinks, and clustered damage (multiple damaged sites). The clustered damage as proposed by Ward (1994) and Goodhead (1994), for example, is presumed to include all the various types of DNA damage within a limited volume. There has been a wide range of quantitative studies for single-strand breaks and double-strand breaks that has assessed the yields of breaks under a variety of conditions, and for a number of radiation qualities for a broad range of species (Roots *et al.* 1990, Teoule 1987, Whitaker *et al.* 1991). It has been broadly shown that the frequency of strand breaks is proportional to the DNA content of a cell for all types of radiation studied (Frankenberg-Schwager 1990). However, the ratio of single-strand breaks to double-strand breaks, and the absolute number of strand breaks per unit of radiation is quite different for radiations of different LET, with generally a higher proportion of double-strand breaks at higher LETs (Goodhead 1994). This would seem to be consistent across a range of species.

Information on the types and frequencies of base damage under various radiation conditions and for different radiation qualities is still quite limited, despite a recent relative flurry of activity. There are a very large number of different types of base damages induced by ionizing radiations (for reviews, see Teoule 1987, Wallace 1988), but the majority of the qualitative and quantitative data are for thymine glycol, 8-hydroxydeoxyguanine and urea, largely because the appropriate detection methods are available. Whether or not the spectrum of changes would be the same for different radiations is moot at this time, although one might expect some differences.

The relationship of the proportion of strand breaks to base damage has been estimated for low and high-LET radiations, and suggests that at low LET the ratio of single-strand breaks: double-strand breaks: base damage is about 25:1:50, but at high LET the ratio of single-strand breaks: double-strand breaks: is closer to 1:1 with little or no information being available for base damage (Goodhead 1994, Ward 1988).

5.1.5.2 Repair of DNA damage

In the past five to ten years there has been a considerable increase in our knowledge of the molecular aspects of DNA repair. This includes a very complete understanding of the nucleotide excision repair pathway for UV-induced DNA damage, of the repair of specific chemical adducts by, for example, methyl guanine methyl transferase, and oxidative DNA damage. More recently, information has been gathered on the repair of double-strand breaks and the relationship of one of these, non-homologous end-joining to recombination of immunoglobulin genes (Jeggo *et al.* 1995, Kanaar *et al.* 1998, Jackson 2002). What is apparent is that for ionizing and nonionizing radiations, the repair pathways are complex and involve multiple enzymes. This is a consequence of both damage recognition and repair pathways being exquisitely accurate, and perhaps because of the derivation of repair pathways from normal cellular housekeeping functions. A

detailed discussion on the various repair pathways for DNA damage induced by ionizing radiations and genetic susceptibility factors is included in Appendix E.

5.1.5.3 Effects of ionizing radiations at the cellular level

Gene alterations involving single base pair changes, either transitions, transversions, frameshifts or deletions, can arise at the sites of double-strand breaks or base damages. They will be generated during ligation or the resynthesis step of excision or recombination repair, or from errors of replication of a damaged template during S phase. On the basis of this mechanism of formation, the dose-response curve is predicted to be linear for acute and chronic exposures for high- and low-LET radiations (Preston 1992). In addition, the relative sensitivity will be determined by the size of the target, the efficiency and fidelity of repair, the nature of the DNA lesions (i.e., as influenced by the quality of the radiation) and the dose rate. This latter is of importance where errors of replication are involved, since the relationship between induction of damage, repair, and time of replication will be different for different rates of damage formation.

All types of chromosome aberrations induced by ionizing radiations also arise as background events. It is generally agreed that all types arise in G_1 or G_2 phases of the cell cycle by errors of repair, either failure to complete repair for terminal deletions and incorrect repair for exchanges and interstitial deletions. For cells in the S phase of the cell cycle, replication of unrepaired DNA damage can lead to errors in the form of chromosome aberrations, and aberrations also can arise from repair errors in the S phase prior to replication. For consideration of genetic events related to cancer induction, it is most appropriate to restrict the discussion to the more pertinent transmissible ones, namely reciprocal translocations, fairly small interstitial deletions, and some inversions. The great majority of dicentrics, rings, and terminal deletions are cell lethal as a result of interference with chromosome segregation of anaphase or loss of large quantities of genetic information in the form of acentric fragments. It has long been suggested and more recently demonstrated that the frequency of induced dicentrics is equal to the frequency of induced translocations (Lucas *et al.* 1992). This allows for conclusions based upon the analysis of dicentrics to be interpreted in terms of translocations. However, recent development of FISH techniques for painting whole chromosomes has allowed direct measurement of translocations in somatic cells to be made much more readily (Lucas *et al.* 1995).

Although it is necessary ultimately for both halves of a DNA double helix to be involved in the formation of chromosome aberrations, it is not necessary for the double-strand breaks to be induced by direct ionizations at the DNA. It is possible to produce double strand interactions through base damage repair via OH-radical interactions with DNA.

In summary, all classes of chromosome aberrations arising from interactions between pairs of DNA damages (strand breaks and damaged bases) and interchanges (dicentrics and reciprocal translocations), interstitial deletions, and rings represent misrepair events, and terminal deletions represent incomplete repair.

5.1.5.4 Potential confounders of dose-response curves

The major aim of extrapolating from data in laboratory animals to humans is to predict responses at low doses for tumors themselves or for genetic indicators of tumors. The current models for extrapolation rely almost exclusively upon the assumption of low dose linearity across the range of responses (NCRP 2001). Several recent observations have led to a reconsideration of low dose linearity, and these will be briefly discussed in the following three sections.

Bystander effects

The bystander effect is described as a response in cells that are not directly traversed by a radiation ionization track. This response can be a genetic one or an epigenetic one. The majority of these bystander responses have been described for high-LET exposures since it is possible to target single cells using microbeams (Zhou *et al.* 2002). For low-LET radiations, targeting single cells is much more difficult, and here the majority of bystander effects have been described using tissue culture medium transfer from irradiated cells to unirradiated cells (Mothersill and Seymour 2001). It is proposed that the bystander effect is mediated by cell signaling pathways being induced, thereby leading to reactive oxygen species production in bystander cells (Lehnert and Iyer 2002). The major question is, what relevance do the *in vitro* cell culture observations have for *in vivo* exposures, where bystander effects are somewhat difficult to predict, especially for low-LET exposures. The implications for predictions of tumor outcomes at low doses are based on the estimation of target size. However, tumor data from animal studies, since they are disease-based, already account for any bystander effects. Extrapolations from animal to human cellular responses should be cognizant of potential bystander effects, and the relative magnitude in different species. Little information on this aspect is currently available.

Genomic instability

The development of widespread genomic instability is a hallmark of tumor development (see Section 5.1.4.3 for further description of the experimental data). In fact, Stoler *et al.* (1999) provide evidence that an early step in sporadic colorectal tumor progression is characterized by several thousand genetic alterations per cell. Cahill *et al.* (1999) proposed that a form of Darwinian selection then selects for specific phenotypes in the tumor progression. This type of instability is both a cause and a consequence of the cancer process. The type of genomic instability described following radiation exposures is really quite different and much more limited in extent (Little 1998). No role for this radiation-induced genomic instability in the cancer process has been described.

Cancer risk assessments based on tumor data already incorporate any role of genomic instability. For extrapolations at the cellular level, it remains necessary to understand better any relationship between cancer-related genomic instability and radiation-induced delayed effects.

Adaptive responses

An adaptive response for radiation exposures has been described for chromosomal alterations and mutations in cellular systems and *in vivo* (UNSCEAR 1994). The phenomenon is one whereby the frequency of chromosome aberrations is lower by a

factor of about two for a small priming dose (e.g., 0.01 Gy) followed by a challenge dose of a Gy or so compared to that for the challenge dose alone. A number of possible explanations has been proffered, but none has convincingly explained the phenomenon. It is highly variable according to cellular (or tissue) system and, in humans, from individual to individual, some showing an adaptive response and others not. Thus, it is not feasible to draw a single conclusion for describing an adaptive response. In addition, there is no definitive evidence for an adaptive response for tumor outcomes. Extrapolations for tumor data from animal models to humans cannot at this time incorporate a component for adaptive response. Certainly, comparisons of underlying mechanisms of an adaptive response in animal and human cellular systems is needed, especially for extrapolation purposes.

5.2 Neutrons

Considerably fewer data are available for the assessment of germ cell and somatic cell effects for neutron exposures than for low-LET radiation discussed above. To a large extent, this is due to the limited availability of neutron sources for research applications and for the lower, albeit increasing, likelihood of medical exposures (Britten *et al.* 2001). In general, neutron radiation has not shown a therapeutic benefit when compared to conventional radiotherapy. The primary sources of neutron exposure include cosmic radiation and the nuclear industry. In the past, nuclear explosions were considered a potential source, but current estimates indicate that only a small percentage of the total dose (≤ 2%) was from neutrons (IARC 2000).

Although neutrons induce a similar spectrum of genetic effects compared to low-LET radiation (see Section 5.1), there are some quantitative differences. Therefore, one area of research has focused on identifying “fingerprints” of genetic damage caused by neutron exposure (Deng *et al.* 2000, Gajendiran *et al.* 2000, Boei *et al.* 2001). The IARC (2000) reviewed the genetic and related effects of neutrons and noted that neutrons induce chromosomal aberrations and gene mutations in mammalian cells more efficiently than X or gamma radiation. Furthermore, while neutrons are comparable to X or gamma radiation in producing double-strand breaks, neutron-induced DNA lesions are repaired less efficiently than those induced by low-LET radiation. The types of genetic damage induced by neutron exposure in humans and in mammalian experimental systems (both *in vivo* and *in vitro*) are reviewed in this section. Where possible, the types of cellular damage and the relative effectiveness are compared to X rays and gamma rays described in Section 5.1.

5.2.1 Human studies

Human exposure to neutrons generally occurs as a minor component of a mixed radiation field. The exceptions are patients treated with neutron radiotherapy and aircraft passengers and crew. Chromosomal aberrations have been reported in the circulating lymphocytes of airline pilots, in workers in nuclear plants, and in patients treated with neutron therapy (IARC 2000).

5.2.1.1 Nuclear explosions and radiation accidents

Atomic bomb survivors were exposed to mixed radiation, including a relatively small percentage of neutrons (see Section 3.2). However, it is now believed that the neutron doses were so small that cancer risk estimates from these data are not reliable for neutron exposure. Genotoxic effects observed in atomic bomb survivors were reviewed in Section 5.1.1.1. These data showed chromosomal aberrations and mutations in the exposed populations but did not show statistically significant associations between parental irradiation and adverse effects, including chromosomal abnormalities and mutations, in subsequent generations (ATSDR 1999, IARC 2000, UNSCEAR 2001).

The IARC (2000) reviewed several studies of men (n = 1 to 8) who were accidentally exposed to mixed gamma radiation and fission neutrons (9% to > 50% of the total dose). Blood samples collected after exposure showed chromosomal aberrations (rings, dicentrics, translocations, deletions, and minutes) and aneuploid cells. The frequency of chromosomal aberrations declined slowly over time, but even after 17 to 19 years the frequency of aberrant cells was 10% to 22% in the most highly exposed individuals. This persistence of unstable chromosome aberrations in peripheral lymphocytes was similar to that reported for the atomic bomb survivors (see Section 5.1.1.1).

5.2.1.2 Occupational and medical exposures

Several studies investigated DNA damage in flight personnel that are exposed to a number of potential genotoxic factors including cosmic rays, airborne pollutants, ozone, and electromagnetic fields. Cosmic radiation is believed to be the main risk factor because of the significantly higher levels encountered at high altitudes compared to ground level. The estimated mean annual exposure on intercontinental flights at an altitude of 10,000 m ranges from 1 to 10 mSv (Cavallo *et al.* 2002a). Cosmic radiation at sea level is about 0.2 mSv/year (Heimers 2000). Some of these studies reported a significant increase of chromosomal aberrations in air-crew (Romano *et al.* 1997, Heimers 2000, Cavallo *et al.* 2002a) compared to ground-based controls while others did not (Zwingmann *et al.* 1998, Wolf *et al.* 1999, Cavallo *et al.* 2002b). Cavallo *et al.* (2002a) concluded that cosmic radiation was not solely responsible for the observed chromosomal aberrations and that other occupational risk factors were likely involved.

A recent paper by Littlefield *et al.* (2000) is the only comprehensive study of the induction and persistence of chromosome aberrations by neutron exposures in a medical setting. The data serve to highlight the difficulties in assessing biological doses from cytogenetic responses in peripheral lymphocytes for partial body, high-LET exposures. Both low- and high-LET radiation conditions result in a highly nonrandom distribution of chromosomal alterations, making dose estimates very equivocal. This study evaluated chromosomal aberrations in lymphocytes exposed *in vitro* to highly efficient 1 MeV monoenergetic neutrons and in patients who received fast neutrons as tumor therapy. FISH (chromosomes 1, 2, and 4) methods and conventional staining methods were used to measure reciprocal translocations and dicentrics and rings, respectively. The 1 MeV neutrons were very effective at inducing all types of chromosome aberrations, with a linear dose-response relationship for all types. For *in vitro* exposure and subsequent *in vitro* culture for 20 cell cycles, the frequency of unstable aberrations was reduced to

background levels, whereas the frequency of reciprocal translocations remained similar to the induced frequency, with a very similar dose-response curve. Conventional staining methods were used to measure the persistence of aberrations in patients who received fractionated neutron therapy to tumors located in many different sites. Cells containing neutron-induced dicentrics and rings were lost from the peripheral lymphocyte pool within 3 years, while reciprocal translocations persisted for more than 17 years. Of particular note for biological dosimetric considerations was the observation that there was a considerable variation in frequency of aberrations among patients who had received similar average bone marrow doses.

In a second medical irradiation study, chromosomal aberrations were analyzed in peripheral lymphocytes of 17 patients who received 14 MeV neutron tumor therapy (Schmid *et al.* 1980). The dose rate was about 0.2 Gy per minute. The treatment consisted of daily doses of 0.65 to 0.80 Gy or of 12 exposures of 1.3 Gy in 3 fractions per week. Gamma-ray contamination was of the order of 5% to 15% depending on the tumor location and size of the irradiation field. The dicentric aberrations were nonrandomly overdispersed, as is generally the case for high-LET exposures. The authors reported a positive correlation between the yield of dicentrics and the total skin dose over a range of individual doses of 0.8 to 15.6 Gy. It should be noted that biological dosimetry for partial body exposures, especially with high-LET exposures, has several limitations related to aberration distribution as discussed in detail by Sasaki (1983).

5.2.1.3 Summary of human studies

Data for human exposures to neutron radiation are limited compared to low LET radiation. Furthermore, human exposures to neutrons usually occur as part of a mixed radiation field. Atomic bomb survivors were exposed to relatively small doses of neutron radiation compared to low LET radiation (see Section 5.1.1.1). Nevertheless, the available data indicate that neutron radiation is highly effective in inducing genetic damage. The data reviewed in this section are summarized in Table 5-7.

Table 5-7. Genetic effects in human populations exposed to neutron radiation

Population	Endpoint	Results[a]	References
Radiation accidents	Chromosomal aberrations Aneuploidy	+	IARC 2000
Airline flight personnel	Chromosomal aberrations	+	Romano *et al.* 1997 Heimers 2000 Cavallo *et al.* 2002a
		–	Zwingman *et al.* 1998 Wolf *et al.* 1999 Cavallo *et al.* 2002b
Tumor therapy patients	Chromosomal aberrations	+	Littlefield *et al.* 2000 Schmid *et al.* 1980

[a] + = positive, - = negative

5.2.2 Human cells

Studies conducted with human mammary cells (Ponnaiya *et al.* 1997), fetal lung fibroblasts (Kadhim *et al.* 1998), lymphocytes (Lukasova *et al.* 1999, Mustonen *et al.* 1999, Schmid *et al.* 2000, Deng *et al.* 2000, Gajendiran *et al.* 2000, Boei *et al.* 2001), and sperm (Kamiguchi and Tateno 2002) demonstrate that neutron irradiation induces chromosomal aberrations, genomic instability, and DNA damage. There is some evidence that the ratios of different chromosomal aberrations may be useful as biomarkers or "fingerprints" of neutron exposure.

5.2.2.1 Chromosomal aberrations

The IARC (2000) reviewed many studies of chromosomal aberrations (dicentrics or dicentrics plus centric rings) induced in human peripheral lymphocytes following exposure to neutron radiation. Maximum RBE values compared to ^{60}Co gamma rays ranged from 4.3 to 83.9 (Table 5-8).

Neutron radiation (0.2 and 0.4 Gy) induced genomic instability in human mammary epithelial cells examined from 5 to 40 population doublings post-irradiation (Ponnaiya *et al.* 1997). Chromosomal dicentrics increased in all irradiated populations at five population doublings. By 10 population doublings, the frequency of dicentrics approached that of controls, but the frequencies of total chromosomal aberrations was higher than controls throughout the experiment. Chromatid-type gaps and breaks were observed at higher frequencies than controls throughout the experiment. Similar results were reported with cells exposed to gamma radiation; however, the significant increases occurred later and were more transient.

Human diploid fetal lung fibroblasts (HF19 and HF12) were irradiated with X rays, neutrons, and alpha-particles (Kadhim *et al.* 1998). After three post-irradiation doublings, the frequency of aberrations in exposed HF19 and HF12 cells was significantly higher than in controls. Chromatid breaks, chromosome fragments, and minutes were the most frequently observed aberrations. After 20 population doublings, the frequency of cells with unstable aberrations decreased but remained significantly higher than control levels in cells exposed to neutrons. After 35 population doublings, the total number of aberrant cells in neutron-exposed cells was significantly greater ($P < 0.001$) compared to control cells. Exposed HF12 cells had significantly greater frequencies of aberrant cells after three post-exposure population doublings. After 20 or 35 population doublings, the frequencies of aberrant cells in exposed HF12 cells were not different from the controls. Thus, chromosomal instability was demonstrated in HF19 cells but not in HF12 cells exposed to high-LET radiation.

Table 5-8. Relative biological effectiveness of neutrons for chromosomal aberrations (dicentrics or dicentrics plus centric rings) induced in human peripheral lymphocytes irradiated *in vitro*

Source	Neutron energy (MeV)	Absorbed dose (Gy/min)	Sampling time (h)	RBE[a] for 2.0-0.02 aberrations per cell	Maximum RBE[a]
d, T					
Japan	~ 14.1	-	-	1.2-5.9	14.5
Germany	~ 15.0 (γ < 4%)	0.12	48	1.1-3.6	9.0
Glasgow, Scotland	~ 14.7 (γ ~ 7.5%)	0.30	48	1.7-6.6	16.7
Harwell, England	~ 14.9 (γ ~ 3%)	0.25	48 (O_2)	2.2-6.6	16.2
			(N_2)	1.2-2.1	4.3
^{3}H(a ,n)^{4}He					
Russian Federation (NG-150M)	14.7 (γ < 10%)	0.36-1.85	50-52	1.7-3.8	9.0
d, Be					
Harwell, England (VEC)	~ 20	~0.50	52-72	1.4-11.3	29.2
Hammersmith, England (cyclotron)	~ 7.6 (γ < 10%)	0.30	48	2.1-11.9	30.4
Louvain, Belgium (cyclotron)	~ 6.2 (γ low)	0.05	48-53	1.0-8.3	21.5
Japan	~ 2.03	-	-	2.2-17.4	43.3
Li/Be					
Russian Federation	~0.04 (γ < 7%)	0.01	50-52	2.4-6.8	16.5
(KG-2.5 accelerator)	~0.09 (γ < 4%)	0.03		1.1-10.8	28.0
Fission					
France (CEA/Crac)	~10 (γ very high)	-	46-53[b]	2.8-22.3	57.4
France (CEN/Triton)	~ 10 (γ ~ 30-50%)	0.03-0.07	46-53[b]	2.7-21.6	55.7
France (CEN/Harmonie)	~1.5 (γ ~ 5%)	0.12	46-53	2.0-16.1	41.3
Sofia, Bulgaria (IRT-2000)	~ 3	-	52	0.8-6.5	16.9
Aldermaston, England	~0.9 (γ < 10%)	0.03	48	2.2-18.0	46.4
Argonne, USA (JANUS)	~0.85 (γ ~ 3%)	0.06	48-50	2.3-18.3	45.6
Russian Federation (BR-10)	~0.85 (γ < 5%)	0.06-2.6	50-52	2.8-19.9	51.1
Harwell, England (BEPO)	~0.7 (γ ~ 10%)	0.50	48	2.6-20.6	53.2
Harwell, England (BEPO)	~0.7 (γ ~ 10%)	0.50	48-56	2.6-21	54.1
Harwell, England (GIEEP)	~0.7 (γ ~ 15%)	0.0005	48-56	2.5-20.4	52.2
		0.0011		3.1-25.2	65.0
Italy (TAPIRO)	~0.4 (γ ~ 10%)	0.002-0.07	48	2.6-22.2	57.1
Russian Federation (BR-10)	~0.35 (γ < 5%)	0.04-0.4	50-52	4.1-32.6	83.9
Russian Federation (BR-10)	Thermal (γ < 5%)	0.005	50-52	1.3-20.6	53.3
National Radiol. Prot. Board (^{252}Cf)	~ 2.13	0.12-0.17	48	1.8-14.8	38.2

Source: IARC 2000
[a]Reference radiation, ^{60}Co gamma rays; constant dose rate of 0.5 Gy/min
[b]Data corrected for gamma (γ) radiation

Lukasova *et al*. (1999) used triple-color FISH to detect exchange aberrations among 10 chromosomes (1, 2, 3, 4, 8, 9, 12, 14, 18, and 22) in human lymphocytes irradiated with 0.5 to 1.5 Gy of fast neutrons (mean energy 7 MeV). The control group showed only five aberrations in the selected group of chromosomes. There were significantly more aberrations observed in chromosomes 8, 14, 18, and 22 after exposure to 1.5 Gy of neutrons ($P < 0.05$, $P < 0.001$, $P < 0.01$, and $P < 0.015$, respectively). Lower doses resulted in more aberrations involving chromosomes 14 and 22. After doses of 1 and 1.5 Gy, there were significantly lower frequencies of aberrations observed in chromosome 2 ($P < 0.01$). The authors noted that the high frequency of exchanges between specific chromosome pairs (14/18, 14/8, 18/8, 8/3, 1/14, 1/8, 3/18, 3/14, and 9/22) corresponded with chromosomes involved in translocations in B-cell non-Hodgkin's lymphoma and chronic myeloid leukemia.

Dicentric chromosomes were induced in human lymphocytes irradiated with neutrons (565 keV) (Schmid *et al.* 2000). The linear dose-response relationship showed that approximately 0.813 dicentrics were formed per cell per Gy over the dose range of 0 to 0.167 Gy.

Deng *et al.* (2000) and Boei *et al.* (Boei *et al.* 2001) conducted comparative studies to identify potential cytogenetic fingerprints for high-LET radiation in human lymphocytes. Compared to low-LET radiation, neutron radiation induced more incomplete exchanges. The ratio of total simple translocations to insertions (I-ratio) was significantly lower ($P < 0.01$) in cells exposed to 0.2 Gy neutrons (8.0 ± 1.1) compared to cells exposed to 3 Gy of ^{60}Co gamma rays (23.1 ± 5.5). In addition, the ratio of complete exchanges to incomplete rejoinings [S(I)-ratio] and dicentrics to interstitial deletions (H-ratio) was significantly lower ($P < 0.05$) in neutron-irradiated cells (Deng *et al.* 2000). Boei *et al.* (2001) reported that, compared to low-LET radiation, exposure to neutron radiation led to a significantly higher frequency of both inter- and intra-arm intrachanges, a higher proportion of complex aberrations, and aberrations with a higher degree of complexity. There was a similar frequency of incomplete exchanges or terminal deletions for both high- and low-LET radiation.

Kamiguchi and Tateno (2002) reported that neutron radiation was more effective than X rays, gamma rays, or beta radiation in inducing chromosomal damage in human spermatozoa. Chromosome breaks occurred more frequently than exchanges.

5.2.2.2 DNA damage

DNA damage was measured using the Comet assay following both gamma and neutron irradiation of a human B-lymphoblastoid cell line, the Raji cells (Mustonen *et al.* 1999). Cultured cells were exposed to neutrons (0.5 to 16 Gy) or gamma rays (1.4 to 44.8 Gy). The results indicated that after one hour, a lower number of normal cells per unit dose was reported for the neutron treatment than for gamma rays; however, the number of damaged cells was comparable between the two treatment groups 24 hours following exposure. DNA repair was less efficient following neutron treatment than gamma-ray exposure, as revealed by the rejoining of DNA breaks. This difference in DNA repair was more pronounced at low doses.

Gajendiran *et al.* (2000) used the Comet assay to distinguish between DNA damage in human peripheral blood lymphocytes induced by monoenergetic neutrons (0.186 to 2.3 MeV) and ^{60}Co gamma rays. Doses ranged between 0.125 and 1 Gy. In contrast to the relatively short and continuous comet tails induced in gamma-irradiated cells, neutron-irradiated cells had longer comet tails consisting of tiny pieces of broken DNA. RBE values were 6.3, 5.4, 4.7, 4.3, 2.6, and 1.7 for 0.37, 0.57, 0.79, 0.186, 1, and 2.3 MeV neutrons, respectively. Compared to low-LET radiation, DNA strand breaks induced by high-LET radiation are more complex, more severe, and longer-lived.

5.2.2.3 Summary of human cell studies

Chromosomal aberrations and DNA damage have been induced in a number of human cell types, particularly peripheral lymphocytes, by *in vitro* exposure to neutron radiation. Results of the studies reviewed in this section are summarized in Table 5-9.

Table 5-9. Genetic effects of neutron radiation in cultured human cells

Cell type	Endpoint	Results[a]	References
Mammary epithelial cells	Chromosomal aberrations Genomic instability	+	Ponnaiya *et al.* 1997
Diploid fetal lung fibroblasts (HF19 and HF12)	Chromosomal aberrations	+	Kadhim *et al.* 1998
Lymphocytes	Chromosomal aberrations	+	Lukasova *et al.* 1999 Schmid *et al.* 2000 Deng *et al.* 2000 Boei *et al.* 2001
Spermatozoa	Chromosomal aberrations	+	Kamiguchi and Tateno 2002
B-lymphoblastoid cell line (Raji cells)	DNA damage	+	Mustonen *et al.* 1999
Lymphocytes	DNA damage	+	Gajendiran *et al.* 2000

[a] + = positive

5.2.3 Experimental animal studies

Most *in vivo* studies of the effects of ionizing radiation are conducted in mice. Mice exposed to neutrons develop both germ-line and somatic cell mutations and cytogenetic damage. Cytogenetic effects include sister chromatid exchanges (SCE), chromosomal aberrations, micronuclei, and translocations.

5.2.3.1 Germ-cell mutations

The data on the induction of mutations in germ cells by neutrons is almost exclusively for the mouse. Mice appear to be more sensitive than humans to germ-cell mutations. Dominant lethal, visible dominant, and recessive visible mutations have been reported in mice exposed to neutron irradiation (Table 5-10). Data comparing effects of fission neutrons and ^{60}Co gamma radiation show that weekly doses of neutron irradiation are more effective than single doses in inducing dominant lethal mutations; whereas, the

opposite is true for gamma radiation (IARC 2000). More specific information on germ-cell mutations is provided in Appendix D.

Table 5-10. Germ-cell mutations observed in mice irradiated with neutrons

Neutron energy or dose	Sex	Mutation type	Mutation rate (per gamete/Gy)[a]	Comment
0.7 MeV	male	visible dominant	2.6×10^{-4}	Background spermatogonial mutation rate was 8×10^{-6}.
nr	male	dominant lethal	0.25[b] 0.04[c]	No effect of dose rate observed after single or weekly exposures.
Up to 1 Gy 0.3–1.2 Gy	male female	recessive visible recessive visible	$1–1.5 \times 10^{-4}$[d] 1.45×10^{-4}[d]	Male mice irradiated at post-spermatogonial stages with no effect of dose rate; female mice received single doses.

Source: IARC 2000.
[a]Unless otherwise noted.
[b]Males treated 4 to 5 weeks before mating with untreated females (postgonial stage).
[c]Males treated in the spermatogonial stem-cell stage.
[d]per locus/Gy.
nr = not reported.

Barber *et al.* (2002) reported that germ-line instability persisted for at least two generations in offspring of irradiated male mice. Initially, five CBA/H and three C57BL/6 mice received chronic, whole-body irradiation with 0.4 Gy fission neutrons delivered at a rate of 0.003 Gy/minute. Other groups of mice were exposed to 1 or 2 Gy of X rays delivered at 0.5 Gy/minute. CBA/H males were mated with untreated females at three and six weeks post-irradiation. C57BL/6 mice were mated with untreated females six weeks after exposure. F_2 and F_3 offspring were produced by random matings of F_1 and F_2 offspring (male or female) with untreated partners. Germ-line mutations were measured by the number of expanded simple tandem repeat (ESTR) DNA loci, which are observed as size changes in the alleles of these loci. The data are summarized in Table 5-11. There was no difference in the transmission of germ-line instability through male or female offspring. There was an elevated mutation rate in the germ line of males that mated six weeks after exposure (premeiotic), but postmeiotic exposure (3 weeks) did not increase ESTR mutations in irradiated males. However, mutation rates were elevated in offspring produced following paternal exposure at three or six weeks prior to mating (data not shown). X ray and neutron exposure gave similar results in CBA/H mice.

Table 5-11. Germ-line mutations in controls and offspring of male mice exposed to 0.4 Gy neutron radiation

Strain	Group	Number		No. of mutations	Mutation rate
		Parents[a]	Offspring		
CBA/H	control	8/8	76	22	0.072
	F_0 (3 wk)	5/0	18	1	0.028
	F_0 (6 wk)	5/0	43	18	0.209***
	F_1	7/9	83	42	0.253***
	F_2	9/7	84	33	0.196***
C57BL/6	control	4/4	98	25	0.064
	F_0 (6 wk)	3/0	45	24	0.267***
	F_1	3/5	58	18	0.155**
	F_2	5/5	63	19	0.151**

Source: Barber *et al*. 2002.
**$P < 0.01$, ***$P < 0.001$ compared to controls.
[a]Number of males/number of females.

5.2.3.2 Somatic cell mutations

Somatic mutations were detected at the *hprt* locus and in *ras* oncogenes. B6CF$_1$ mice were exposed to whole-body irradiation with fission neutrons either as a single dose (1.5 Gy) or six fractionated doses (0.25 Gy) given over a two-week period. *Hprt* mutant frequencies in splenic lymphocytes were $6.0 \pm 1.5 \times 10^{-5}$ and $8.7 \pm 5.4 \times 10^{-5}$ for the single and fractionated doses, respectively (Kataoka *et al.* 1993). N-*ras* mutations were detected at a higher frequency in the spleens of CBA/Ca mice that developed myeloid leukemia following exposure to neutron radiation than mice exposed to low-LET radiation (Rithidech *et al.* 1996). A thymic lymphoma induced by neutron radiation in an RF/J mouse contained a K-*ras* gene activated by a mutation at codon 146. This mutation had not previously been detected in any human or animal tumor. Overall, neutron radiation induced a different spectrum of *ras* mutations than gamma radiation (Sloan *et al.* 1990).

5.2.3.3 Cytogenetic effects

Poncy *et al.* (1988) reported an increase in SCE in bone marrow cells in three-month-old rats exposed to 2 Gy of whole-body radiation with 1-MeV fission neutrons. There were two distinct phases of increased SCE. The first occurred in the days following exposure but then dropped back to control levels from 15 to 150 days after exposure. A second increase occurred between days 150 and 240 and then leveled off. The authors suggested that the first increase was due to DNA damage that was rapidly repaired while the second increase coincided with tumor growth. No tumors occurred in bone marrow but were observed in skin, lung, mammary gland, adrenal gland, liver, kidney, and bladder.

Mouse splenocytes were cultured following irradiation with 1-MeV neutrons *in vivo* (Darroudi *et al.* 1992). Micronuclei and chromosomal aberrations increased linearly with

dose. The RBE values ranged from 6 to 8 in the dose range of 0.25 to 3 Gy for dicentrics and rings and was about 8 for micronuclei at doses between 0.25 and 2 Gy.

Van Buul (1989) exposed rhesus monkeys to 0.25 Gy of 2-MeV neutrons or 1 Gy of gamma radiation. Reciprocal translocations were induced in stem-cell spermatogonia. The RBE for neutrons was 2.1 compared to an RBE of 4 in mice.

5.2.3.4 Summary of experimental animal studies

Neutron radiation induces mutations in germ cells and somatic cells in mice and cytogenetic effects in mice, rats, and monkeys. The data reviewed in this section are summarized in Table 5-12.

Table 5-12. Genetic effects of neutron radiation in experimental animals

Test animal	Endpoint	Results[a]	References
Mice	Germ cell mutations Germ-line instability	+	IARC 2000 Barber *et al*. 2002
Mice	Somatic cell mutation (*hprt*, *ras*)	+	Sloan *et al*. 1990 Kataoka *et al*. 1993 Rithidech *et al*. 1996
Mice	Chromosomal aberrations Micronuclei	+	Darroudi *et al*. 1992
Rats	Sister chromatid exchange	+	Poncy *et al*. 1988
Rhesus monkeys	Reciprocal translocations	+	Van Buul 1989

[a] + = positive

5.2.4 Experimental animal cells

Many studies of mammalian cellular systems have been conducted. Genotoxic effects include DNA damage, chromosomal aberrations, genomic instability, gene mutations, and cell transformation. DNA lesions induced by radiation exposure have been classified into three categories: rapidly repaired breaks, slowly repaired breaks, and unrepairable breaks. Compared to equal doses of low-LET radiation, neutron radiation does not induce as much rapidly repaired damage, induces similar amounts of slowly repaired damage, and induces more unrepairable damage. Neutron radiation also is more efficient in inducing chromosomal aberrations, mutations, and cell transformations in mammalian cells than equivalent doses of X rays or gamma rays (IARC 2000). The studies reviewed in this section are summarized in Table 5-13.

Table 5-13. Genetic effects of neutron radiation in cultured animal cells

Cell type	Endpoint	Results[a]	References
Hamster V-79	Chromosomal aberrations Genomic instability	+	Trott *et al.* 1998
CHO	Mutation (*hprt)*	+	Kinashi *et al.* 1997, 2000
Mouse C3H10T½	Cell transformation	+	Miller *et al.* 1995

[a] + = positive

Ionizing radiation induces genomic instability in the form of chromosomal aberrations that occur several cell generations after exposure (IARC 2000). Trott *et al.* (1998) exposed V-79 hamster cells to different doses of X rays, alpha particles, and neutrons. The progeny of surviving cells were examined for delayed reproductive death, delayed micronuclei, delayed dicentrics, and delayed apoptosis for up to four weeks after exposure. The authors reported a similar dose-response relationship for all endpoints with an initial steep rise at low doses and reaching a plateau at doses > 3 Gy. The authors concluded that chromosome instability was not due to damage inflicted on individual chromosomes at the time of irradiation but was likely due to an increased level of a non-specific clastogenic factor in the progeny of all surviving irradiated cells.

Kinashi *et al.* (1997) investigated the mutagenicity of neutron irradiation on Chinese hamster ovary (CHO) cells in the presence and absence of boric acid. The theory behind boron neutron capture therapy (BNCT) is that as the tumor cells accumulate boron compounds and are exposed to thermal neutrons, the $^{10}B(n,\alpha)^{7}Li$ reaction releases an alpha-particle and a recoiling ^{7}Li ion. These particles have the characteristics of high-LET radiation, thus, increasing the efficiency of tumor-cell killing. In this study, CHO cells were grown and irradiated in the late exponential phase. Mutagenicity at the *hprt* locus was evaluated at various boric acid levels (0, 5, 10, 20, and 30 ppm). The presence of boric acid increased the mutation frequency resulting from neutron irradiation, and the RBE for neutron exposure was correlated with the boron concentration (3.8, 4.7, 5.1, 5.7 and 6.2 for 0 ppm, 5 ppm, 10 ppm, 20 ppm, and 30 ppm of boric acid, respectively). Moreover, the RBE for mutagenicity correlated with increasing LET. The results indicated that neutron double-strand breaks are associated with a high level of lethality because of misrepair.

Kinashi *et al.* (2000) exposed CHO cells to thermal, epithermal, and mixed thermal and epithermal neutrons. Epithermal neutrons penetrate deeper into tissue than thermal neutrons and are more suited for treatment of deep-seated tumors. Mutant frequencies were dependent on dose, but there were differences for the three modes. Epithermal neutrons were more mutagenic. The mutation frequency at the *hprt* locus following exposure to epithermal neutrons was about 5-fold higher compared to thermal neutrons and about 1.5-fold higher compared to the mixed mode. Total and partial deletions accounted for 84.4% to 94.7% of all mutations. Partial deletions were more prevalent with the thermal neutron and mixed mode treatments, whereas total deletions were more prevalent with epithermal neutron treatment. The fraction of total deletions was increased by boron for both the thermal neutron and mixed mode treatments; however, boron did

not significantly increase total mutant frequency. Epithermal neutrons were not tested with boron.

Cell transformation studies with neutron radiation in mammalian cells have reported that the RBE values depend on the energy of the neutrons. Dose-response curves are nearly linear for various neutron energies but are curvilinear for reference X rays. RBE values for cell transformation decrease with increasing dose and have been reported as low as 3 for high dose rates up to 35 for low dose rates. Lower RBE values occurred in a study using a less pure source of neutrons (IARC 2000). Miller *et al.* (1995) also reported that, following neutron irradiation, mouse C3H10T½ cells were more sensitive to oncogenic transformation during the G_1 phase while the X rays primarily affect G_2 cells. However, cell-cycle effects are not as pronounced for neutrons as for X rays (Redpath *et al.* 1995).

5.2.5 Mechanistic considerations

There are a number of similarities between the induction of DNA damage and DNA repair for X and gamma rays and neutrons. Thus, much of what has been described in Section 5.1.5 (X and gamma rays mechanistic considerations) is pertinent to this section on neutrons (and other high-LET radiations).

5.2.5.1 DNA damage

The types of DNA damage induced by neutrons are similar to those induced by low-LET radiations (see Section 5.1.5.1). The spectra of damage however, is different, with neutrons having a higher proportion of double-strand breaks and multiple damaged sites than for low-LET radiations. In general, the damage is more severe for neutrons as indicated by the reduced rejoining efficiency of the double-strand breaks and the complexity of the multiple damaged sites (Pogozelski *et al.* 1999, Boei *et al.* 2001). Of interest is the observation that clustered damage induced by neutron tracks results in a high proportion of complex aberrations and in non-reciprocal interactions of chromosome breaks. Most of the exchanges observed occurred within one neutron track, and little interaction seems to take place between the breaks formed in different tracks (Boei *et al.* 2001). This provides an explanation for the linearity of the dose-response curve for chromosomal alterations induced by neutrons. The fact that damage from more than one low-LET track can interact, in turn, helps explain the linear-quadratic nature of the dose-response curve for chromosome aberrations. The comparison of the types of DNA damage induced by high- and low-LET radiations and their mode of repair has recently been reviewed by Stenerlow *et al.* (2002).

5.2.5.2 Induction of gene mutations and chromosome aberrations

The majority of mutations induced by high-LET radiations are large deletions, produced by failure to repair or misrepair multiple damaged sites or double-strand breaks. The multiple damaged sites have been shown to be difficult to repair (Boei *et al.* 2001). The higher proportion of multiple damaged sites with neutrons compared to X rays and gamma rays is a major reason for the higher RBE for neutrons.

For neutron-induced chromosome aberrations, the dose-response curve is linear over the complete dose range studied, and presumably at very low doses, also. This leads to the

conclusion that DNA double-strand lesions (including multiple damaged sites) are involved in their formation and that interacting pairs of these are produced by one radiation track (Mustonen *et al.* 1999). The specific molecular nature of the lesions involved has not been established, nor has the mode of their repair, although some form of recombination repair is most likely. Given the single track nature of formation of chromosome aberrations by neutrons, there is little or no reduction in aberrations frequency compared to that at high dose rates.

The difference in the spectrum of DNA damage and its cellular distribution between high- and low-LET radiations, has led to the search for specific biomarkers of high-LET radiation. Two recent studies exemplify this search. Brenner *et al.* (2001b), based on theoretical and experimental studies, have suggested that a so-called H value, which is the ratio of inter-chromosomal aberrations and intra-arm aberrations, should differ by a factor of about 3 between high- and low-LET radiations. Anderson *et al.* (2003) proposed, based on experimental studies, that the most effective biomarker of high-LET effects is the "profile of damage" that relies on the presence of insertions, a low frequency of stable simple reciprocal translocations, and the complexity of the chromosome damage initially produced.

Genomic instability persists for many years following high-LET exposures (Kadhim *et al.* 1998). Bystander effects are much easier to demonstrate for high-LET than low-LET radiations, in particular because of the ability to delineate the exposed cell population (Mothersill and Seymour 2001).

5.3 Summary

The preceding sections summarize the current state of knowledge on the induction of DNA damage by radiations of different qualities. It appears that double-strand breaks and some base damage quite possibly at multiple damaged sites (or sites of clustered damage) are the most important for the induction of chromosomal alterations and point mutations. These genetic end-points are largely the consequence of misrepair during one of the several known DNA repair processes, although errors of DNA replication can occur for DNA damage remaining at the time of replication. A number of cellular components and functions are involved in ensuring efficient and accurate repair. Mutations in one or more of these processes will result in increased sensitivity to the induction of genetic damage.

5.3.1 X radiation and gamma radiation

The extensive data base on the assessment of genetic effects in somatic cells following the A-bomb detonations and various radiation accidents and occupational exposures show that low-LET-radiations induce chromosomal alterations and gene mutations. The dose-response curve is predictable on the basis of induction by one or two radiation tracks, and the frequency at unstable chromosome aberrations is a good predictor of dose received. The other cytogenetic endpoints and gene mutational endpoints are less consistent biodosimeters. The induction of genetic alterations in germ cells is much less clear-cut. To date, it appears that the only suggestion of an induction and transmission of a mutation in human germ cells is for minisatellite alterations in males exposed as a consequence of the Chernobyl accident; however, this conclusion is equivocal.

Much of the data on the genotoxic effects of ionizing radiation was generated from *in vitro* systems. Evidence of chromosomal aberrations of various types is well documented and constitutes the primary effect of ionizing radiation exposure. Moreover, a differential effect has been demonstrated on germ cells and somatic cells. Studies of germ cell irradiation point to potentially heritable mutagenic effects such as minisatellite mutations. X rays and gamma radiation results in various germ cell effects in mice, including dominant lethal mutations, recessive autosomal mutations, and sex-linked recessive lethal mutations. Gene mutations in somatic cells also have been demonstrated from *in vivo* and *in vitro* studies in humans and experimental animals following ionizing radiation exposure.

5.3.2 Neutrons

The genetic effects induced by neutron radiation are qualitatively similar to the effects of X rays and gamma rays, but there are some quantitative differences. Several investigators have identified some potential cytogenetic fingerprints of neutron radiation based on these quantitative differences. These include the ratios of simple translocations to insertions (I-ratio), complete exchanges to incomplete rejoinings (S[I]-ratio), and dicentrics to interstitial deletions (H-ratio). In general, chromosomal aberrations, mutations, and DNA damage are induced more efficiently; DNA lesions are more severe and repaired less efficiently; and there are higher proportions of complex aberrations compared to low-LET radiation.

Studies of individuals accidentally or medically exposed to neutron radiation show that some chromosome aberrations can persist for decades, and some *in vitro* studies show genomic instability in progeny of irradiated human cells. Many *in vitro* studies conducted in the U.S., Europe, Russia, and Japan consistently demonstrate that neutron radiation induces chromosomal aberrations in human peripheral lymphocytes more effectively than gamma radiation. In contrast to studies in mice, the human data do not show statistically significant effects of parental exposure on chromosomal abnormalities and mutations in subsequent generations. Germ-line mutations observed in mice exposed to neutrons include dominant lethal, visible dominant, and recessive visible. Germ-line instability in mice has persisted for at least two generations. Somatic cell mutations have been detected at the *hprt* locus and in *ras* oncogenes and various cytogenetic effects, including chromosomal aberrations, SCE, and micronuclei, have been reported in mice. Reciprocal translocations were reported in rhesus monkeys. DNA damage, chromosomal aberrations, genomic instability, gene mutations, and cell transformations occurred in mammalian cells exposed to neutrons *in vitro*.

6 Other Relevant Data

6.1 Introduction

In order for ionizing radiation to alter biological tissues, the photon or particle must interact with cellular molecules, particularly DNA. The transmission and absorption of ionizing radiation in biological tissues are reviewed briefly in Sections 6.2 through 6.4 below.

Two types of radiation effects may be a consequence of ionizing radiation. The deterministic effects, which include both early and late effects, are summarized in Section 6.5. The stochastic effects of ionizing radiation have already been discussed in Section 3 (Human Cancer Studies), Section 4 (Studies of Cancer in Experimental Animals), and Section 5 (Genetic and Related Effects). Additional discussion on proposed mechanisms for radiation-induced cancer is included in Section 6.6.

Much information on cellular responses to radiation damage and the mechanisms for maintaining genomic stability has been gained from rare diseases that are recessively inherited and are known collectively as radiosensitive disorders. These "Experiments of Nature" are reviewed briefly in Section 6.7, and additional details on many of the topics discussed in that section are available in Appendix F.

6.2 Transmission and absorption of ionizing radiation in biological tissues

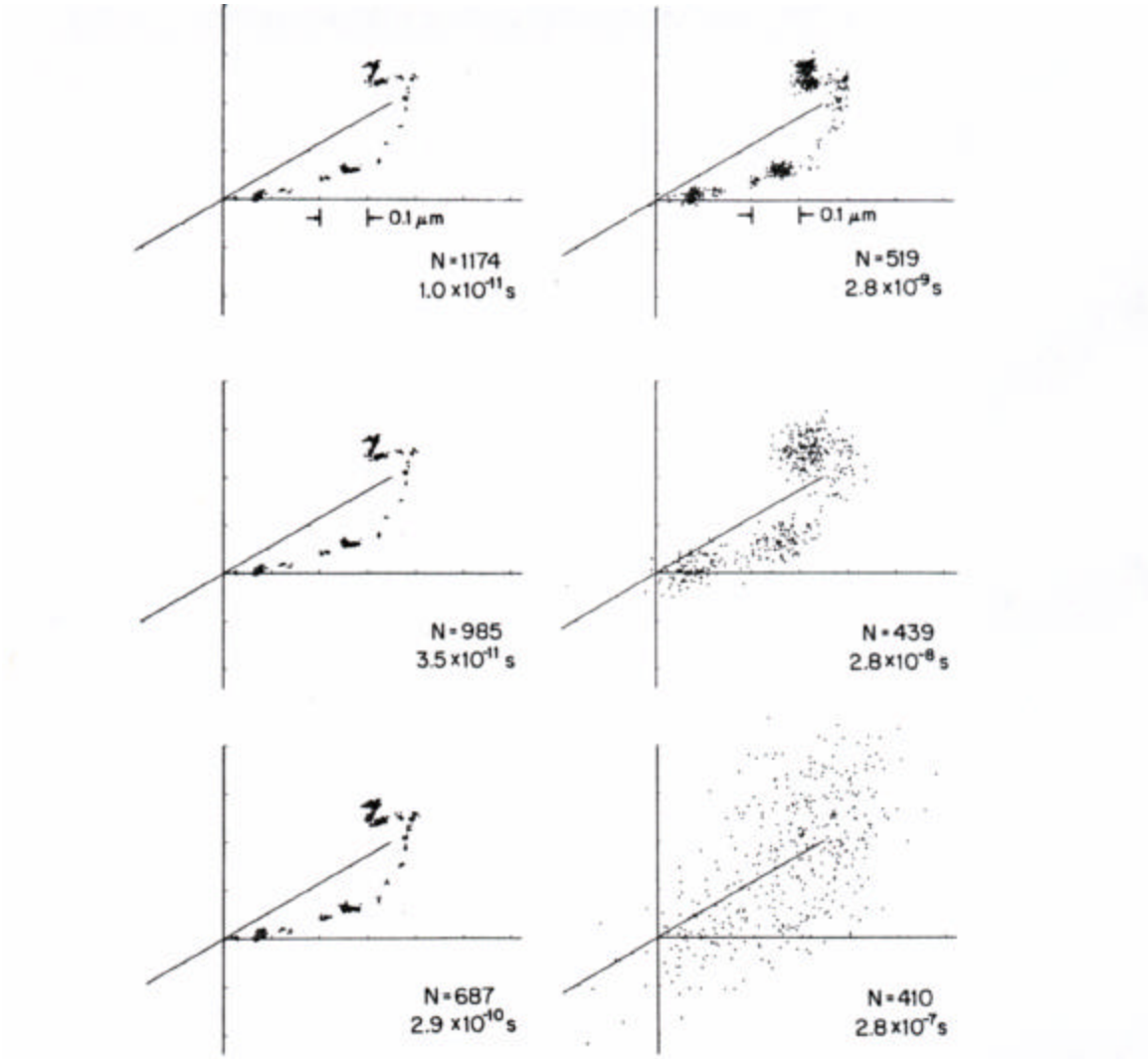
Ionizing radiation deposits energy in matter (including biological tissues) through interactions with the atoms in the material. The energy is deposited in discrete packages that are scattered throughout the medium non-uniformly, in random patterns. Characterization of the amount of radiation absorbed involves quantifying the amount of energy absorbed per unit mass of the target material or per unit path length of the radiation. Directly ionizing particles (electrons and alpha particles) ionize and excite molecules in the medium (mostly water molecules, in biological tissues); the ionized and excited species interact with each other and with other molecules. This may result in damage to critical molecules, such as strand breaks and base damage in DNA (Ward 1994), which may or may not be ultimately corrected by intracellular repair mechanisms. Other types of radiation, namely photons, including both gamma rays and X rays, and neutrons, are termed 'indirectly ionizing,' because they cause few ionizing events, principally with orbital electrons or protons (H atoms in water molecules), which themselves cause the vast majority of the ionization and excitation of species in the material.

Relating the amount of radiation absorbed to the ultimate biological effects observed continues to be a matter of intense scientific investigation. The classic, and mathematically easiest approach, is to average the energy absorbed over a large amount of mass, perhaps the entire mass of an organ or the entire mass of the organism. This averaging over large tissue masses, however, may cause difficulty in interpreting the subsequent biological effects if the patterns of energy distribution are non-uniform relative to the dimensions of the critical targets within the tissue. Identification of these critical targets continues to be a matter of study, but they are generally thought to be of

cellular or nuclear dimensions (i.e., micrometers, μm). Characterizing the radiation dose over dimensions smaller than the whole organ requires more effort, often involving the use of computer simulations involving Monte Carlo methods. Study of the stochastic nature of the energy deposition patterns at the cellular or subcellular level involves the science of microdosimetry (ICRU 1983), initially developed by Rossi and colleagues and currently of considerable interest in studying both dosimetry and the biological effects from radiation dose to cells in both *in vitro* and *in vivo* studies (e.g., Kramer and Kraft 1994, Kvinnsland *et al.* 2001, Pignol *et al.* 2001, Cruz *et al.* 2001, Wilson *et al.* 2001).

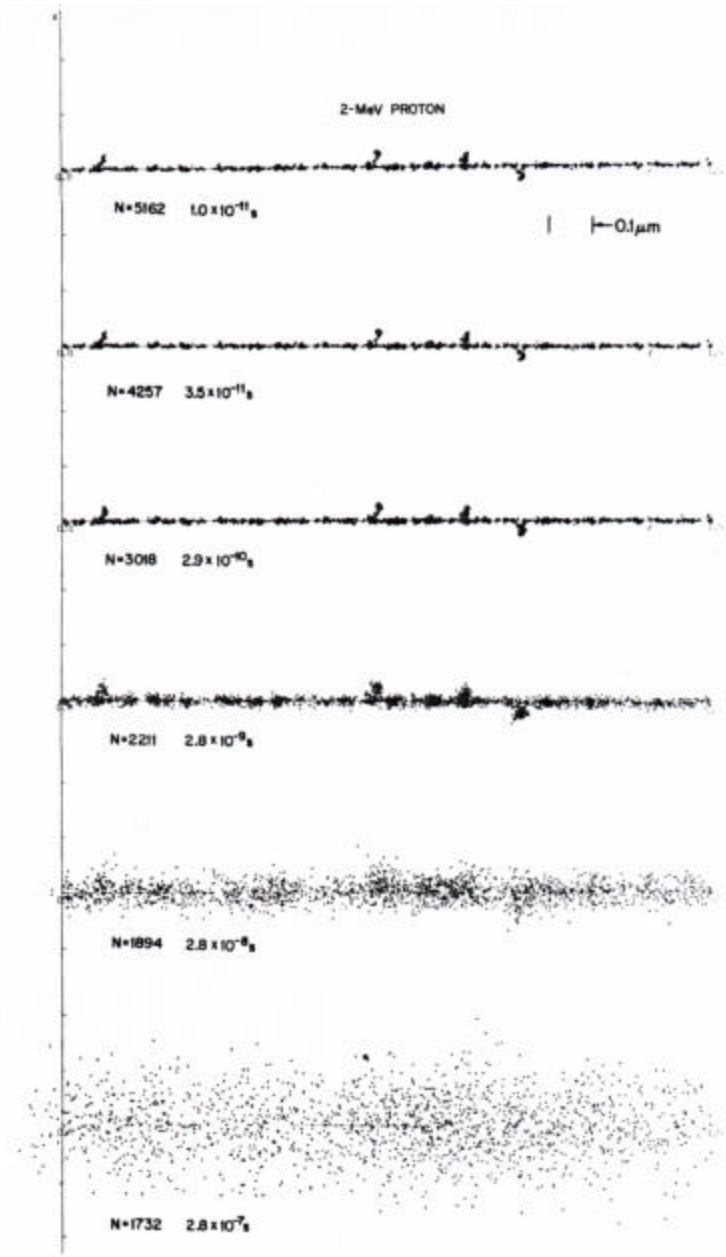
As briefly described above, all ionizing radiation deposits energy primarily through ionization or excitation of the atoms and molecules in the material through which it travels. Such ionizations and excitations can occur directly in a critical molecule, such as DNA, or in nearby molecules such as water (Nikjoo *et al.* 1997). Depending on their charge and mass, different types of particles will deposit their energy at different rates in tissue. Particles are roughly categorized as "high-LET" or low-LET" radiation. The more tortuous tracks in matter produced by low-LET radiation are illustrated in Figure 6-1, while the straight-line tracks produced by high-LET radiation are shown in Figure 6-2.

The choice of scale over which energy deposition is averaged from a given exposure scenario may have a profound impact on both the physical and biological interpretation of the results. In Figure 6-3, Fisher (1986) shows a hypothetical proton track in an aqueous medium. The proton, a high-LET particle, travels in a straight line, with numerous interactions (marked as x's) with atoms in the medium. Electrons, produced when individual atoms were ionized, themselves go on to ionize other atoms, with secondary interactions shown as dots in the tortuous paths leading away from the primary track. Note the dramatic difference in the absorbed dose calculated by choosing target volumes of 5, 10, 20, or 50 nm diameters (the definition of the absorbed dose values will be given below). Much conventional dosimetry, as noted above, averages dose over structures whose dimensions are of the order of cm, so the averaged doses become even several orders of magnitude smaller still. In some cases, doses averaged over such large dimensions can be reasonably correlated with biological effects. In other cases, usually involving doses from high-LET particles, good correlations can be obtained only by studying the doses on a more microscopic scale.



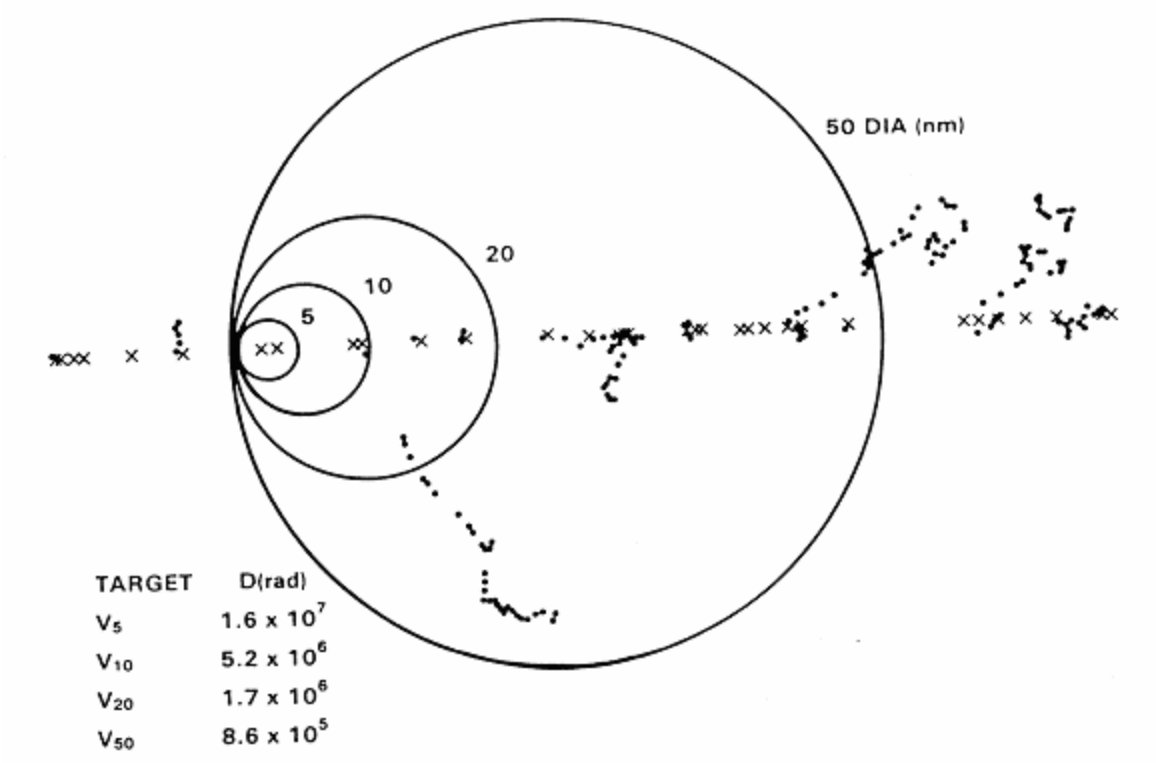
Source: Turner 1986.

Figure 6-1. Hypothetical tracks from a low-LET (5 keV electron) particle in water, as simulated by a Monte Carlo program. Individual dots represent reactive species (see section 6.3). The number of reactive species at any time given as N, their diffusion, and elimination by interaction are shown over time.



Source: Turner 1986.

Figure 6-2. Hypothetical tracks from a high-LET particle (2 MeV proton) in water, as simulated by a Monte Carlo program. Individual dots represent reactive species (see section 6.3). The number of reactive species at any time given as N, their diffusion, and elimination by interaction are shown over time.



Source: Fisher 1986.

Figure 6-3. Comparison of doses to microscopic spheres along the path of a hypothetical proton track

The detailed spatial and temporal properties of the initial physical features of radiation energy deposition influence the final biological consequences, despite the physical, chemical, and biological processes that eliminate the vast majority of the initial damage. Ionizing radiation produces many different possible clusters of spatially adjacent damage, and analysis of track structures from different types of radiation has shown that clustered DNA damage, including, but not limited to, double-strand breaks can occur at biologically relevant frequencies with all types of ionizing radiation and at any dose. Such clustered damage can be produced by a single track of ionizing radiation, with a probability that increases with ionization density but is not zero even for sparsely ionizing radiation such as X and gamma rays (Goodhead and Brenner 1983, Brenner and Ward 1992, Goodhead 1994).

6.3 Effects of radiation following energy absorption

Ionizing radiation also may interact with other molecules in the vicinity of DNA to produce reactive species that may produce modifications of DNA molecules that are similar to those resulting from direct interaction with ionizing radiation. The BEIR V (1990), ATSDR (1999), and the IARC Working Group (2000) describe the formation of reactive products of radiation degradation of water. These include free electrons (e-), ionized water molecules (H2O+), hydroxyl ions (OH-), hydrogen free radicals (H·), hydrogen ions (H+), and hydroxyl radicals (OH·). In the presence of molecular oxygen,

additional highly reactive molecules may be formed, e.g., hydrogen peroxide (H2O2), hydroperoxy radicals (HO2·), and hydroperoxy ions (HO2-). Since water makes up > 70% of cells, most of the energy absorption will involve water.

$$H_2O + IR \rightarrow e^- + H_2O^+$$

$$e^- + H_2O \rightarrow H_2O^- \rightarrow OH^- + H^{\chi}$$

$$H_2O^+ \rightarrow H^+ + OH^{\chi}$$

Source: ATSDR 1999.

Figure 6-4. Radiolysis of water

The most important reactive species formed, i.e., aqueous electrons, H, and OH radical, are produced in the proportions 45%, 10%, and 45%, respectively. The OH radical is an oxidizing agent that can produce a highly reactive site on DNA molecules by removing a H atom from the deoxyribose sugar of the DNA. Exposure to ionizing radiation causes the creation of ionized and excited species in biological tissue (shown as dots in Figure 6-1). These species are very reactive and thus short-lived, combining with each other or atoms and molecules within the medium and within a few microseconds of being formed. Below is a list of some of the important reactive species and a list of the diffusion coefficients (Table 6-1) and interaction coefficients (Table 6-2) of these reactive species in water (OH and H are hydroxyl and hydrogen radicals, e_{aq}^- is a hydrated electron).

Table 6-1. Diffusion coefficients of several reactive species in water

Species	D (10^{-5} cm^2 per sec)
OH	2.5
H_3O^+	9.5
e_{aq}^-	5.0
H	8.0
OH^-	5.3
H_2O_2	1.4

Table 6-2. Comparison of reaction coefficients for several reactive species in water

Reaction	k (10^{10} per M per sec)
$H + OH \rightarrow H_2O$	2.0
$e_{aq}^- + OH \rightarrow OH^-$	3.0
$e_{aq}^- + H + H_2O \rightarrow H_2 + OH^-$	2.5
$e_{aq}^- + H_3O^+ \rightarrow H + H_2O$	2.2
$H + H \rightarrow H_2$	1.0
$OH + OH \rightarrow H_2O_2$	0.55
$2e_{aq}^- + 2H_2O \rightarrow H_2 + 2OH^-$	0.5
$H_3O^+ + OH^- \rightarrow 2H_2O$	14.3
$e_{aq}^- + H_2O_2 \rightarrow OH^- + OH$	1.2
$OH + OH^- \rightarrow H_2O + O^-$	1.2

Source: Stabin *et al.* 1997.

6.4 Effects of dose rate and fractionation of low- and high-LET radiation

The dose rate may alter the effect of a given dose, particularly for low-LET radiation (BEIR V 1990). Delivery of low-LET radiation such as gamma rays and X rays at a low dose rate reduces the effectiveness of the dose as a result of several potential factors. These factors include repair of sublethal damage, the distribution of cells within the mitotic cycle, and the ability of cellular proliferation to compensate for the detrimental effects when exposure is protracted. The attenuation of damage resulting from high-LET radiation such as neutrons is much less than that for low-LET radiation (IARC 2000). This difference is attributed to a differing ability of cells to repair the damage induced by the different qualities of radiation.

6.5 Effects of neutrons on tissues

Neutrons interact with tissues in the body through five basic processes: elastic scattering, inelastic scattering, nonelastic scattering, capture reactions, or spallation processes. Elastic scattering describes an interaction in which the neutron interacts primarily by collisions with nuclei, with the nucleus remaining unchanged. The most important interaction of neutrons in soft tissues irradiated with neutrons at energies below 20 meV is with hydrogen. Inelastic scattering consists of interaction of a neutron with the nucleus resulting in prompt re-emission with reduced energy. The nucleus is left in an exited state and emits gamma rays. Nonelastic scattering is a process by which the neutron interacts with the nucleus to emit particles other than a single neutron, such as alpha particles and protons. Spallation occurs when the neutron-nucleus interaction results in the fragmentation of the nucleus and the emission of several particles and nuclear fragments.

The most important reactions in tissue for neutrons in the fission energy range are elastic and nonelastic scattering and the capture process. Inelastic and nonelastic scattering begin at about 2.5 and 5 MeV, respectively, and become important at an energy of about 10 MeV. As the neutron energy goes higher, nonelastic scattering and spallation reactions

increase in importance and elastic scattering becomes less important for energies greater than 20 MeV (BEIR V 1990).

6.6 Deterministic and stochastic effects of radiation

The reactions undergone in critical targets within living cells may lead to unrepaired damage. Deterministic effects tend to occur at radiation absorbed doses greater than 100 rads [1 Gy]. In high enough doses (> 5 to 50 Gy to individual tissues, depending on the system), this will be expressed within hours or days as clearly observable deterministic effects. Deterministic effects are radiation effects whose severity is dose-related, and which are not seen below a threshold dose level. Deterministic effects include erythema and other skin injuries, bone marrow depression, decreased fertility, and acute radiation syndrome.

Stochastic effects, including cancer induction, are known to occur at lower levels of dose. These levels are still high (> 0.5 Gy to the whole body) relative to the doses typically encountered in diagnostic medical examinations. Stochastic effects are those effects for which the probability of occurrence, and not the severity, is dose related. There is no known threshold for stochastic effects. The medical conditions resulting from stochastic radiation effects cannot be distinguished from similar conditions that arise spontaneously. Stochastic effects include cancer induction, teratogenesis, and mutagenesis. They are assumed to occur at doses lower than those typically observed for deterministic effects, but are generally not observed at radiation doses less than 10 rads. Radiation has been associated with most forms of leukemia and with cancers of many organ systems, increasing the occurrence of these cancers in certain exposed populations above the natural incidence. These cancers are generally expressed years or decades after the radiation exposure. Based on studies in animals, radiation also is thought to be capable of inducing hereditary effects in the offspring of exposed persons, but this effect has never been demonstrated in any human population. Radiation also can cause observable effects in children born to mothers who were exposed while pregnant to doses of radiation exceeding 0.15 Gy. As noted above, stochastic effects have been discussed in Sections 3, 4, and 5.

Whether “low” doses of radiation (such as occur in diagnostic medical examinations, occupational radiation exposure, and other minor exposures) can ultimately cause stochastic effects continues to be a subject of much debate. Although damage to cellular DNA within the cell in the form of double-strand breaks is not the only cellular damage of interest to the study of observable biological effects of radiation, it continues to be considered the main initiating event by which radiation may cause stochastic effects (UNSCEAR 2000). Damage to other cellular components may, however, influence the process of the expression of malignant disease in the exposed organism. Mechanisms exist for intracellular repair of DNA double-strand breaks, but they are error-prone, and are affected by dose, dose rate, and radiation quality (LET). Many data have been published showing an adaptive response in cells or organisms exposed to low levels of radiation and subsequently exposed again to a (generally) higher dose. This suggests that exposure to certain low levels of radiation may actually induce a response that reduces the risk of long-term expression of stochastic effects (i.e., ‘hormesis’). The absence of

any consistent departures from linear relationships between radiation dose and incidence of cancer, the activity of well characterized error-prone DNA repair pathways, and evidence on the spontaneous DNA damage in mammalian cells, however, continue to argue against establishment of a threshold for radiation effects at low doses, at least in general (UNSCEAR 2000). Further issues surround the possibility that communication processes between cells cause damage to be recorded in cells that did not undergo direct interactions with the radiation, the so-called "bystander effect" (See Section 5).

The early effects that follow exposure to ionizing radiation have been related primarily to cell death. The relative radiosensitivity of cells is determined by their reproductive capacity and degree of differentiation. Rubin and Casarett (reviewed by Hobbs and McClellan 1986) classified cells into five classes with varying degrees of radiosensitivity (Table 6-3).

The initial effects of whole-body irradiation at a relatively high dose (≥ 0.5 Gy) comprise a set of acute symptoms of gastrointestinal and neuromuscular symptoms known as the prodromal syndrome that occurs within one to two hours of the exposure (Hobbs and McClellan 1986). Estimates have been made of the approximate dose that should produce specific symptoms in 50% of exposed individuals. In increasing order of dose these are: anorexia, 1 Gy; nausea, 1.2 Gy; vomiting, 1.8 Gy; and diarrhea, 2.3 Gy. As the dose increases the most prominent clinical manifestations involve different organ systems. Although the effects of gamma rays and X rays may occur at a different range of doses compared to those produced by neutrons, the effects themselves are qualitatively similar.

At whole-body doses of approximately 0.5 to 10 Gy, the primary injury is to bone, resulting in the hematopoietic syndrome. Cells with shorter life spans in the peripheral circulation are most affected in this syndrome. A drastic fall in the number of circulating lymphocytes is one of the earliest observable changes and is seen one to two days after exposure even with doses as low as 0.5 to 1 Gy. Blood platelet counts also are likely to decrease, increasing the likelihood of hemorrhage. Frank anemia is not a common symptom at this level of exposure as erythrocytes are more radioresistant and have a longer lifespan than the leukocytes and platelets. The mean survival time is on the order of days to weeks.

Table 6-3. Classification of cellular radiosensitivity

Radiosensitivity	Class	Characteristics	Representative cell types
Highly radiosensitive	vegetative intermitotic cells	short-lived individual cells in a primitive undifferentiated state that divide to produce daughter cells	hematopoietic stem cells, dividing cells in intestinal glands, type A spermatogonia, granulosa cells of the ovarian follicles, germinal cells of the epidermis, gastric and holocrine glands, large- and medium-sized lymphocytes
Less radiosensitive	differentiating intermitotic cells	cells undergoing differentiation	differentiating hematopoietic series in intermediate stages of differentiation in bone marrow, more differentiated spermatogonia and spermatocytes, and oogonia
Intermediate radiosensitivity	multipotential connective tissue cells	cells that divide regularly or sporadically in response to specific stimuli	endothelial cells, fibroblasts, mesenchymal cells
Relatively radioresistant	reverting postmitotic cells	long-lived cells that do not divide at a high rate unless specifically stimulated	epithelial parenchymal cells, duct cells of salivary glands, liver, kidney, and pancreas; cells of the adrenal, thyroid, parathyroid, and pituitary gland
Most radioresistant	fixed postmitotic cells	normally non-dividing cells that are well differentiated and specialized in function	long-lived neurons, some muscle cells, neutrophils, erythrocytes, spermatids, spermatozoa, superficial cells of the alimentary tract, and epithelial cells of sebaceous glands

Source: Hobbs and McClellan 1986.

Higher doses of whole-body irradiation, i.e., approximately 10 to 50 Gy, are associated with shorter survival times of 5 to 10 days. The gastrointestinal syndrome to which these deaths are ascribed is characterized by bloody diarrhea and destruction of gastrointestinal mucosa.

The lethal effects of extremely high doses of whole-body radiation in the range of 50 Gy or greater are usually due to the central nervous system syndrome. The neurologic and cardiovascular degeneration that characterize this syndrome may be fatal within minutes to hours (usually less than 48 hours).

6.6.1 IARC review of deterministic effects of gamma radiation and X radiation

The IARC Working Group (2000) reviewed the deterministic effects of gamma rays and X rays. Most of the general description of deterministic effects of ionizing radiation described above are applicable to these low-LET forms of radiation since exposure to these forms through medical uses of radiation primarily involve gamma rays and X rays.

The IARC monograph on X radiation and gamma radiation discussed the radiation syndromes described above, together with the cutaneous radiation syndrome and the chronic radiation syndrome. All radiation syndromes depend on cell death, which may occur either during mitosis as a consequence of chromosomal alterations or during interphase through apoptosis. The Working Group reviewed in some detail what they referred to as the late deterministic effects of radiation based on patients that have undergone radiotherapy and survivors of the atomic bombs in Japan. Medical uses of radiation often involve repeated exposure to gamma rays or X rays. Thus, the observed effects are believed to depend on multiple factors of total dose, fraction size, and the interval between fractions. In general, higher doses can be tolerated with reduced or no effects on specific organs or tissues when the same total dose is received as fractionated doses rather than as a single dose.

Effects on skin, lung, gonads, kidney, gastrointestinal tract, hematopoietic system, central nervous system, thyroid, and eye are often evident several months after exposure to radiation. The underlying cause of these late effects of radiation is not completely understood, but likely involves loss of function through depletion of parenchymal cells. Effects of radiation on the skin are seen within hours after exposure to 5 Gy or greater of radiation (2 Gy or greater when the exposed fields are large). The lungs are susceptible to radiation-induced effects of pneumonitis and fibrosis that become evident several months to a year after the exposure. Gonadal tissues in both sexes are sensitive to the effects of radiation resulting in impaired fertility from effects on germinative cells and in decreased production of sex hormones by endocrine cells. The late deterministic effects on the kidneys, which include nephritis, nephrosclerosis, tissue necrosis and fibrosis, can lead to hypertension and loss of renal function when the total fractionated dose exceeds 23 Gy in adults or 12 to 14 Gy in children.

As noted above, the gastrointestinal tract is one of the most sensitive sites in the body for the acute effects of ionizing radiation; however, the sensitivity and response to radiation vary considerably in different parts of the gastrointestinal tract with different structure and kinetics of cell turnover. Damage to the intestinal epithelium may be detected in experimental animals exposed to > 1 Gy, but severe effects on the stomach may be seen at doses of 20 to 65 Gy. The most prominent effects are nausea, vomiting, and diarrhea associated with acute radiation syndrome. Acute effects of radiation exposure on the hematopoietic system have been described above. The most sensitive period for radiation effects on the central nervous system is during development when cells are proliferating. While the thyroid is subject to damage by radiation, most of the damage is a result of internal exposure to radionuclides of iodine rather than to external exposure to gamma rays or X rays. The most important radiosensitive structure within the eye is the lens, although the cornea, lachrymal gland, retina, and conjunctiva also may suffer damage at higher doses.

6.6.2 IARC review of deterministic effects of neutrons

The IARC Working Group (2000) also reviewed the deterministic effects of neutrons; however, the Working Group noted that less information is available about these effects compared to those of low-LET radiation such as gamma rays and X rays. The main

reason for this scarcity of relevant data is the much lesser use of neutrons in medical applications. In addition, the neutron component of the radiation released at Hiroshima was confounded by the other components of the radiation. Thus, most of the available information on the biological effects of neutrons has been derived from studies of those patients treated with neutrons and experimental studies of animals exposed to neutrons. As a high-LET radiation, neutrons produce effects that differ in important ways from those of low-LET radiation such as gamma rays and X rays. The modifying effects of dose rate, dose fractionation, tissue oxygenation, and cell cycle stage are less for neutrons than for low-LET radiation, and the radiosensitivity of oxygenated cells and variation in radiosensitivity across the cell cycle also are much less pronounced for neutrons. Finally, neutrons have a very narrow therapeutic window since high-LET radiation does not spare normal tissues from damage relative to tumor tissue.

Tissues with a high rate of cell division, including skin, gastrointestinal tract, and hematopoietic system, are generally more susceptible to the acute effects of ionizing radiation. While the responses and time courses of effects of high- and low-LET radiation on skin and gastrointestinal tract are qualitatively similar, the RBE for neutrons is generally > 1. The RBEs for the effects of neutrons on neural tissues range from approximately 5 to 10. RBEs greater than 2 but exceeding 5 in some instances also have been reported for spermatogonia, kidney, and lungs in mice exposed to neutrons. Induction of lens opacities, i.e., cataracts, in mice is associated with RBEs were < 10 at doses ≥ 1 Gy the, while much lower doses of ≤ 10 mGy were associated with RBEs > 100.

6.7 Proposed mechanisms for radiation-induced cancer

Ionizing radiation may induce cell death, immediate or delayed reproductive incapacitation, chromosomal aberrations, mutations, and oncogenic transformation (Iyer and Lehnert 2000). The effects of ionizing radiation involve DNA damage, including single-strand breaks, double-strand breaks, modifications of deoxyribose rings and bases, intra- and interstrand DNA-DNA cross-links, and DNA-protein cross-links. The genotoxic effects of ionizing radiation are discussed in Section 5.

Although a paradigm of radiation biology has long attributed most effects of ionizing radiation to DNA damage caused by interaction of ionizing radiation with cell nuclei, evidence is accumulating that suggests that cancer development may be related to effects of ionizing radiation that do not involve direct irradiation of cell nuclei. Thus, the potential exists for DNA damage to result from both direct and indirect effects of ionizing radiation. Evidence for a direct action of ionizing radiation on DNA was published half a century ago. Taylor *et al.* (1948) and Alper (1954) reported that exposure of DNA molecules *in vitro* to ionizing radiation (X rays) resulted in a decrease in the viscosity of the solution, which Taylor *et al.* (1948) interpreted as a reduction in the molecular weight of the DNA due to the introduction of DNA strand breaks. The experiment by Alper (1954) also showed that bacteriophage could be inactivated in a dose-responsive manner by ionizing radiation.

Two alternative pathways begin from the point at which damage to DNA is sensed by an irradiated cell (Szumiel 1998). One pathway leads to an attempt to restore the structure of DNA by recruitment of DNA repair enzymes to the damaged site. The other response leads to initiation of signaling cascades and activation of gene expression that leads to either cell cycle arrest or to cell death. Enzymes that are potentially activated by DNA damage, and thus may be part of a damage-sensing system, include poly(ADP-ribose) polymerase (PARP), DNA-dependent protein kinase (DNA-PK), the protein product of the ataxia telangiectasia mutated (ATM) gene, and the tumor suppressor, p53.

Ionizing radiation has been proposed to cause cancer by either a genetic, i.e., an alteration of cellular DNA, or an epigenetic, i.e., an alteration of the expression of genomic information, mechanism (Trosko 1996). Although a role for radiation-induced DNA damage and its associated repair mechanisms have been referred to by some as the "central dogma" of radiation biology (Szumiel 1998), some data are not yet easily explainable in terms of genetic damage. The review below focuses on mechanisms by which ionizing radiation may either alter the genomic information contained in DNA (genetic mechanism) or the transmission of this information (epigenetic mechanism). No attempt is made to relate these mechanisms to proposed models of carcinogenesis, e.g., the stem cell theory, the initiation, promotion/progression theory, the "oncogeny as partially blocked ontogeny theory," or the nature and nurture theory (Trosko 1996).

6.7.1 Mutations

The interactions of ionizing radiation with DNA have been summarized by the Agency for Toxic Substances and Disease Registry (ATSDR 1999). If a particle or photon of ionizing radiation has a direct interaction with DNA, the DNA molecule may be ionized as a result of the impact. The ionization will result in the separation of the molecule at the point of impact into two chemically reactive pieces. Because of their proximity, these pieces may recombine to recreate the original molecular structure, which does not result in any lingering effect of the ionizing radiation. Alternatively, the two chemically reactive parts of the molecule may be stabilized by chemical interaction with nearby molecules. The resulting molecular species will differ from the original DNA structure. The changes in structure may include strand breaks or modification of the nucleotide structure, e.g., point mutations. A variety of lesions may be induced in DNA by exposure to radiation; these include single- and double-strand breaks, crosslinks, and various types of base damage (Liber and Phillips 1998). The lesions may be caused directly by free radicals, including reactive oxygen intermediates (ROI), derived from the ionization of water molecules by radiation, e.g., hydroxyl radical, hydrogen radical, superoxide radical, and hydrogen peroxide. The variety of damage to DNA from exposure to ionizing radiation may result in a wide range of mutations, including base pair substitutions, small insertions or deletions, larger kilobase to megabase deletions, and chromosomal alterations. The latter include DNA amplification and homologous and nonhomologous recombination. The various types of DNA lesions were described in Section 5.

6.7.2 Initiation and promotion

The possible roles for ionizing radiation at various stages of a multi-step carcinogenic process have been discussed by Trosko (1996). Trosko (1996) reviewed the few studies

that were available at that time in which ionizing radiation had been tested in an initiation/promotion/progression bioassay. The overall conclusion of these studies was that ionizing radiation acts as a weak initiator but is an effective promoter. Trosko (1996) noted that one study by Jaffe *et al.* (1987) indicated that "there might be a small but finite probability that it [ionizing radiation] could act as a progressor of carcinogenesis." Trosko (1996) further discussed possible involvement of tissue-specific stem cell populations with varying sensitivities to ionizing radiation and the potential role of gap junctionally coupled syncytia in modulating responses to radiation. He speculated that stem cells, which may lack gap junctional communication, could be more sensitive to radiation-induced changes in cell redox state through generation of reactive oxygen species. His final summation on the state of knowledge about the role of ionizing radiation in initiation/promotion/progression concluded that further studies were needed to test these mechanisms since so few experiments to test these ideas had been designed and executed prior to 1996.

Although the above discussion represents one way of describing the development of cancer cell genotypes, other ways of looking at the transformation from a normal cell to a malignant cell have been proposed. Recently, (Hanahan and Weinberg 2000) proposed that the following six essential alterations in cell physiology are necessary for malignant growth: 1) self-sufficiency in growth signals, 2) insensitivity to growth-inhibitory (antigrowth) signals, 3) evasion of programmed cell death (apoptosis), 4) limitless replicative potential, 5) sustained angiogenesis, and 6) tissue invasion and metastasis. Although Hanahan and Weinberg suggested that the same altered functions are necessary, they acknowledged that the specific physiologic changes likely differ in number and type for different cancer cells.

6.7.3 Delayed and indirect effects of ionizing radiation on DNA

Although a direct interaction between ionizing radiation and DNA molecules has been postulated as the mechanism underlying mutation and carcinogenesis, a number of researchers have published data that suggest that irradiation of cells *in vitro* may result in mutations in cells that do not display immediate or direct damage due to radiation. Several mechanisms that have been proposed by which these delayed or indirect effects of ionizing radiation could alter the genetic makeup of cells include 1) induction of mutations by cytoplasmic irradiation, 2) radiation-induced genomic instability, and 3) "bystander effects" in irradiated cell populations; the latter two topics were discussed in Section 5.

Mothersill and Seymour (1998) discussed the delayed effects of ionizing radiation exposure, including genomic instability. Since one common outcome of the delayed response of cells to radiation is an increased death rate, i.e., a decrease in reproduction, after generations of normal cell reproduction, the authors proposed that an aftereffect of exposure to ionizing radiation that ultimately resulted in the death of affected cells, even generations later, is more likely a protective effect against carcinogenesis. Cells affected by this phenomenon are often able to divide for many generations at the same rate as unirradiated cells, then at some point they have a failure in clonogenicity. The effect

appears to be random and is by its nature non-clonal, since it occurs in cells that previously reproduced at a rate consistent with that of control cells.

Induction of mutations by cytoplasmic irradiation were described by Wu *et al.* (1999) based on microbeam (alpha particles) irradiation of either nuclei or cytoplasm of human-hamster hybrid (A_L) cells. Cytoplasmic irradiation induced minimal cellular toxicity under these conditions, and mutant cells were detected by loss of function of the *CD59* locus, which allowed cells to survive exposure to an anti-CD59 antibody that killed wild-type cells in the presence of complement. The mutant spectrum of these *CD59*$^-$ cells consisted primarily of small alterations involving only the *CD59* gene and were similar to spontaneous mutations. In contrast, nuclear irradiation induced primarily multilocus deletions. The authors suggested that the genomic effects of cytoplasmic irradiation were most likely mediated by reactive oxygen species. Since cytoplasmic irradiation has a low toxicity, the authors estimated that irradiation of the cytoplasm could induce seven times as many mutants as an equitoxic dose of nuclear irradiation.

6.8 Cellular responses to radiation damage and the radiation-sensitive disorders

The IARC Working Group (2000) and UNSCEAR (2001) reviewed some of the genetic disorders that increase the sensitivity to radioactivity at the cellular level. The risk for development of cancer is enhanced for individuals with specific types of genetic disorders that increase the sensitivity to radiation at the cellular level. Ataxia-telangiectasia (A-T) is the prototype for radiation sensitive disorders and has already taught us much about how cells respond to radiation damage since the ATM gene, defective in A-T patients, was cloned seven years ago. A brief review of radiation sensitive disorders and cancer risk follows.

6.8.1 Background

Awareness of the radiation-sensitive disorders (RSD) developed essentially from the early observations of Gotoff *et al*. (1967) who noted an "untoward response" to gamma radiation in a child with A-T undergoing radiotherapy for cancer. The corollary observation in the laboratory was that fibroblasts from A-T patients were radiosensitive to < 1 Gy (Taylor *et al.* 1975). This radiosensitivity was attributed to a DNA repair defect, and was later shown to be associated with repair of *double* strand DNA breaks. Unlike cells of patients with xeroderma pigmentosum, which were sensitive to UV radiation but not ionizing radiation, A-T cells showed the reverse pattern, radiosensitivity but not sensitivity to UV light, the latter creating primarily *single* strand DNA breaks (Cleaver 1968). Of the many laboratory assays that were subsequently established for characterizing cellular responses to ionizing radiation, two have prevailed: colony survival (CSA) (Taylor *et al.* 1975, Paterson *et al.* 1985) and radioresistant DNA synthesis (RDS) (Young and Painter 1989). Virtually all patients with A-T were radiosensitive in both assays (Painter 1983).

Most other chromosomal instability syndromes also are radiosensitive by CSA, including: Nijmegen Breakage Syndrome (NBS), Mre11 deficiency (aka ATLD), ligase IV (LIG4) deficiency, Fanconi anemia, and several immunodeficiencies (Sun *et al.*

2002), but not Bloom syndrome. The other form of recessive early-onset ataxia, Friedreich's ataxia, is not radiosensitive by CSA.

From 1981 to 1995, an international effort was made to identify the gene responsible for A-T (ATM) (Gatti *et al.* 1988, Lange *et al.* 1995, Savitsky *et al.* 1995) since this gene appeared to play a pivotal role in both cancer genetics and radiation biology. The ATM protein was found to be a high molecular weight PI-3 kinase, phosphorylating serine or threonine residues in many target substrates that are important in cell cycle control, DNA repair, and responses to oxidative stress (Jongmans and Hall 1999, Shiloh and Kastan 2001).

Of particular interest to this report, clinical correlates of *in vitro* radiosensitivity have recently been reported for NBS and LIG4 deficiency (Bakhshi *et al.* 2003, Riballo *et al.* 1999), further extending the observations of Gotoff *et al.* (1967). Knockout mouse models for the RSD genes result mostly in embryonic lethals, i.e., the embryo does not live to full term, with the notable exceptions of the *atm* (Barlow *et al.* 1996) and *H2AX* (Celeste *et al.* 2002) knockout mouse models.

6.8.2 General concepts linking RS with cancer and immunodeficiency

Each of the RS disorders is very rare in the general population; however, it also is important to consider the impact of radiosensitivity in *heterozygotes or carriers*. Here again, present understanding stems primarily from studies of cells from patients with A-T and related disorders. In general, A-T patients' parents, who are obligate heterozygotes, do not manifest 'untoward responses' to radiation therapy (Weissberg *et al.* 1998), although a few have developed telangiectasias and other skin changes over the areas that were exposed to radiation. In the laboratory, fibroblasts from A-T heterozygotes are intermediate in radiosensitivity (Lavin *et al.* 1995, Lavin 1998, Jongmans and Hall 1999); ATM protein levels also are intermediate (Gatti, unpublished data). When patients with severe acute radiation reactions have been analyzed for mutations in the ATM gene, no convincing correlations have been revealed (for example, see Clarke *et al.* 1998).

Although A-T carriers are at an increased risk of cancer (Swift *et al.* 1991), Atm+/- 'knockout' mice do not develop more tumors than normal mice of the same genetic background. On the other hand, a newer 'knock-in' mouse model has been designed to address the *in vivo* effects of missense ATM mutations and possible dominant negative effects: delSRI+/- mice (with deletion of three amino acid residues – serine, arginine, and isoleucine --from the ATM protein) *do* manifest an increased frequency of tumors, and their cells are intermediate in radiosensitivity (Spring *et al.* 2002, Concannon 2002). Thus, rather than considering only conventional heterozygosity or carrier status for RSD genes, 'dominant negative' mutations may be causing more radiosensitivity, and cancer, in the general population than is presently appreciated. NBS heterozygotes also are at an increased risk of cancer (Sperling *et al.* 2002). Once any of these carriers become cancer patients who receive radiation therapy, they represent a population of potentially radiosensitive individuals who may manifest radiation reactions and skew the "safe" dosage ranges for the radiotherapy of future patients.

Since unphysiological (unrepaired) gene rearrangements are associated with many cancers, it could have been anticipated that radiosensitivity, cancer susceptibility, immunodeficiency, and DNA repair defects would appear together and be common to most chromosomal instability disorders. Most of the RSD genes discussed herein also are considered to be tumor suppressor genes of the caretaker variety, i.e., genes that induce neoplasia only indirectly (Kinzler and Vogelstein 1996, 1997, Levitt and Hickson 2002). Mutations in tumor suppressor genes generally result in tumor formation only after both alleles have been inactivated; thus, a dominantly inherited mutation (such as in the BRCA1 gene) does not usually manifest as cancer until a second somatic mutation occurs, leaving the cell without either functioning copy of that protein (Knudson 1971). The IARC Working Group (2000) and UNSCEAR (2001) discussed a number of tumor suppressor genes for which mutations might be expected to lead to a predisposition to cancer, such as in retinoblastoma, Li-Fraumeni syndrome (p53 and CHEK2 mutations), nevoid basal-cell carcinoma syndrome (*patched* gene), and breast cancer (*BRCA1* and *BRCA2)*.

6.8.3 Ataxia-Telangiectasia, a prototype for radiosensitivity and cancer susceptibility

6.8.3.1 Phenotype

A-T is primarily an early onset, and relentlessly progressive, cerebellar ataxia (Boder and Sedgwick 1963, Boder 1985, Gatti *et al.* 1991, Gatti 2001a) that is transmitted as an autosomal recessive disorder. It occurs in approximately 1 per 40,000 live births in the U.S. This frequency varies considerably from country to country depending upon the degree of inbreeding. Infants appear normal and walk at a normal age (one year), begin to stagger by age 3, and generally require a wheelchair by age ten. Oculocutaneous telangiectasias appear several years after onset of neurological symptoms. Ocular apraxia and dysarthria become apparent early. Frequent sinopulmonary infections are common. Cancer, usually lymphoid, occurs in one-third of A-T patients during their lifetimes (see below). In young children, the neurological diagnosis is often difficult to establish. Laboratory findings include: (1) elevated serum alphafetoprotein (AFP), (2) immunological deficiencies, (3) characteristic chromosomal aberrations, such as t(7;14) translocations and telomeric fusions; the rate of telomeric shortening also is increased, (4) decreased or absent intracellular ATM levels by western blotting, (5) deficient ATM phosphorylation of many substrates, such as p53-Serine 15, (6) ATM mutations, and (7) *in vitro* radiosensitivity, such as colony survival and radioresistant DNA synthesis (Gatti 2001b). There is presently no treatment for A-T, although some of the secondary symptoms, such as drooling, are amenable to supportive therapy. Patients who do not die of early incurable cancers or overwhelming infections, sometimes survive into their forties and even fifties.

6.8.3.2 Immunodeficiency

The most consistent immune defects of A-T patients are those of IgA, IgE, IgG2, or IgG4 deficiencies (Ammann *et al.* 1969, Oxelius *et al.* 1982, Rivat-Peran *et al.* 1981, Gatti *et al.* 1982). In general, however, about one-third of A-T patients do not manifest any obvious immunodeficiency, nor do they have increased infections. Only one immune parameter is consistently abnormal in A-T – the thymus is dystrophic, with poor corticomedullary differentiation, and no Hassall's corpuscles (Peterson *et al.* 1964,

Amromin *et al.* 1979). This probably reflects a perturbation in the maturation of T cells, as they try to rearrange the T cell receptor (TCR) genes, a form of nonhomologous recombination. A similar situation probably arises during the differentiation of B cells. Reguiero *et al.* (2000) exhaustively reviewed the immunological literature of A-T.

6.8.3.3 6.7.3.3. ATM mutations

Over 400 unique mutations in the ATM gene have been described in A-T patients worldwide (www.vmresearch.org/atm.htm). Most A-T patients are compound heterozygotes, i.e., they inherit a different mutation from each parent. These mutations do not cluster to any particular region of the gene, and only about a dozen mutations have been observed with > 1% global frequency. These recurring genes appear to be ancestrally related (Campbell *et al.* 2003) rather than recurring mutational events ('hot spots'); they are associated with specific ethnicities and haplotypes (as defined by both short tandem repeat and single nucleotide polymorphism markers) and some mutations appear to be thousands of years old. However, even these dozen recurring mutations account for only about one-third of all ATM mutations in A-T patients, the other mutations being of very low frequency.

6.8.3.4 Cancer risk

ATM homozygotes

Much has been written on the cancer profile of A-T homozygotes, based on just a few well-documented studies (Gatti and Good 1971, Spector *et al.* 1982, Morrell *et al.* 1986). These patients "develop new cases of cancer at approximately 100 times the age-specific population rate" (Su and Swift 2000). Most cancers (85% for patients under 20 years) involve the lymphoid system, either as lymphomas or leukemias; lymphomas are more common. A characteristic T-cell leukemia (T cell prolymphocytic leukaemia [T-PLL]) occurs in older A-T patients in which TCL-1 protooncogene is upregulated (Chun *et al.* 2002). Younger A-T patients typically develop T-cell acute lymphoblastic leukemia (T-ALL). In patients over 20 years of age, approximately half of the cancers are non-lymphoid, occurring in the following order of decreasing frequency: stomach cancer, breast cancer, medulloblastoma, basal cell carcinoma, ovarian dysgerminoma, hepatoma, and uterine leiomyoma (Su and Swift 2000).

ATM heterozygotes

ATM mutations and 11q22-23 loss of heterozygosity (LOH) have been reported in association with breast, prostate, and ovarian cancer, head and neck cancer, lymphoma and leukemia (Carter *et al.* 1994, Koike *et al.* 1999, Laake *et al.* 1997, Stilgenbauer *et al.* 1999, Lu *et al.* 2001). Clearly, the ATM gene plays a role in oncogenesis. In the knockout ATM -/- mouse, almost every animal develops a thymic lymphoma by four months of age (Barlow *et al.* 1996). With further scrutiny, however, several caveats become apparent. First, ATM mutations for some human cancers are somatic, not inherited. Such individuals would not be at an increased risk for cancer, at least with regard to the ATM gene (Stilgenbauer *et al.* 1997). With T-PLL and chronic lymphocytic leukemia (CLL), while it is clear that the ATM gene and protein are involved in the pathogenesis, only about one-third of patients were ATM heterozygotes (Vorechovsky *et al.* 1997, Stankovic *et al.* 1999). Two mechanisms are implicated in such patients: 1) a

'second hit' to disable the remaining normal allele, or 2) a dominant negative mutation. For non-carriers, two hits would have to occur: however, these individuals would not constitute an 'at risk' population.

6.8.3.5 Molecular studies of ATM function

Efforts to understand A-T have turned towards unraveling the function of ATM, the protein that is absent or non-functional in all A-T patients. In general, the complexity of the role of ATM in cells parallels the multi-faceted phenotype of the disorder. What also is becoming clear is that this otherwise basic research on a rare disorder is unraveling new therapeutic strategies for cancer patients and for individuals exposed to ionizing radiation.

ATM is involved in sensing DNA double-strand breaks that are caused by metabolic/cellular events. Once damage has occurred, ATM is activated and proceeds to activate numerous proteins involved in different signaling pathways participating in cell cycle checkpoints, DNA damage repair, and stress-activated apoptosis. ATM is involved in initiating the mechanisms necessary to maintain the cell's genomic integrity, making it a crucial component in the immediate response to potentially damaging events. The role of ATM-dependent cell signaling in response to radiation damage is discussed in more detail in C.3.5.

The disease was linked to chromosome 11q23.1, and this eventually led to the cloning of a single ATM gene (Gatti *et al.* 1988, Lange *et al.* 1995, Savitsky *et al.* 1995). Based on protein sequence homology, ATM is a member of a family of high molecular weight kinases that share the phosphoinositide-3 kinase (PI3K) domain in the C-terminal end. ATM-deficient cells are sensitive to ionizing radiation (IR) and radiomimetic agents and unaffected by UV exposure, although some investigators report defective UV-mediated pathways in A-T cells

6.8.3.6 Apoptosis

ATM induction of apoptosis is presumed to occur when DNA damage is too severe to repair. Stress-activated protein kinase (SAPK) activity is defective in A-T cells when induced by IR, whereas UV- and anisomycin-treated A-T cells exhibit a normal SAPK response (Shafman *et al.* 1995).

6.8.3.7 Telomere and Chromosome Maintenance

ATM participation in the maintenance of telomere structure occurs through its influence on telomeres rather than of telomerase activity. Telomeres are DNA repeats (TTAGGG)n placed in tandem at the ends of chromosomes to protect important DNA sequences from loss or damage produced by exonucleolytic activity, breakage of chromosome ends, or incomplete replication (reviewed by Zakian (1995) and Pandita (2002)). Incorrectly maintained telomeres are exposed and resemble the ends of double strand DNA breaks. A-T cells have shortened telomeres and telomere fusions, characteristic of telomere instability. Accelerated telomere shortening occurs in A-T cells (Metcalfe *et al.* 1996).

6.8.3.8 Other ATM-dependent phosphorylation pathways

ATM plays a complex role in many different aspects of the cellular response to radiation damage. ATM's primary focus most likely involves the repair of regularly broken DNA strands that must be rapidly rejoined. It plays a major role in the chromatin remodeling that is necessary for transcription. Some A-T patients show insulin-resistant and glucose intolerant diabetes, indicating a possible ATM-related defect in insulin-signaling pathway. Judging from the severe ataxia that occurs in A-T patients, ATM also must be important in neurogenesis. In neuronal cells, in which ATM has a predominantly cytoplasmic localization, progress has been slow due to the lack of good neurodegeneration models and stem cell research restrictions (Soares *et al.* 1998, Barlow *et al.* 2000). Perhaps the role of ubiquitin in synaptic function will provide the key to unraveling how ATM protects neuronal integrity (Wilson *et al.* 2002, Ehlers 2003).

6.8.4 Nijmegen breakage syndrome (A-T variants 1 and 2)

NBS was first considered a variant of A-T because the patients were immunodeficient, cancer prone, t(7;14) translocations were noted during karyotype analyses, and the cells were radiosensitive (Weemaes *et al.* 1981, Jaspers *et al.* 1988, Sun *et al.* 2002). However, Sendai virus-fused fibroblasts from A-T and NBS patients corrected ('complemented') the radiosensitivity of both, suggesting that two distinct genes were involved (Jaspers *et al.* 1988). Molecular studies have corroborated the close phenotypic relationships between A-T and NBS; nibrin, the protein lacking in NBS patients, is responsible for the nuclear localization of the Rad50/Mre11/NBS1 (R/M/N) complex.

6.8.5 A-T_{Fresno}

This phenotype describes a small subset of patients with symptoms of both A-T and of NBS (Curry *et al.* 1989). Unlike most A-T patients, these children are microcephalic, growth retarded, and often mentally retarded. The serum alphafetoprotein level is elevated. CSA shows a radiosensitivity similar to that of classical A-T (Huo *et al.* 1994). These patients carry mutations in the ATM gene, which vary from site to site, and appear to progress clinically as typical A-T patients (Gilad *et al.* 1998, Becker-Catania *et al.* 2000). Considering that nibrin is a phosphorylation target of ATM, it is not surprising to see both A-T and NBS symptoms in these patients. It is possible that some A-T_{Fresno} patients may exist who have mutations in NBS1 rather than ATM.

6.8.6 MRE11 deficiency (aka ATLD= AT-like disorder)

During the positional cloning of the ATM gene, a family with AT-like symptoms in two siblings did not link to chromosome 11q22.3-23.1 (Hernandez *et al.* 1993, Stewart *et al.* 1999). Subsequently, it was found that these patients had normal amounts of ATM protein but lacked the Mre11 protein of the Rad50/Mre11/nibrin complex. Progression of the neurological symptoms was somewhat slower than in A-T. Telangiectasiae were not present; the AFP remained normal.

6.8.7 Ligase IV deficiency

Ligase IV (LIG4) forms a complex with XRCC4 as the final step in the pathway of non-homologous end joining. LIG4 deficiency was first observed in a 14-year-old patient

(180BR cell line) with leukemia who dramatically over-responded to radiation therapy (Riballo *et al.* 1999). The most striking finding in LIG4 patients has been that cell cycle checkpoints are normal, suggesting that sensitivity to ionizing radiation in mammalian cells arises primarily from problems in the sensing or repair of double-strand breaks and not from cell cycle checkpoint defects.

6.8.8 BRCA1 and BRCA2

The BRCA1 and BRCA2 genes were identified by positional cloning, tracking, and analyzing genetic linkages in families with multiple affected breast cancers (Miki *et al.* 1994, Wooster *et al.* 1995). Mutations in BRCA1 also predispose to ovarian cancer. After several false starts, it was established that the BRCA1 gene plays a major role in maintaining genome stability. Mouse embryos carrying a BRCA1 null mutation are hypersensitive to gamma irradiation. Despite much evidence implicating BRCA1/2 in radiation sensitivity, when Leong *et al.* (2000) analyzed these genes in 22 cancer patients who had experienced severe normal tissue reactions after radiation therapy, no mutations were found. Perhaps future genetic studies of such patients also will screen the NBS1 and LIG4 genes.

6.8.9 Fanconi anemia

Fanconi anemia (FA) has typically been viewed as a childhood disorder, with bone marrow failure and cancer manifesting within the first decade of life. The cancers include acute myeloid leukemia and squamous cell carcinoma of the head and neck. These children also have growth retardation, skeletal defects, such as microcephaly and absent thumbs or radial bones, and abnormal skin pigmentation. FA is an autosomal recessive disorder that can result from mutations in one of at least eight distinct complementation groups or FANC genes, the products of which function as a complex.

6.8.10 Radiosensitivity associated with primary immunodeficiency

The association of RS with immunodeficiency is not a new concept, especially since the majority of patients with A-T and NBS manifest both. Vorechovsky *et al.* (1989, 1990) reported RS in cells from a patient with common variable immunodeficiency. Lymphoblastoid cell lines (LCLs) from patients with X-linked agammaglobulinemia are RS (Huo *et al.* 1994). DNA-PK deficient cells from severe combined immunodeficiency (SCID) mice are RS (Taccioli *et al.* 1994). RS also has been noted in cells from patients with other forms of SCID (Gatti *et al.* 2001). In most of these disorders, the underlying genetic defect has been identified. Despite this, the relationship between abnormal maturation of the immune system and hypersensitivity to ionizing radiation remain unclear. Most of these genetic disorders also are associated with increased cancer risk (Gatti and Good 1971, Spector *et al.* 1982). The prevailing wisdom suggests that unrepaired DNA damage, especially of double-strand breaks, leads to downstream consequences, such as translocations and microdeletions that either delete gatekeeper and caretaker cancer genes (Kinzler and Vogelstein 1996, 1997) or displace/dissociate them from their normal transcriptional control elements.

6.8.11 Conclusions - radiosensitive disorders

The radiosensitive disorders are rare (orphan) diseases that are recessively inherited, in that both copies of the gene must be defective in order to manifest symptoms. As "Experiments of Nature", they are helping to elucidate cellular responses to radiation damage and the mechanisms for maintaining genomic stability. Radiosensitivity is often found in association with immunodeficiency, cancer, and DNA instability. Certain types of missense mutations can cause symptoms when only a single defective copy in inherited, suggesting an alternative dominant mode of inheritance. This has profound public health implications since substantial numbers of people carry only a single defective copy of such genes; these individuals also are cancer susceptible and may be sensitive to lower doses of ionizing radiation than are presently considered safe for the general public. These individuals, if they become cancer patients, may be distorting the "safe effective dosages" of some radiation therapy protocols. Developing cost-effective laboratory methods for identifying such individuals will be important when radiation exposure beyond normal levels is anticipated. Effective agents for treating children with radiosensitive disorders, such as A-T, may someday also prove useful for treating individuals exposed to increased levels of radiation.

6.9 Summary

Exposure to ionizing radiation results in two broad categories of effects, deterministic and stochastic. Deterministic effects, which include skin burning, blood count effects, and cataracts, have a definite threshold dose, above which the severity of the effect increases with increasing dose. Stochastic effects, which include cancer and hereditary effects, are random in nature and do not have a threshold dose. While the probability of a stochastic effect increases with dose, the severity of the effect in an individual does not.

Effects of ionizing radiation on biological tissues requires that the energy associated with the radiation be deposited within the tissue through interactions with the atoms in the material. The rate at which the ionizing radiation deposits its energy in matter is different for different types of radiation. This energy transfer is termed the linear energy transfer (LET) of the radiation and differs for X rays, gamma rays, and neutrons. X rays and gamma rays as photons are categorized as low-LET radiation while neutrons are high-LET radiation. X rays, gamma rays, and neutrons are considered indirectly ionizing radiations because they most frequently cause ionization of water molecules with production of reactive products that may produce modifications of DNA molecules. These reactive products include free electrons, ionized water molecules, hydroxyl ions, hydrogen free radicals, hydrogen ions, hydroxyl radicals, and, in the presence of molecular oxygen, hydrogen peroxide, hydroperoxy radicals, and hydroperoxy ions. When reactions of these products with living cells produce unrepaired damage, deterministic and stochastic effects may result. It is not clear if "low" doses of radiation received during diagnostic medical examinations, for example, can ultimately cause stochastic effects.

The early effects of ionizing radiation are deterministic effects that relate primarily to cell death and vary with the radiosensitivity of cell populations. The prodromal syndrome comprises a set of acute symptoms of gastrointestinal and neuromuscular symptoms that

are seen as the initial response to whole-body irradiation. Increasing doses are associated with decreased survival time and with primary lethal effects that range from the hematopoietic syndrome through the gastrointestinal syndrome to the central nervous system syndrome. The IARC Working Group reviewed the deterministic effects of X rays and gamma rays in 2000. The Working Group reviewed the effects of these low-LET ionizing radiations on the skin, lungs, gonads, kidneys, gastrointestinal tract, hematopoietic system, central nervous system, thyroid, and eye. The Working Group also reviewed the deterministic effects of neutrons, which are less well described because of the lack of information. As a high-LET radiation, neutrons produce effects that differ in important ways from those of the low-LET X rays and gamma rays. One difference is the higher relative biological effect (RBE) of neutrons compared to low-LET radiation.

While exposure to ionizing radiation has been related to the later appearance of cancer, several mechanisms by which ionizing radiation could cause cancer have been proposed. While ionizing radiation may induce DNA damage directly, resulting in single-strand breaks, double-strand breaks, modifications of deoxyribose rings and bases, intra- and interstrand DNA-DNA cross-links, and DNA-protein cross-links. While ionizing radiation has been proposed to cause cancer by this alteration of cellular DNA, or genetic mechanism, some researchers have proposed epigenetic mechanisms in which the expression of genomic information is altered. These proposed mechanisms include radiation-induced genomic instability, induction of mutations by cytoplasmic irradiation, and “bystander effects,” which are based on mutational events occurring in cells that do not directly receive exposure to ionizing radiation.

Certain genetic disorders predispose affected individuals to radiation sensitivity and cancer. These disorders include ataxia-telangeictasia (A-T), Nijmegen breakage syndrome, Mre11 deficiency, and ligase IV deficiency. A-T is an early onset and progressive cerebellar ataxia that occurs in 1 out of 40,000 live births. By age ten, most affected individuals require the use of a wheelchair. Mutations of the A-T gene have been associated with breast and prostate cancer, head and neck cancer, lymphoma, and leukemia.

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8 Glossary

Absorbed dose: the radiation energy absorbed per unit mass of an organ or tissue and is used in studies examining radiation damage to the human body.

Activity: the traditional unit of radioactivity is the curie (Ci) where 1 Ci is equal to 3.7×10^{10} disintegrations per second. The SI unit is the becquerel (Bq). 1 Bq is equal to 1 disintegration per second.

Alpha particles: a positively charged particle consisting of two protons and two neutrons emitted during radioactive decay.

Ataxia telagiectasia: a slowly progressive multisystem disorder characterized by several manifestations, including telangiectasias (spots formed on the skin by a dilated capillary or terminal artery) of the conjunctiva and skin of the face, neck, and ears and failure of muscular coordination.

Becquerel: a unit of activity of a radionuclide equal to one spontaneous nuclear transformation per second.

Beta particles: an electron or positron emitted from a nucleus during beta decay.

Brachytherapy: the placement of internally implanted sealed sources of radioactive material within or in close proximity to tumors, with the intent of killing malignant or hyperplastic cells.

Bystander effect: radiation-induced effects in unirradiated cells.

Chernobyl accident: a disaster that occurred at the Chernobyl nuclear power plant in the Ukraine in April 1986 as a result of a flawed reactor design coupled with serious mistakes made by the plant operators. The accident destroyed the Chernobyl-4 reactor and killed 30 people, including 28 from radiation exposure. A further 209 on site were treated for acute radiation poisoning and among these, 134 cases were confirmed (all of whom recovered). Nobody off-site suffered from acute radiation effects. However, large areas of Belarus, Ukraine, Russia and beyond were contaminated in varying degrees. The Chernobyl disaster was the only accident in the history of commercial nuclear power where radiation-related fatalities occurred.

Chromosome rings: a mutation event in which both telomeres are removed and the ends of the chromosome sealed together forming a ring chromosome.

Computed tomography: the process of producing a picture showing human body organs in cross section by first electronically detecting the variation in X-ray transmission through the body section at different angles, and then using this information in a digital computer to reconstruct the X-ray absorption of the tissues as an array of points representing the cross section.

Cosmic radiation: electrons and nuclei of atoms, mostly hydrogen, that impinge upon the earth from all directions of space with nearly the speed of light.

Curie: a unit of radioactivity defined as 3.7×10^{10} disintegrations per second.

Deterministic radiation effects: radiation effects whose severity is dose-related, and which are not seen below a threshold dose level.

Diagnostic radiology: the acquisition and interpretation of diagnostic medical images and the diagnosis and treatment of human disease by measures and agents applicable to the science of radiology.

Dicentric chromosome aberrations: having two centromeres.

Directly ionizing radiation: charged particles (electrons, positrons, protons, alpha particles, heavy ions) with sufficient energy to ionize or excite atoms or molecules.

Double-strand breaks (DSB): a complete break in the DNA molecule.

Dysarthria: a disturbance of speech and language.

Energy: the SI unit for energy is the joule (J). The energy of ionizing radiation is more commonly expressed in electron-Volt (eV) units. One eV represents the energy gained by a single-charged particle, e.g. electron or proton, in a potential differential of 1 V, and is equal to 1.6×10^{-19} J.

Equivalent dose: the equivalent dose (H) to an organ or tissue is obtained by weighting the absorbed dose in an organ or tissue by a radiation weighting factor that reflects the biological effectiveness of the particles that produce damage in the tissue.

Erythema: redness of the skin resulting from congestion of the capillaries.

Excess relative risk (ERR): the ratio of the excess risk of a specified stochastic effect to the probability of the same effect in the unexposed population, i.e., the relative risk minus one.

Exposure: an outdated quantity providing a measure of ionizing radiation (limited to photons) in terms of ionization in air. The unit of exposure is the roentgen (R). Exposure is not applicable to particulate radiation, photons with energies exceeding 3 MeV and media other than air.

Fanconi's anemia: a rare hereditary disorder, transmitted in a recessive manner, and characterized by pancytopenia, hypoplasia of the bone marrow, and patchy brown discoloration of the skin due to deposition of melanin.

Fast neutrons: neutrons resulting from fission that have lost relatively little of their energy by collision, etc.; having energies exceeding 0.1 Mev.

Gamma rays: high energy photons, especially those emitted by a nucleus in a transition between two energy levels.

Genomic instability: a state in which the rate of introduction of genomic changes, including point mutations, chromosomal aberrations, aneuploidies, and gene amplifications, become grossly elevated in comparison with the normal condition.

Goiania (Brazil) accident: a serious radiation accident that occurred in the Brazilian town of Goiania in September 1987. A radiation source (caesium-137) for medical therapy equipment was stolen and sold to a scrap dealer who broke up the metal casing exposing the source. Approximately half of the radiation source, which had a strength of 100 TBq, was spread around the town. Four people died in a couple of months of radiation injuries and a further ten were seriously incapacitated. A total of 50 people were hospitalized and about 250 were estimated to have received high radiation doses.

Gray: a unit of absorbed dose, equal to the energy imparted by ionizing radiation to a mass of matter corresponding to 1 joule per kilogram; symbol Gy.

Homologous recombination: breakage of two homologous duplex DNA molecule, exchange of both strands, and resolution of the two duplexes so that no tangles remain.

Hypocenter: the location on the ground vertically below the air burst point of an atomic bomb.

Indirectly ionizing effects: uncharged particles (photons, neutrons) that set in motion directly ionizing radiation (charged particles) or that can initiate nuclear transformations.

Ionizing radiation: particles or photons that have sufficient energy to produce ionization directly in their passage through a substance.

Isotopes: atoms having the same atomic number but different mass numbers.

Kerma: (kinetic energy released in matter) the sum of the initial kinetic energies of all charged particles released by indirectly ionizing radiation in a volume element of a given material, divided by the mass of this element. The dimension is energy per unit mass, kerma is therefore a density type quantity. Its use is limited to ionizing radiation, i.e. X rays, gamma rays and neutrons, and has been used in epidemiological studies of the survivors of the atomic bombings in Japan. The SI unit of kerma is the gray (Gy), 1 Gy = 1 J/kg. An older unit previously used is the rad, 1 rad = 100 erg/g.

Linear energy transfer (LET): the energy lost by a charge particle passing through a substance per unit length of path.

Mantle irradiation: a circumscribed area of irradiation around the shoulders and chest, including the neck, clavicular regions, axillae, and mediastinum.

Mayak nuclear complex: a nuclear bomb manufacturing and fuel reprocessing plant in Ozersk, Russia. Between 1949 and 1956 amounts of liquid radioactive waste from Mayak

were discharged into the river Techa, and continued operation of the reprocessing facility led to further routine discharges to the environment. On September 29th 1957, a cooling pipe overheated and exploded, releasing 740 PBq of radioactivity and contaminating parts of neighbouring Sverdlovsk and Tyumen regions and Chelyabinsk, home to one million people.

Minisatellites: polymorphic variation in DNA sequences due to a variable number of tandem repeats of a short DNA sequence.

Mutagenesis: production of genetic alterations by chemicals or radiation.

Neutron: an elementary particle with approximately the same mass as a proton but lacking an electric charge.

Neutron capture reactions: absorption of a neutron by a nucleus, with the new nucleus emitting one or more gamma rays.

Neutron elastic scattering: a neutron colliding with a nucleus rebounds in a different direction. The energy the neutron loses is gained by the target nucleus which moves away at an increased speed; the total kinetic energy of the neutron and the nucleus remains unchanged by the collision.

Neutron inelastic scattering: a reaction in which a neutron interacts with a nucleus and is promptly reemitted with reduced energy and usually with a changed direction. The scattering nucleus is left in an excited state and emits a nuclear de-excitation gamma ray.

Neutron interactions: elastic scattering, inelastic scattering, nonelastic scattering, capture reactions, spallation processes.

Neutron nonelastic scattering: interactions between neutron and nucleus in which particles other than a single neutron are emitted, e.g., alpha particles or protons.

Neutron spallation processes: a nuclear reaction in which the energy of the incident particle is so high that more than two or three particles are ejected from the larger nucleus and both its mass number and atomic number are changed.

Nijmegen breakage syndrome: a syndrome characterized by short stature, progressive microcephaly with loss of cognitive skills, ovarian failure in females, recurrent sinopulmonary infections, and an increased risk for cancer, particularly lymphoma.

Non-homologous end joining (NHEJ) repair pathway: direct joining of the broken ends of a complete (double strand) break in the DNA molecule. Proteins recognize and bind to the exposed ends and bring them together for ligating. Complementary nucleotides are not required for this type of joining.

Nonhomologous recombination: recombination between DNA molecules that does not require homology between the recombining molecules.

Nuclear medicine (diagnostic): the administration of small amounts of radioactive material to patients, and the subsequent determination of the metabolic fate of the radioactive material using non-invasive *in vitro* or external imaging techniques, in order to obtain diagnostically useful information.

Ocular apraxia: a congenital inability impairing the ability to control eye movement to redirect the line of sight.

Oculotaneous telangiectasias: telangiectasias of the eyes and skin.

Particle radiation: radiation consisting of physical particles, e.g., alpha or beta particles, neutrons, or protons.

Person-Sv: unit of cumulative radiation dose to a specified population.

Photons: quantum particles of electromagnetic radiation, lacking in mass but carrying energy.

Premature chromosome condensation (PCC): a method of studying chromosomes in the interphase stage of the cell cycle.

Rad: a unit of absorbed dose, equal to the energy absorption of 100 ergs per gram (0.01 joule per kilogram); equal to 0.01 gray.

Radiation sensitive disorders: specific types of genetic disorders that increase the sensitivity to radiation at the cellular level resulting in an increased risk for development of cancer.

Radioactive decay: the spontaneous transformation of a nucleus into one or more different nuclei by emission of particles from the nucleus, nuclear capture or ejection of orbital electrons, or fission.

Radiopharmaceuticals: a chemical or pharmaceutical preparation labeled with a radionuclide. Radiopharmaceuticals are administered to accumulate in specific tissues, to deliver highly absorbed doses and to kill cells. Most therapeutic radiopharmaceuticals emit beta particles, which travel only a few millimeters in tissue.

Radon Spa: a health resort in Bad Gastein, Austria where radon gas coming from deep cracks in the mountain (Gastein Thermal Springs) together with air saturated with humidity has been used as a therapy since the Middle Ages.

Reactive oxygen intermediates: molecules of oxygen that are in altered chemical states, thereby making them capable of oxidatively injuring cells, tissues, and, in some instances, DNA.

RBE_m (RBEM) (RBE maximum): the ratio of the initial slopes of the dose-responses of the radiation under study and the reference radiation.

Reciprocal chromosome translocations: exchange of exactly the same length and area of DNA between a pair of chromosomes resulting in a shuffling of genes.

Relative biological effectiveness (RBE): the ratio of the dose from 200 keV X rays required for a given biological effect to the dose that would produce the same effect with that radiation. RBEs also can be defined for specific scenarios that compare the effects of different radiation types on producing the same endpoint.

Rem: a unit of ionizing radiation equal to the amount of radiation that produces the same damage to humans as one roentgen of high-voltage x rays.

Roentgen: the unit of exposure. The roentgen is equivalent to ionization in dry air of 2.58×10^{-4} C/kg.

Sievert: a unit of dose equivalent equal to the dose equivalent when the absorbed soe of ionizing radiation multiplied by stipulated dimensionless factors is 1 joule per kilogram; symbol Sv.

Single strand breaks (SSB): breaks in a single strand of the DNA molecule.

Stochastic radiation effects: those effects for which the probability of occurrence, and not the severity is dose related.

Teletherapy: the use of external beams of energetic photons or subatomic particles to irradiate tumors within the body, with the intent of killing malignant cells or infectious agents.

Telomeres: the distal end of a chromosome arm consisting of long strands of DNA composed of a six-base repeating sequence TTAGGG.

Teratogenesis: disturbed growth processes involved in the production of a malformed neonate.

Terrestrial radiation: long wave radiation emitted by the Earth, including its atmosphere.

Thermal neutrons: neutrons in equilibrium with their environment. Their most probable energy is about 0.025 eV; or the speed of a gas molecule at room temperature.

Three-Mile Island: On March 28, 1979, the most serious accident at a U.S. commercial nuclear power plant occurred at the Three Mile Island Unit 2 (TMI-2) nuclear power plant near Middletown, Pennsylvania. A sequence of equipment malfunction, design-related problems and worker errors led to significant damage to the TMI-2 reactor core but only very small off-site releases of radioactivity. No deaths or injuries to plant workers or members of the nearby community resulted from the accident.

Tissue weighting factor (quality factor): a modifying factor that is used to derive dose equivalent from absorbed dose.

Track structure of radiation: a description of spatial and temporal variations in energy deposition.

UNSCEAR: United Nations Scientific Committee on the Effects of Atomic Radiation, established by the General Assembly of the United Nations in 1955 with a mandate to assess and report levels and effects of exposure to ionizing radiation.

X rays: an ionizing electromagnetic radiation produced by the excitation of the inner orbital electrons or an atom by either bombardment of the target anode of an X-ray tube with a stream of electrons from a heated cathode or by other processes, such as nuclear decay.

XRCC4: the factor that complemented XR-1 radiosensitivity and V(D)J recombination deficiency.

Appendix A: Further Details on Medical Uses of Ionizing Radiation

A.1 Diagnostic radiology

A.1.1 Overview

Diagnostic radiology refers to the use of X rays to visualize structures within the human body and to obtain useful information concerning organ morphology, the anatomical relationships between various structures, and the abnormal presence of gas, fluid or foreign bodies. X-ray production is generally accomplished by bombardment of a heavy-metal target (typically tungsten) with an energetic beam of electrons within a vacuum to produce so-called "Bremmsstrahlung" radiation. The X-ray beam is then collimated and directed toward the anatomic structures of interest. To produce an image, an X-ray detector is placed to intercept the X-ray beam after it has penetrated the patient. To increase the mean energy of the beam (and thus reduce the contribution of low-energy X rays to skin entrance absorbed dose), thin metallic filters are interposed between the X-ray source and the patient. The variation in intensity of the transmitted beam, as measured at the position of the detector, reflects variations in electron density within the patient. This basic principal of image formation has remained unchanged since the first medical use of X rays over a century ago. Since that time, a number of X ray detectors have been employed, including glass plates coated with scintillating materials, photographic film, electronic image intensifiers, ionization chambers and solid state radiation detectors.

Different geometric relationships between the X-ray beam, the patient and the detector have been developed over the years. The oldest and simplest of these is a fan-shaped X-ray beam in conjunction with a planar detector such as photographic film. This is the most common modality employed in diagnostic X-ray procedures in the U.S. In many cases, simple planar imaging can produce diagnostically adequate images at a relatively low cost. Examples of "plain film" (PF) studies include chest and abdominal X rays, dental X rays, and a variety of procedures designed to detect fractures or other skeletal abnormalities. A variation of this technique uses a high-speed electronic image intensifier as the detector, enabling the radiologist to view moving structures, or quickly produce multiple views in real time and record them on cinegraphic film or videotape. The use of intra-arterial, intravenous or intraluminal radio-opaque contrast agents permits visualization of pulmonary, cerebral, and cardiovascular systems; the gastrointestinal tract; the renal collecting system; and the ureters and bladder.

Mammography is a diagnostic plain film technique in which only the breast is irradiated, in order to detect abnormal tissue or microcalcifications within the breast that may indicate the presence of breast cancer. To achieve the required tissue contrast, the maximum energy of the X-ray beam is typically lower than for ordinary plain film procedures (typically 23 to 35 kVp for mammograms as opposed to 80 to 120 kVp for ordinary plain films), and metals other than tungsten and aluminum, such as molybdenum and rhodium, are used for electron beam target and X-ray beam filtration, respectively.

Beginning in the early 1970s, highly-collimated "pencil" beams of X rays have been employed in conjunction with solid state detectors and digital computers. In this modality, referred to as computerized tomography (CT), thousands of X-ray beams are directed toward the patient at multiple angles, and the intensity of the transmitted beams is measured and recorded digitally. The numerical data representing the transmitted X ray

beam intensities are mathematically reconstructed by computer to produce multiple thin transaxial planar images. The acquisition of data at multiple positions and angles may be accomplished by rotating the X-ray source in the transaxial plane and translating the supine patient in the sagittal plane, or by irradiating an annular target with an electron beam. CT permits the visualization of anatomic structures in three dimensions at 1 to 2 mm resolution, with exceptional tissue contrast compared to planar procedures.

A.1.2 Range of expected radiation dose

Diagnostic X-ray procedures typically irradiate specific regions of the body. Tissues and organs within the primary X-ray beam sustain the highest radiation absorbed doses; however, tissues distant from the irradiated field also are exposed to scattered radiation from the primary beam. For this reason, it is possible that stochastic radiation effects may be seen in tissues not directly irradiated. For that reason, a measure of radiation detriment that takes into account variations in radiation dose among tissues and organs as a consequence of partial body irradiation is required to assess risks. One such index is the *effective dose equivalent* (EDE), which is calculated as a weighted average of radiation doses from selected tissues. The EDE due to a given diagnostic procedure reflects the same radiation detriment from stochastic effects as a numerically-equivalent total body dose.

The approximate EDE values for some common plain film diagnostic X-ray procedures are shown in Table A-1. The actual radiation dose and EDE for a particular procedure depends upon many variables, including the irradiated field size, X-ray beam energy spectrum and intensity, exposure time, patient body habitus, and the number of multiple exposures required to produce an image of acceptable quality; the variability in actual dose is great. The EDE values below are not precise, but are representative of the magnitude of the radiation exposure. Organ doses and EDE for procedures involving the distal extremities (ankle, wrist, etc.) are very low (< 5 millirem). The EDE values for other plain film procedures range from 6 to 8 millirem for chest X rays to 145 to 230 millirem for a typical lumbar spine series.

Table A-1. Organ doses[a] and EDE[b] for selected plain film diagnostic radiology procedures

Procedure	Female breast (millirads)	Bone marrow (millirads)	Gonads, m/f (millirads)	EDE, m/f (millirem)
Chest	15	4	0.0/0.1	6/8
Lumbar spine	0	110	6/346	145/230
Hip	0	17	357/79	117/47
Abdomen	0	41	15/194	49/94
Pelvis	0	22	48/126	65/85
Cervical spine	0	98	0/0	35/35
Intravenous pyelogram	0	98	43/560	101/230
Shoulder	68	9	0/0	10/18
Skull	0	33	0/0	42/42

[a]Organ doses are computed from typical skin entrance exposures, beam qualities, and number of films for each procedure for the United States population (NCRP 1989), and dose conversion factors obtained from Monte Carlo studies in mathematical phantoms that relate skin entrance exposure to radiation absorbed dose for various tissues, beam geometries, and beam filtration (Gorson *et al*. 1984).

[b]EDE is computed according to methods and tissue weighting factors prescribed by the United States Nuclear Regulatory Commission for the documentation of compliance with annual occupational exposure limits (Federal Register 1993).

Radiation absorbed dose to the glandular tissue of the female breast from mammography procedures depends on many factors, including the choice of target and filtration materials, the skin entrance exposure required to produce an image of diagnostic quality, the proportion of glandular tissue relative to adipose tissue in the breast, and the thickness of the compressed breast tissue. For a molybdenum target with molybdenum filtration, 0.265 mm aluminum-equivalent filtration, a maximum photon energy of 25 KeV, 50% glandular tissue, and a breast thickness range of 2 to 8 cm, a range of glandular tissue radiation dose of about 250 to 360 millirads for a single mammography exposure can be computed using parameterized data from Sobol and Wu (1997).

Organ doses and EDE due to CT procedures will vary widely, depending upon the geometry of the X-ray source and detector, the area of the body irradiated, the technique used, and the patient's body habitus. In general, however, organ doses and EDE are higher than those associated with plain film procedures. For example, the radiation absorbed dose to the female breast from CT of the chest is about of 0.8 to 3.3 rads (Murphy and Heaton 1985, Evans *et al.* 1989, McCollough and Liu 1995). Estimates of EDE from chest and abdominal CT may be computed for different patient weights (Ware *et al.* 1999, Huda *et al.* 2000). Using a typical multiplane scanner, operated at 120 kVp and assuming a patient mass of 70 kg, the effective dose is about 400 millirem from an abdominal CT and about 540 millirem for a chest CT for a single exposure. If the examination is repeated using intravenous or intraluminal contrast agents, the dose from the entire procedure will be doubled.

Currently, there are approximately 10,000 CT scanners in use in the U.S. Since the introduction of helical 4-slice multi-detector CT scanners in 1998, we have today newer helical scanners that can provide eight (8) and even sixteen (16) slices simultaneously, and in the next few years they will probably replace most of the axial-only models. Approximately 29% of all CT scanners today in the U.S. can do multi-slice helical scanning. Recent advances in CT technology have been rapidly adopted into clinical practice and have led to an explosive growth in the number of applications, to a capability of examining patients quickly and to a high rate of use. The preliminary results of the 2000 to 2001 NEXT survey (NEXT 2001) indicate that the total number of annual CT exams is approximately 58 million, where 79% of all exams consist of scanning in six anatomical regions or combinations of regions: brain, abdomen-pelvis, chest, abdomen, chest-abdomen-pelvis, and pelvis alone. The effective dose average for the six exam regions is approximately 6.2 millisievert (620 mrem), and the product of this average and the number of exams corresponds to a collective annual dose of approximately 284,000 person-sievert per year. According to the U.K. ImPACT group (Lewis 2001), dose contribution from CT scanning has increased from 20% in 1989 to 40% in 1999; however, the percent CT examinations has only marginally increased from 2% to 4% during this period. They project a dose contribution increase from CT scanning to 80% in 2009; however, this dose contribution is expected to continue to come from a small number of CT examinations (approximately 8%) as a percent of all diagnostic radiological procedures.

A.1.3 Expected deterministic and stochastic radiation effects

For individual procedures, the patient radiation doses in diagnostic radiology are generally within the range where no deterministic radiation effects would be observed. The EDE is generally below the doses where stochastic effects have been observable in humans (i.e., less than about 10 rem). However, there have been some instances in the past where extended diagnostic fluoroscopic procedures, performed multiple times during the course of the patient's illness, have been shown to result in the induction of cancer later in life. An example is the increased incidence of breast cancer in women who underwent multiple fluoroscopic studies for the evaluation of tuberculosis (Miller *et al.* 1989). In this case, the increased relative risk of breast cancer was directly related to cumulated radiation absorbed dose to the breast, was inversely related to the patient's age, and peaked approximately 20 years following exposure. Although a statistically significant increase in cancer incidence was not observed at radiation absorbed doses to the breasts below about 50 rads, the data do not exclude the possibility that stochastic effects could have been produced at lower doses, due to the relatively small number of patients in each dose group. For that reason, repeated radiation exposures to the breast, such as occur with mammography or chest CT, should be minimized in number and optimized in technique to yield the best diagnostic information and the minimum radiation dose.

A.1.4 Measures to reduce patient radiation exposure

Since the introduction of X rays into the healing arts, many technological improvements and refinements in technique have been developed to reduce patient radiation dose while increasing the diagnostic value of the images and the procedure as a whole. These include:

- Improvements in the resolution and sensitivity of X-ray detectors. These permit the production of high-quality images with fewer X-ray photons and hence less radiation dose.
- Introduction of filters to remove lowest-energy components of the X-ray beam. Filtration reduces skin dose from low-energy X rays while preserving the diagnostic information afforded by the more penetrating components of the beam.
- Introduction of so-called "Buckey grids" to reduce the contribution of scattered radiation to the image. This improves image contrast, which in turn permits lower beam energies and intensities to be used.
- Optimizing beam energy and intensity to the patient's body habitus.
- Minimizing the size of the irradiated field to include only the areas of interest.
- Reducing the exposure time per film to the minimum consistent with adequate diagnostic quality.
- Careful positioning of the patient to minimize the number of "retakes."
- Gonadal shielding for women of procreative potential.
- Minimizing the irradiation time during fluoroscopic procedures.
- Restricting the use of X-ray procedures to those cases where there is a clear benefit to the patient, and where the desired diagnostic information cannot be obtained using modalities that do not employ ionizing radiation, such as ultrasound or magnetic resonance imaging.

A.2 Interventional radiology

A.2.1 Overview

Interventional radiology refers to the use of diagnostic radiology procedures in conjunction with invasive therapeutic techniques. In interventional radiology, X-ray imaging is used as a guide in visualizing the performance of the therapeutic procedure. Examples include percutaneous transhepatic cholangiography, endoscopic retrograde cholangiopancreatography, transjugular intrahepatic portosystemic shunt, percutaneous transluminal angioplasty, stent or filter placement, arterial embolization, percutaneous urinary and billiary drainage and stone removal, and tissue ablation using cryogenic or radiofrequency probes. Interventional radiology procedures are typically performed under

fluoroscopic guidance. Unlike diagnostic fluoroscopic procedures, in which the fluoroscopy time is limited to a few seconds or minutes, interventional procedures may require up to ninety minutes or more of fluoroscopy time.

A.2.2 Range of expected radiation dose

Due to the use of extended fluoroscopy time, radiation doses to tissues and organs in interventional radiology can be expected to be considerably higher than for those associated with diagnostic procedures. Radiation doses will vary greatly with the nature of the procedure, the difficulty of the technique, the experience of the operator and the patient's body habitus. As an example of the magnitude of the radiation doses that might be encountered in interventional radiology, we can compute the dose for a radio frequency ablation of aberrant cardiac conducting tissue from tables relating organ absorbed dose, beam filtration, angiographic view, and skin entrance exposure provided by the U.S. FDA. For a total fluoroscopy time of 90 minutes, with 30 minutes spent in each of three conventional views (anterior, LAO 45, and RAO 30), the approximate dose to the female breast, lungs, and skin of the posterior thorax is 4 rads, 18 rads, and 160 rads, respectively. The EDE is approximately 7 rem.

A.2.3 Expected deterministic and stochastic radiation effects

The risk of deterministic and stochastic radiation effects is increased for interventional procedures. The most common observed adverse deterministic effect is transient erythema and epilation of the skin within the irradiated field following long fluoroscopic procedures. Occasionally, more severe skin injury, including dry and moist desquamation, dermal atrophy, fibrosis, late erythema, ulceration, and necrosis can occur. Patients that are most at risk for severe skin injuries are those for whom the fluoroscopy time exceeds ninety to one hundred minutes.

The potential for stochastic effects is present, especially for radiation-sensitive tissues such as the breast or thyroid. The risk is further increased in adolescent or pediatric patients whose life expectancy (and therefore the time available for late occurrence of stochastic effects) may be many decades.

A.2.4 Measures to reduce patient radiation exposure

In general, the methods used to reduce patient radiation dose in diagnostic procedures apply to interventional procedures. Other methods recommended by the U.S. FDA (FDA 1994) include the following:

- Establishment of standard protocols, operating procedures, and a quality assurance program designed to minimize exposure time and optimize beam collimation, and monitor and document radiation output of fluoroscopic units.

- Operator awareness of radiation dose rates in various modes of operation.

- Use of real-time indications of cumulative exposure time and cumulative skin dose-area product.

- Judicious use of "last-image hold" and "freeze-frame" display modes, and efficient automatic brightness control algorithms.

- Avoiding extended fluoroscopy time in a single view.

A.3 Positron emission tomography

A.3.1 Overview

As in conventional diagnostic nuclear medicine, Positron Emission Tomography (PET) involves the administration of small amounts of radioactive material, followed by imaging studies that yield quantitative information on organ or tumor function. In PET, the radionuclides administered are always positron-emitters. Following nuclear transformation within the tissues of the patient, the positrons travel a few millimeters and are converted into high-energy photons (0.511 MeV) by annihilation with electrons. These photons, which are emitted in temporal coincidence and travel co-linearly, are detected by the PET tomograph, which reconstructs millions of such events into a three-dimensional image. The high energy of the photons and the ubiquitous presence of the positrons result in higher energy deposition in tissue with PET radiopharmaceuticals, compared to the radionuclides used in conventional diagnostic nuclear medicine. This higher energy deposition, however, is offset by the very short half-life of PET radionuclides, yielding radiation doses that are comparable to those encountered with conventional nuclear medicine imaging techniques.

An advantage of PET radionuclides is that they include elements that are interesting from a biochemical standpoint. For example, short-lived radioisotopes of carbon, oxygen and nitrogen may be produced by on-site cyclotrons. Using these precursor radionuclides, biologically interesting compounds may be synthesized that have the same biophysical and biochemical properties as their non-radioactive counterparts. The ability to study fundamental biological processes in situ makes PET a powerful tool for basic biomedical research. In clinical practice, a fluorinated analog of glucose, fluorine-18 labeled fluorodeoxyglucose (FDG), has been found to be effective in the detection of primary and metastatic breast and prostate carcinoma and the evaluation of response to therapy.

Illustrative examples of PET radiopharmaceuticals used in research and clinical practice are listed in Table A-2. The development of radiopharmaceuticals for PET is a major field of research; the number of PET radiopharmaceuticals being investigated far exceeds the examples cited here. Currently, the majority of clinical PET studies in the U.S. are performed using fluorine-18 FDG.

Table A-2. Radiopharmaceuticals employed in positron emission tomography

Radionuclide and chemical form	Radionuclide half-life (min)	Organ systems or processes evaluated
Carbon-11 amino acids (methionine, etc.)	20.4	organ and tumor amino acid metabolism; tumor imaging
Carbon-11 dopamine, serotonin, and nicotine analogs	20.3	brain receptor mapping and functional studies
Fluorine-18 choline analogs	109.8	tumor localization
Fluorine-18 fatty acids	109.8	myocardial fatty acid metabolism
Fluorine-18 fluorodeoxyglucose	109.8	brain glucose metabolism (Alzheimer's Disease, seizure disorders); tumor localization and response to treatment; myocardial viability
Nitrogen-13 amino acids (glutamate, glutamine, valine, etc.)	10.0	organ and tumor amino acid metabolism; tumor imaging
Nitrogen-13 ammonia	10.0	myocardial perfusion
Oxygen-15 carbon monoxide	2.0	tissue blood volume
Oxygen-15 oxygen	2.0	tissue oxygen utilization
Oxygen-15 water	2.0	cerebral blood flow
Rubidium-82 rubidium chloride	1.3	myocardial perfusion

A.3.2 Range of expected radiation dose

The EDE values for PET studies are comparable to those for conventional diagnostic nuclear medicine. The EDE values for some of the commonly-employed PET radiopharmaceuticals are listed in Table A-3.

Table A-3. Effective dose equivalent[a] values for common PET procedures

Radiopharmaceutical and procedure	Effective dose equivalent (rem)	Percent of annual occupational limit[b]
Fluorine-18 fluorodeoxyglucose	1.3	26%
Nitrogen-13 ammonia	0.2	4%
Rubidium-82 rubidium chloride	0.2	4%
Oxygen-15 water	0.2	4%
Oxygen-15 oxygen (inhalation)	0.2	4%

[a]Organ doses and EDE per unit administered activity computed by the MIRD method (Snyder *et al*. 1975, Stabin *et al*. 1996) were multiplied by administered activities typically used in clinical practice.

[b]Percentage of U.S. Nuclear Regulatory Commission annual occupational dose limit for radiation workers (5 rem).

A.3.3 Expected deterministic and stochastic radiation effects

Patient radiation doses in PET are virtually always within the range where no deterministic radiation effects would be observed. The EDE for PET procedures is below the doses where stochastic effects have been observed in humans (i.e., less than about 10 rem).

A.3.4 Measures to reduce patient radiation exposure

Because the EDE is directly proportional to the amount of radioactive material administered, patient radiation dose may be reduced by administering the smallest amount of PET radiopharmaceutical possible, while still obtaining images of high diagnostic quality.

A.4 Teletherapy

A.4.1 Overview

Teletherapy refers to the use of external beams of energetic photons or subatomic particles to irradiate tumors within the body with the intent of killing malignant cells or infectious agents. Teletherapy is best suited for the treatment of solitary, well-circumscribed tumors. It is not well-suited for widely disseminated metastatic disease or diffuse disease; however, it may be a useful adjunct to chemotherapy in some cases. As with systemic radionuclide therapy, the objective of teletherapy is to maximize the adverse radiation effect on malignant cells and minimize the effect on neighboring normal tissues. Table A-4 lists the forms of ionizing radiation that have been employed in teletherapy and the means of their production.

Table A-4. Forms of ionizing radiation employed in teletherapy

Photon or subatomic particle	Mode of production	Target tissues
X rays (energies < 150 KeV)	X-ray generator	superficial skin cancers
X rays (energies > 1 MeV)	betatron or linear accelerator	deep tumors
Gamma rays	sealed radionuclide sources (Cs-137, Co-60)	deep tumors
Electrons	linear accelerator	deep tumors
Protons	linear accelerator	deep tumors
Neutrons	linear accelerator or cyclotron	deep/hypoxemic tumors
Pions / Mesons / heavy charged particles	linear accelerator	deep tumors (research applications only)

The objective of using heavy charged particles such as protons is to take advantage of the so-called “Bragg peak,” which describes the large increase in energy transfer per unit path length to tissue by the particles near the end of their range. By adjusting the initial energy of the particle beam, the position of the Bragg peak within the patient may be

varied so that the maximum energy loss, and therefore maximum radiation dose deposition, coincides with the location of the tumor.

Uncharged, indirectly-ionizing particles such as neutrons have been employed for hypoxic tumors. The biological effectiveness of low-LET radiation (photons, electrons) is reduced as local tissue oxygen concentration decreases, making treatment of poorly vascularized, hypoxemic tumors difficult. This effect is not present for neutrons; the effectiveness of the radiation is not reduced by tumor hypoxemia.

In order to maximize the radiation dose to the tumor tissue and minimize dose to normal tissues, a variety of collimated radiation beam configurations have been employed. These include multiple beams directed from multiple angles and continuously rotating single beams. A modern refinement is Intensity Modulated Radiation Therapy, where multi-leaf collimators in conjunction with a rotating beam under computer control precisely deliver radiation dose to an area that closely coincides with the tumor's margins.

A.4.2 Range of expected radiation dose

Radiation doses required to completely eradicate tumor cells are on the order of 5,000 to 8,000 rads. Local radiation doses from teletherapy are among the highest employed in the medical uses of ionizing radiation. Doses to normal tissues will depend upon the proximity of the tissue to the external beam, and in many cases are likely to be near the range where both deterministic and stochastic effects would be expected.

A.4.3 Expected deterministic and stochastic radiation effects

Deterministic effects on normal tissues associated with teletherapy include skin injury (erythema, epilation, fibrosis, ulceration, etc.), symptoms of acute radiation syndrome (nausea, vomiting, and diarrhea), bone marrow depression, insufficiency fractures of bone, stomatitis, esophagitis, cystitis, bowel or bladder perforation and fistula formation, meningitis, encephalitis, and cognitive impairment.

The major stochastic effect associated with teletherapy is the induction of secondary cancers. Teletherapy for post-partum mastitis (Shore *et al.* 1986) and Hodgkin's disease (Hancock *et al.* 1993) has been associated with increased risk for the development of breast cancer. X-ray therapy for tinea capitis (ringworm of the scalp) in children is a risk factor for development of thyroid cancer (Ron and Modan 1980). Radiation therapy for carcinoma of the cervix has been found to be a risk factor for the late occurrence of several types of cancer (Boice *et al.* 1985). Cases of sarcoma arising in bone within prior radiation fields have been reported (Calham *et al.* 1948, Amendola *et al.* 1989).

A.4.4 Measures to reduce patient radiation exposure

One effective measure for reducing stochastic radiation effects and the genetically significant dose to the U.S. population has been the elimination of teletherapy as a mode of treatment for non-malignant conditions such as tinea capitis, acne vulgaris, ankylosing spondylitis, and mastitis. The development of antibiotic therapy has replaced teletherapy as the treatment of choice for some of these conditions.

Another measure has been the development of dose-fractionation schemes. Dividing the tumoricidal dose (5,000 to 8,0000 rads) into smaller fractions (200 to 300 rads) separated by varying time intervals can deliver an effective cumulated dose to the tumor, while permitting the normal tissues time to repair sub-lethal damage. This can assist in minimizing adverse deterministic and stochastic effects.

Other methods for reducing unnecessary radiation exposure from teletherapy include:

- Establishment of rigorous quality management programs with regard to the radiation dose delivered, the anatomic location of dose delivery, and the delivery schedule. Administration of teletherapy quality management programs is under the oversight of state and federal regulatory agencies.

- Continued research directed toward improved shaping of the teletherapy beam to avoid unnecessary irradiation of normal tissues.

A.5 Appendix A References

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Appendix B: Ionizing Radiation Regulations

Table B-1. Department of Energy (DOE) regulations

Regulatory citation	Regulatory action
10 CFR 60 - PART 60 - DISPOSAL OF HIGH-LEVEL RADIOACTIVE WASTES IN GEOLOGIC REPOSITORIES. Promulgated: 46 FR 3980, 2/5/81. U.S. Codes: 42 U.S.C. 2071, 2073, 2092, 2093, 2095, 2111, 2201, 2232, 2233.	Rules governing the receiving and storing of radioactive materials in geological repositories.
10 CFR 835 - PART 835 - OCCUPATIONAL RADIATION PROTECTION. Promulgated: 58 FR 65485, 12/14/93. U.S Codes: 42 U.S.C. 2201.	Establishes radiation protection standards, limits, and program requirements.

Table B-2. Department of Transportation (DOT) regulations

Regulatory citation	Regulatory action
49 CFR 172 - PART 172 - HAZARDOUS MATERIALS TABLE, SPECIAL PROVISIONS, HAZARDOUS MATERIALS COMMUNICATIONS, EMERGENCY RESPONSE INFORMATION AND TRAINING REQUIREMENTS. Promulgated: 41 FR 15996, 4/15/76. U.S. Codes: 49 U.S.C. 5101–5127.	Sets forth shipping papers, marking, and labeling requirements for radioactive materials.
49 CFR 173 - PART 173, SUBPART I - SHIPPERS - GENERAL REQUIREMENTS FOR SHIPMENTS AND PACKAGINGS - CLASS 7 (RADIOACTIVE) MATERIALS. Promulgated 60 FR 50307, 9/28/95. U.S. codes: 49 U.S.C. 5101–5127.	Sets forth requirements for the packaging and transportation of radioactive materials.
49 CFR 177 - PART 177 - CARRIAGE BY PUBLIC HIGHWAY. Promulgated 32 FR 5606, 4/5/67. U.S. codes: 49 U.S.C. 5101–5127.	Sets forth requirements for shipping radioactive materials by public highways.

Table B-3. Environmental Protection Agency (EPA) regulations

Regulatory citation	Regulatory action
40 CFR 61 - PART 61, SUBPART H - NATIONAL EMISSION STANDARDS FOR RADIONUCLIDES OTHER THAN RADON FROM DEPARTMENT OF ENERGY FACILITIES. Promulgated: 54 FR 51695, 12/15/89. U.S. Codes: 42 U.S.C. 7401, 7412, 7413, 7414, 7416, 7601, 7602.	Emissions of radionuclides to the air from DOE facilities shall not exceed those amounts that would cause any member of the public to receive in any year an effective dose equal to 10 mrem.
40 CFR 61 - PART 61, SUBPART I - NATIONAL EMISSION STANDARDS FOR RADIONUCLIDE EMISSIONS FROM FEDERAL FACILITIES OTHER THAN NUCLEAR REGULATORY COMMISSION LICENSEES AND NOT COVERED BY SUBPART H. Promulgated: 54 FR 51697, 12/15/89. U.S. Codes: 42 U.S.C. 7401, 7412, 7413, 7414, 7416, 7601, 7602.	Emissions of radionuclides, including iodine, to the air from a facility regulated under this subpart shall not exceed those amounts that would cause any member of the public to receive in any year an effective dose equal to 10 mrem.

Regulatory citation	Regulatory action
40 CFR 61 - PART 61, SUBPART K - NATIONAL EMISSION STANDARDS FOR RADIONUCLIDE EMISSIONS FROM ELEMENTAL PHOSPHORUS PLANTS. Promulgated: 54 FR 51699, 12/15/89. U.S. Codes: 42 U.S.C. 7401, 7412, 7413, 7414, 7416, 7601, 7602.	Emissions of polonium-210 to the air at an elemental phosphorus plant shall not exceed a total of 2 Ci/yr.
40 CFR 61 - PART 61, SUBPART FF - NATIONAL EMISSION STANDARDS FOR BENZENE WASTE OPERATIONS. Promulgated: 55 FR 8346, 3/7/90. U.S. Codes: 42 U.S.C. 7401, 7412, 7413, 7414, 7416, 7601, 7602.	Stack monitoring using sample collection methods detailed in the rule are required for radionuclides as particulates, the radionuclide tritium, and radionuclides of iodine, argon, krypton, xenon, oxygen, carbon, nitrogen, and radon.
40 CFR 141 - PART 141, SUBPART B - MAXIMUM CONTAMINANT LEVELS FOR RADIUM-226, RADIUM 228, AND GROSS ALPHA PARTICLE RADIOACTIVITY IN COMMUNITY WATER SYSTEMS. Promulgated: 41 FR 28404, 7/9/76. U.S. Codes: 42 U.S.C. 300f *et seq*.	Maximum contaminant levels are: combined radium-226 and radium-222 = 5 pCi/L gross alpha particle activity (including radium-226 but excluding radon and uranium) = 15 pCi/L beta particles and photon activity = 4 mrem/yr
40 CFR 141 - PART 141, SUBPART C - MONITORING AND ANALYTICAL REQUIREMENTS. Promulgated: 41 FR 28404, 7/9/76, as amended at 65 FR 26022, 5/4/00. U.S. Codes: 42 U.S.C. 300f *et seq*.	The average annual concentrations of tritium shall be < 20,000 pCi/L and strontium shall be < 8 pCi/L, provided that the sum of both of their annual dose equivalents to bone marrow do not exceed 4 rem/yr.
40 CFR 9, 141, 142 - PARTS 9, 141, AND 142 - AMENDED MAXIMUM CONTAMINANT LEVELS FOR RADIONUCLIDES. Promulgated: 65 FR 76708, 12/7/00. U.S. Codes: 42 U.S.C. 300f *et seq*.	The monitoring requirements for beta particles and photon activity are no longer applicable.
40 CFR 146 - PART 146, SUBPART A - UNDERGROUND INJECTION CONTROL PROGRAM: CRITERIA AND STANDARDS. Promulgated: 45 FR 42500, 6/24/80. U.S. Codes: 42 U.S.C. 300f *et seq*.	Injection control regulations are in effect for wells that inject for the *in situ* production of uranium.
40 CFR 190 - PART 190, SUBPART B - ENVIRONMENTAL RADIATION PROTECTION STANDARDS FOR NUCLEAR POWER OPERATIONS. Promulgated: 42 FR 2860, 1/13/77. U.S. Codes: 42 U.S.C. 2011–2296 as amended, 5 U.S.C. app.1, 42 U.S.C. 10101–10270.	The annual dose equivalent shall not exceed 25 mrem to the whole body, 75 mrem to the thyroid, 25 mrem to any other organ. The total quantity of radioactive materials entering the general environment shall be < 50,000 Ci of krypton-85, 5 mCi of iodine-29, 0.5 mCi combined of plutonium-239 and other alpha-emitting transuranic radionuclides with half-lives greater than 1 year.
40 CFR 191 - PART 191, SUBPART A - ENVIRONMENTAL RADIATION PROTECTION STANDARDS FOR MANAGEMENT AND DISPOSAL OF SPENT NUCLEAR FUEL, HIGH-LEVEL AND TRANSURANIC RADIOACTIVE WASTES - MANAGEMENT AND STORAGE. Promulgated 50 FR 38084, 9/19/85. U.S. Codes: 42 U.S.C. 2011–2296 as amended, 5 U.S.C. app.1, 42 U.S.C. 10101–10270.	Management and storage of spent nuclear fuel or high-level transuranic radioactive wastes shall provide assurance that the combined annual dose equivalent to any member of the general population shall be < 25 mrem to the whole body, < 75 mrem to the thyroid and 25 mrem to any other critical organ.

Regulatory citation	Regulatory action
40 CFR 191 - PART 191, SUBPART B - ENVIRONMENTAL RADIATION PROTECTION STANDARDS FOR MANAGEMENT AND DISPOSAL OF SPENT NUCLEAR FUEL, HIGH-LEVEL AND TRANSURANIC RADIOACTIVE WASTES - ENVIRONMENTAL STANDARDS FOR DISPOSAL. Promulgated 50 FR 38084, 9/19/85, as amended at 58 FR 66414, 12/20/93. U.S. Codes: 42 U.S.C. 2011–2296 as amended, 5 U.S.C. app.1, 42 U.S.C. 10101–10270.	Disposal systems for spent nuclear fuel of high-level or transuranic radioactive wastes shall have a release limit per 1,000 metric tons of heavy metal as follows: americum-241, 243 = 100; carbon-14 = 100; cesium-135,137 = 1,000; iodine-129 = 100, neptunium-237 = 100; plutonium-238, 239, 240, 242 = 100, radium-226 = 100; strontium-90 = 1,000; technetium-99 = 10,000; thorium-230, 232 = 10; tin-126 = 1,000; uranium-233, 234, 235, 236, 238, and any other alpha-emitting radionuclide with a half-life > 20 years = 100; any other radionuclide with a half-life > 20 years that does not emit alpha particles = 1,000.
40 CFR 191 - PART 191, SUBPART C - ENVIRONMENTAL RADIATION PROTECTION STANDARDS FOR MANAGEMENT AND DISPOSAL OF SPENT NUCLEAR FUEL, HIGH-LEVEL AND TRANSURANIC RADIOACTIVE WASTES - ENVIRONMENTAL STANDARDS FOR GROUND WATER PROTECTION. Promulgated 58 FR 66415, 12/20/93. U.S. Codes: 42 U.S.C. 2011–2296 as amended, 5 U.S.C. app.1, 42 U.S.C. 10101–10270.	Disposal systems for waste shall be designed to provide a reasonable expectation that 10,000 years of undisturbed performance after disposal shall not cause the levels of radioactivity in any underground source of drinking water to exceed the limits in 40 CFR 141.
40 CFR 192 - PART 192, SUBPART A - STANDARDS FOR THE CONTROL OF RESIDUAL RADIOACTIVE MATERIALS FROM INACTIVE URANIUM PROCESSING SITES. Promulgated 60 FR 2866, 1/11/95. U.S. Codes: 42 U.S.C. 2022.	Control of residual radioactive materials shall be designed to be effective for up to 100 years, and to the extent reasonably achievable - 200 years. Releases of radon-222 shall not be greater than an average release rate of 20 pCi/m^2/sec or increase the average concentration of radon-222 in the air by more than one-half pCi/L. The concentration of a contaminant in groundwater shall not exceed: combined U-234 and U-238 = 230 pCi/L; gross alpha particle activity (excluding radon and uranium) = 15 pCi/L.
40 CFR 192 - PART 192, SUBPART B - STANDARDS FOR THE CLEANUP OF LAND BUILDINGS CONTAMINATED WITH RESIDUAL RADIOACTIVE MATERIALS FROM INACTIVE URANIUM PROCESSING SITES. Promulgated 48 FR 602, as amended at 60 FR 2867, 1/11/95. U.S. Codes: 42 U.S.C. 2022.	Remedial actions shall be conducted so as to provide assurance that the concentration of radium-226 in land over an area of 100 square miles shall not exceed the background level by more than 5 pCi/g - averaged over the 1st 15 cm of soil below the surface and 15 pCi/g averaged over the 1st 15 cm thick layers of soil more than 15 cm below the surface. In any occupied or habitable building, the objective shall be an annual average radon decay product concentration (including background) not to exceed 0.02 working level. The radon decay product concentration (including background) shall not exceed 0.03 working level and the level of gamma radiation shall not exceed the background level by more than 20 microroentgens per hour.

Regulatory citation	Regulatory action
40 CFR 192 - PART 192, SUBPART C - STANDARDS FOR THE CLEANUP OF LAND BUILDINGS CONTAMINATED WITH RESIDUAL RADIOACTIVE MATERIALS FROM INACTIVE URANIUM PROCESSING SITES - IMPLEMENTATION. Promulgated 48 FR 602, 1/15/83, as amended at 60 FR 2868, 1/11/95. U.S. Codes: 42 U.S.C. 2022.	The following contaminants are also listed for screening purposes: combined radium-226 and 228, combined uranium-234 and 238, gross alpha particle activity (excluding radon and uranium).
40 CFR 192 - PART 192, SUBPART E - STANDARDS FOR MANAGEMENT OF THORIUM BYPRODUCT MATERIALS PURSUANT TO SECTION 84 OF THE ATOMIC ENERGY ACT OF 1954. Promulgated 48 FR 45947, 10/17/83, as amended at 58 FR 60356, 11/15/93. U.S. Codes: 42 U.S.C. 2022.	Provisions applicable to uranium shall provide a reasonable assurance that the annual dose equivalent does not exceed 25 mrem to the whole body, 75 mrem to the thyroid, and 25 mrem to any other organ as a result of planned discharges of radioactive materials to the general environment.
40 CFR 197 - PART 197 - PUBLIC HEALTH AND ENVIRONMENTAL PROTECTION STANDARDS FOR YUCCA MOUNTAIN, NV. Promulgated 66 FR 32074, 6/13/01. U.S. Codes: 42 U.S.C. 2011-2296, 5 U.S.C. Appx. 1.	Standards consisting of containment requirements will limit the total amount of radionuclides entering the environment over 10,000 years.
40 CFR 268 - PART 268 - LAND DISPOSAL RESTRICTIONS. Promulgated 51 FR 40638, 11/17/86, as amended numerous times. U.S. Codes: 42 U.S.C. 6905, 6912, 6921, 6924.	Radioactive waste is prohibited from land disposal.
40 CFR 300, PART 300, SUBPART L - NATIONAL OIL AND HAZARDOUS SUBSTANCES POLLUTION CONTINGENCY PLAN: INVOLUNTARY ACQUISITION OF PROPERTY BY THE GOVERNMENT. Promulgated 62 FR 34602, 6/26/97. U.S. Codes: 33 U.S.C. 1321 and 42 U.S.C. 9601–9657.	Radionuclides are ranked in the hazard ranking system.
40 CFR 302 - PART 302 - DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated 50 FR 13474, 4/4/85. U.S. Codes: 42 U.S.C. 9602, 9603, 9604 and 33 U.S.C. 1321, 1361.	Reportable quantities have been set for a number of radionuclides.

Table B-4. Food and Drug Administration (FDA) regulations

Regulatory citation	Regulatory action
21 CFR 179 - PART 179, SUBPART B - IRRADIATION IN THE PRODUCTION, PROCESSING, AND HANDLING OF FOOD - RADIATION AND RADIATION SOURCES. Promulgated: 42 FR 14635, 3/15/77. U.S. codes: 21 U.S.C. 321, 342, 343, 348, 373, 374.	Ionizing radiation for the treatment of foods may be used as follows: Gamma rays from sealed units of the radionuclides cobalt-60 or cesium-137, electrons generated from machine sources at energies not to exceed 10 million electron volts, and X rays generated from machine sources at energies not to exceed 5 million electron volts. For control of *Trichinella spiralis* in pork carcasses - not to exceed 0.3 kGy to 1 kGy (30 to 100 krad); for growth and maturation inhibition of fresh food - not to exceed 1 kGy (100 krad); for disinfection of arthropod pests in food - not to exceed 1 kGy (100 krad); for microbial disinfection of dry or dehydrated enzyme preparations - not to exceed 10 kGy (1Mrad); for microbial disinfection of a number of dry or dehydrated aromatic vegetable substances when used as ingredients in small amounts solely for flavoring or aroma - not to exceed 30 kGy (3 Mrad); for control of food-borne pathogens in fresh or frozen uncooked poultry products - not to exceed 3 kGy (300 krad); for sterilization of frozen packaged meats used solely in the National Aeronautics and Space Administration space flight program - minimum dose 44 kGy (4.4 Mrad); for control of foodborne pathogens in, and extension of the shelf-life of, refrigerated or frozen uncooked meat, meat byproducts, or meat food products - not to exceed 4.5 kGy (450 krad) maximum for refrigerated products and 7.0 kGy (700 krad) maximum for frozen products; for control of Salmonella in fresh shell eggs - not to exceed 3.0 kGy (300 krad); for control of microbial pathogens on seeds for sprouting - not to exceed 8.0 kGy (800 krad). Radiofrequency radiation, including microwave frequency, may be used for heating foods. Ultraviolet radiation may be used for processing and treatment of foods if radiation sources consist of low pressure mercury lamps emitting 90% of the emission at a wavelength of 253.7 nanometers (2,357 angstroms). Pulsed light may be used to treat food providing that radiation sources consist of xenon flashlamps designed to emit broadband radiation, wavelengths covering the range of 200 to 1,100 nanometers and the pulse duration is no longer than 2 milliseconds.
21 CFR 179 - PART 179, SUBPART C - PACKAGING MATERIALS FOR IRRADIATED FOODS. Promulgated: 42 FR 14635, 3/15/77. U.S. codes: 21 U.S.C. 321, 342, 343, 348, 373, 374.	Packaging material identified in the rule may be subject to a dose of radiation not to exceed 10 kGy, incidental to the use of gamma, electron beam, or x-radiation in the radiation treatment of prepackaged food.

Regulatory citation	Regulatory action
21 CFR 579 - PART 579, SUBPART B - IRRADIATION IN THE PRODUCTION, PROCESSING, AND HANDLING OF ANIMAL FEED AND PET FOOD, RADIATION AND RADIATION SOURCES. Promulgated 51 FR 5993, 2/19/86, as amended numerous times. U.S. Codes: 21 U.S.C. 321, 342, 343, 348, 371.	Ionizing radiation is limited to: gamma rays for sealed units of the radionuclides cobalt-60 or cesium-137 and electrons generated from machine sources at energy levels not to exceed 10 million electron volts. The absorbed dose for laboratory animals should not exceed 50 kGy (5Mrad). For the treatment of poultry feed and poultry feed ingredients, the ionizing radiation is limited to gamma rays from sealed units of cobalt-60. For single treatment for rendering poultry diets or poultry feed ingredients salmonella negative: the minimum dose is 2.0 kGy (0.2 Mrad) and the maximum dose is 25 kGy (2.5 Mrad)
21 CFR 1020 - PART 1020 - PERFORMANCE STANDARDS FOR IONIZING RADIATION EMITTING PRODUCTS. Promulgated 38 FR 28632, 10/15/73. U.S. Codes: 21 U.S.C. 351, 352, 360e-360j, 360gg-360ss, 371, 381.	Performance standards have been set for cold-cathode gas discharge tubes, diagnostic X-ray systems and their major components, radiographic equipment, fluoroscopic equipment, computed tomography (CT) equipment, and cabinet X-ray systems.
21 CFR 1040 - PART 1040 - PERFORMANCE STANDARDS FOR LIGHT-EMITTING PRODUCTS. Promulgated 44 FR 52195, 9/7/79, as amended at 53 FR 11254, 4/6/88. U.S. Codes: 21 U.S.C. 351, 352, 360e-360j, 371, 381; 42 U.S.C. 263b–263n.	Performance standards have been set for laser products, sunlamp products and ultraviolet lamps intended for use in sunlamp products and high-intensity mercury vapor discharge lamps.

Table B-5. Nuclear Regulatory Commission (NRC) regulations

Regulatory citation	Regulatory action
10 CFR 20 - PART 20 - SUBPARTS A, B, D-O - STANDARDS FOR PROTECTION AGAINST RADIOACTIVITY. Promulgated 56 FR 23391, 5/21/91. U.S. codes: 42 U.S.C. 2073, 2093, 2095, 2111, 2133, 2134, 2201, 2232, 2236, 2297f, 5841, 5842, 5846.	Standards for surveys and monitoring, control of exposure from external sources in restricted areas, respiratory protection and controls to restrict internal exposure in restricted areas, storage and control of licensed material, waste disposal, records, reports, and enforcement.
10 CFR 20 - PART 20 - SUBPART C - STANDARDS FOR PROTECTION AGAINST RADIOACTIVITY - OCCUPATIONAL DOSE LIMITS. Promulgated 56 FR 23396, 5/21/91. U.S. codes: 42 U.S.C. 2073, 2093, 2095, 2111, 2133, 2134, 2201, 2232, 2236, 2297f, 5841, 5842, 5846.	Limits for adults: total effective dose = 5 rem/yr or the sum of the deep-dose equivalent and the committed dose equivalent to any individual organ or tissue other than the lens of the eye = 50 rems eye-lens dose equivalent = 15 rems shallow-dose equivalent to the skin or any other extremity = 50 rems soluble uranium intake = 10 mg/week limit for minors = 10% of annual dose for adult workers limit for embryo/fetus during the entire pregnancy = 0.5 rem
10 CFR 20 - PART 20 - APPENDIX B - STANDARDS FOR PROTECTION AGAINST RADIOACTIVITY. Promulgated 56 FR 23409, 5/21/91, 56 FR 61352, 12/3/91, as amended at 57 FR 57879 12/8/92, redesignated at 58 FR 67659, 12/22/93. U.S. codes: 42 U.S.C. 2073, 2093, 2095, 2111, 2133, 2134, 2201, 2232, 2236, 2297f, 5841, 5842, 5846.	Presents Annual limits of Intake (ALIs) and Derived Air Concentrations (DACs) of Radionuclides for Occupational Exposure.
10 CFR 20 - PART 20 - APPENDIX C - STANDARDS FOR PROTECTION AGAINST RADIOACTIVITY Promulgated 56 FR 23465, 5/21/91, 56 FR 61352, 12/3/91, redesignated and amended at 58 FR 67659, 12/22/93, 60 FR 20186, 4/25/95. U.S. codes: 42 U.S.C. 2073, 2093, 2095, 2111, 2133, 2134, 2201, 2232, 2236, 2297f, 5841, 5842, 5846.	Presents quantities of licensed radioactive material requiring labeling.
10 CFR 35 - PART 35 - MEDICAL USE OF BY-PRODUCT MATERIAL. Promulgated 51 FR 36951, 10/16/86. U.S. Codes: 42 U.S.C. 2111, 2201, 2232, 2233, 5841.	Presents requirements and provisions for the medical use of radioactive material and for the issuance of specific licenses authorizing the use of this material.
10 CFR 71 - PART 71 - PACKAGING AND TRANSPORTATION OF RADIOACTIVE MATERIAL. Promulgated 60 FR 50264, 9/28/95. U.S. Codes: 42 U.S.C. 2073, 2077, 2092, 2093, 2111, 2201, 2232, 2233, 2297f, 5841, 5842, 5846.	Presents requirements for packaging, preparing for shipment, and transportation of licensed radioactive material.

Table B-6. Occupational Safety and Health Administration (OSHA) regulations

Regulatory Citation	Regulatory action
29 CFR 1910 - PART 1910, SUBPART H - HAZARDOUS MATERIALS. Promulgated 54 FR 9317, as amended numerous times. U.S. Codes: 29 U.S.C. 653, 655, 657.	Monitoring shall be carried out for ionizing radiation.
29 CFR 1926 - PART 1926, SUBPART D - SAFETY AND HEALTH REGULATIONS FOR CONSTRUCTION. Promulgated 44 FR 8577, 2/9/79 and 44 FR 20940, 4/6/79. U.S. codes: 40 U.S.C. 333.	Requirements have been set out for use of sources of ionizing radiation in construction.

The regulations in all of the tables in this Appendix have been updated through the 2001 Code of Federal Regulations, December 31, 2001.

Appendix C: Human Cancer Studies: Summary Table

Table C-1. Recent human cancer studies of X and gamma radiation

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Military Exposures					
A-bomb survivor studies					
Pierce and Preston 2000	Solid cancer incidence	Dose estimates based on Dosimetry System 86 (RERF 1987).	A-bomb survivors who received doses less than 0.5 Sv and had been within 3,000 m of the hypocenter of the bombs. Solid cancer incidence data for 1958–1994 provided data for 7,000 cancer cases among 50,000 survivors in this low dose and close distance range. About 35,000 persons (presenting 5,000 cancer cases) received doses in the range of 0.005–0.2 Sv.	Linear extrapolation of risk estimates from the wider dose ranges (0 to 2 Sv or 0 to 4 Sv) provides useful risk estimates for doses as low as 0.05 to 0.1 Sv and does not overestimate risk estimates at lower ranges. Statistically significant risks in the 0–0.1 Sv dose range, upper confidence limit for any possible threshold about 0.06 Sv. Solid cancer rates increase about 5% per 0.10 Sv. Solid cancer radiation risks persisted even 50 years after exposure and, given sex-and age at exposure, acute radiation exposure increased normal age-specific solid cancer rates by a dose dependent factor throughout life.	

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Cologne *et al*. 1999	Primary liver cancer incidence	Dose estimates based on Dosimetry System 86 (RERF 1987).	Cohort of atomic bomb survivors. A comprehensive pathology review of known or suspected liver neoplasms diagnosed between 1958 and 1987 generated a total of 518 incident, first primary cases of mostly hepatocellular carcinoma (cholangiocarcinoma and hemangiosarcoma cases were rare in this cohort).	Relative risk due to radiation exposure estimated to be linear, RR = 1.81 per 1 Sv weighted liver dose; 95% CI = 1.32–2.43. Males and females: similar size relative risks, but the radiation-related excess incidence substantially higher in males due to a threefold higher background liver cancer incidence in male A-bomb survivors. No excess risk observed for those exposed before age 10 or after age 45.	

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Atmospheric nuclear testing					
Dalager *et al*. 2000	Cancer mortality	Personal radiation monitoring devices (mostly film badges).	1,010 veterans who received gamma radiation doses of ≥ 5 rem (out of 2,870 participants of U.S. atmospheric nuclear weapons tests conducted in the Pacific in 1958 (HARDTACK I)). Compared cancer mortality with 2,870 Navy veterans who received no or minimal radiation doses (≤ 0.25 rem). Mortality follow-up from 1958–1996; identified 814 deaths among 3,880 total cohort members.	Increased mortality from all cancers (cohort exposed to ≥ 5 rem compared with unexposed Navy controls): RR = 1.29; 95% CI = 0.97–1.72; n = 94 exposed all lymphopoietic cancers: RR = 3.72; 95% CI = 1.28–10.83; n = 11 exposed Largest contributor to cancer deaths were respiratory tract cancers: RR = 1.41; 95% CI = 0.91–2.18; n = 39 exposed.	Age, rank, and Navy assignment. Navy exposed and Navy unexposed personnel very similar in age, rank, and assignment except for radiation dose. According to the authors, confounding due to other risk factors was unlikely to have been a major problem.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Medical Uses					
Secondary cancers after medical treatment for a primary cancer in adults					
Brenner *et al*. 2000	Cancer incidence at all sites after primary prostate cancer .	Exposure to radiation therapy recorded in SEER database; due to the prevalent treatment techniques (prior to 1993 ^{60}Co irradiation) the lung dose was estimated at ~0.6 Gy, the pelvic region dose (bladder and rectum) at ~6 Gy, and the kidney dose at ~2 Gy.	Primary prostate cancer incidence cases from the Surveillance, Epidemiology, and End Results Program cancer registry 1973–1993: 51,584 men (mean survival time after diagnosis, 4.2 years) who received radiotherapy (3,549 subsequently developed second malignancies) and 70,539 men (5,055 subsequently developed a second primary cancer) who underwent surgery for prostate cancer without radiotherapy (mean survival time after diagnosis, 4.4 years).	Radiotherapy for prostate carcinoma associated with an overall small increase in the risk of solid tumors: RR = 1.06; 95% CI = 1.01–1.11, relative to treatment with surgery. Among patients who survived for > 5 years, increased relative risk for all solid cancers reached 15%: RR = 1.15; 95% CI = 1.06–1.24 Increased further to 34% for patients surviving > 10 years: RR = 1.34; 95% CI = 1.14–1.57 Strongest increase in risk for cancers within close proximity to the radiation treatment field: carcinomas of the bladder, (10 years after treatment): RR = 1.77; 95% CI = 1.14–2.63 rectum (10 years after treatment): RR = 2.05; 95% CI = 1.09–3.92 sarcomas within the field, all year RR = 1.85; 95% CI = 1.15–3.01 No increase in leukemia observed: RR (first 5 years after treatment) = 1.05; 95% CI = 0.76–1.44 Although dose estimates for the lung were low (~0.6 Gy), they produced an increased risk, for lung 10 years after treatment, RR = 1.42; 95% CI = 1.05–1.93.	Authors found no indication that differential smoking behavior might have introduced bias for the lung cancer results reported.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Yap *et al*. 2002	Sarcoma cancer incidence following a primary breast cancer	Exposure to radiation therapy recorded in SEER database.	Primary invasive breast cancer incident cases from the Surveillance, Epidemiology, and End Results Program cancer registry 1973–1997: 263 secondary sarcomas (87 with and 176 treated without radiation therapy) that developed in 274,572 incident breast cancer cases (82,296 (30%) of whom received radiotherapy); median latent time to diagnosis of sarcoma was 6 years.	Cumulative incidence of sarcoma at 15 years post diagnosis 3.2 per 1,000 (SE = 0.4) with radiation therapy vs. 2.3 per 1,000 (SE = 0.2) without radiation treatment; $P = 0.001$); angiosarcomas accounted for 56.8% of those sarcomas occurring within the field of radiation compared to only 5.7% angiosarcoma cases not treated with radiotherapy (cumulative incidence at 15 years post diagnosis: 0.9 per 1,000 with and 0.1 per 1,000 without radiation treatment). The cumulative sarcoma incidence started to differ clearly 5 years past the primary diagnosis when comparing radiation treated with untreated patients.	All female, no difference in age or race distribution in radiation treated and untreated breast cancer patients, but more patients received radiation treatment after 1993 than between 1973 and 1993.
Huang *et al*. 2001	Thyroid carcinoma after first diagnosis of invasive primary breast carcinoma	Exposure to radiation therapy recorded in SEER database.	Primary invasive breast cancer incident cases from the Surveillance, Epidemiology, and End Results Program cancer registry 1973–1993: 48,495 women treated with radiation developed 28 and 146,303 women not treated with radiation developed 112 secondary thyroid carcinoma.	No increase in the risk of thyroid carcinoma in the radiation treatment (RT) and the non-treatment (non-RT) cohort compared with the general population; SIR = 1.1; 95% CI = 0.8–1.6 for the RT cohort SIR = 1.2; 95% CI = 1.0–1.4 for the non-RT cohort.	All female, adjusted for age and calendar period.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Galper *et al*. 2002	All second cancers after clinical Stage I or II primary breast cancer	Radiation dose estimates were derived from medical records. Those treated with radiation received a median dose of 63 Gy to the tumor bed and 57%, in addition, received supraclavicular/axil-lary radiation (median dose 45 Gy, range 20 to 60).	Primary breast cancers incident case (n = 1,884) treated between 1968 and 1987, median follow-up to second cancer occurrence 10.9 years. Expected number of cases derived from the Surveillance, Epidemiology, and End Results Program; all 1884 patients followed at Brigham and Women's Hosptial received radiation treatment to the tumor site.	SIR 1.15 (P = 0.05) for any secondary malignacy; first 5 years after treatment of the primary cancer, the observed and expected rates of all second cancers were identical (47 vs. 46.9); after 5 years, 24% more second cancers were observed than expected (100 vs. 80.8, P = 0.02). In younger patients (< 50 years of age at breast cancer diagnosis) the excess observed was larger than in older patients (43% vs. 7% increase). Lung cancers excess of 52% above expected (P = 0.01); most of these lung cancers occurred > 5 years after treatment (n = 23), in women who were > 50 years at the time of breast cancer diagnosis (n = 27), and a larger percentage had received third-field radiation. No increase in colorectal cancers and lymphomas.	All female, adjusted for age and calendar period.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Kleinerman *et al*. 1995	Cancer incidence at all sites after primary cervical cancer	Due to the prevalent treatment techniques, radiation therapy doses were estimated as > 30 Gy for the pelvic region dose (proximity to field greatest), 2 Gy for the kidney, 20 Gy for the bone and 7 Gy for the active bone marrow, and only as ~0.1-0.3 Gy for the lung and thyroid.	86,193 primary cervical cancer patients reported to 13 population-based cancer registries in 5 countries (7,543 developed second cancers). Focus on long-term survivors (> 30 years after diagnosis of primary cancers and treatment). Among 49,828 women treated with radiation, 3,750 survived > 30 years. Expected numbers calculated based on 5-year age and calendar year incidence rates in the general female population.	7,543 second cancers observed versus 6,015 cancers expected based on population rates ([SIR 1.25; 95% CI = 1.22–1.28]). Lung cancers accounted for nearly half of the excess cancers. Two-fold risk of cancers in heavily irradiated organs. Most of excess risk found for pelvic organs in close proximity to the field of irradiation: cancers of the rectum, SIR = 4.0; [95% CI = 3.0–5.1] vagina, SIR = 39.4; [95% CI = 17.2–78.8] vulva, SIR = 7.9; [95% CI = 2.7–16.3] ovary, SIR = 1.7;[95% CI = 1.0–2.6] bladder, SIR = 6.2; [95% CI = 4.7–7.9] At lower doses, increases found for non-chronic lymphocytic leukemia in the first 10 years after radiation treatment only: SIR = 1.89; 95% CI = 1.21–2.82 1–4 years after treatment SIR = 1.69; 95% CI = 1.05–2.58 5–9 years after treatment Cancers of the bone: SIR = 3.0, 95% CI = 1.7–4.8 Kidney: SIR = 1.3, 95% CI = 1.0–1.5; SIR = 1.9 > 30 yrs after radiation treatment. Small increased risks for stomach, esophageal, and laryngeal cancers. Breast cancer occurred less often than expected.	Age, calendar time (and sex) stratification for SIR calculations

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Travis *et al*. 2000	Leukemia incidence after primary testicular cancer	Information about treatment type, dose, and duration of treatment abstracted from medical charts. The estimated mean dose to the active bone marrow was 12.6 Gy.	Case-control study of leukemia in a cohort of 18,567 1-year or more survivors of primary testicular cancer diagnosed between 1970–1993 selected from 8 population-based registries. 36 men developed leukemia and 106 control survivors without leukemia. Secondary leukemia developed on average 6.8 years after the diagnosis of testicular cancer.	Radiotherapy without chemotherapy (≥ 7.5 Gy): threefold elevated risk of leukemia: RR = 3.1 (95% CI = 0.7–22 based on n = 22 cases) Only abdominal/pelvic radiotherapy (mean dose to active bone marrow 10.9 Gy): RR = 2.9 (95% CI = 0.6–21 Additional chest radiotherapy (mean dose to bone marrow, 19.5 Gy): RR = 11.2 (95% CI = 1.5–123).	Controls matched on age, registry, calendar year of and survival time without leukemia after diagnosis.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Secondary cancers after medical treatment for a primary cancer in children					
Garwicz *et al*. 2000	Any second malignant neoplasm after cancer in childhood.	Radiotherapeutic charts provided information on target volume, target dose, number of days, and of fractions and radiation quality employed for treatment. The high-dose local radiation group received a maximum dose of >30 Gy at any volume, low dose group received ≤ 30 Gy.	Case-control study (1:3 matching ratio) of patients diagnosed and treated for a first malignant neoplasm before the age of 20 in 5 Nordic countries between 1960 and 1987. Follow-up through December 1991 and average follow-up time 7.5 years (range 0-32).	The relative risk of developing second malignant neoplasm in the irradiated volume was 4.3 (95% CI = 3.0–6.2). Risks of secondary cancers due to local irradiation were increased for cancers of the bone and connective tissue (RR 19.8; 95% CI = 4.5–86.7), breast (RR = 11.5; 95% CI = 3.2–40.6), leukemia (RR = 2.6; 95% CI = 0.8–8.5), lymphoma (RR = 5.1; 95% CI = 1.0–25.9) brain (RR = 2.8; 95% CI = 1.4–5.5). The risk was highest in children diagnosed and treated before the age of 5 years, and increased with the dose of radiation and with increasing follow-up time after first malignant neoplasm (i.e., risks were much greater after more than 10 years of follow-up) (RR 0–9 years = 1.7; 95% CI = 0.8–3.9 and 10-30 years RR = 4.3; 95% CI = 2.2–8.3). Chemotherapy alone was not associated with an increased RR, but significantly potentiated the effect of radiotherapy at low doses (interaction RR for low dose local radiation and chemotherapy = 7.0; 95% CI = 1.5–32.9).	Controls were sampled from the registries, matched by sex, age, and calendar year of diagnosis and length of follow-up.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Bhatia *et al*. 2002	Any second neoplasm after primary diagnosis for acute lympho-cytic leukemia (ALL).	The standard ALL treatment protocol required the treating institutions to record radiation doses and assigned fields which ranged from 0 to 1,800 cGy to the cranium (for CNS prophylaxis) and 2, 400 cGy to the cranium and 600 to 1, 200 cGy to the spine for treatment of CNS disease.	8,831 children diagnosed with ALL at 122 institutions throughout the U.S. and Canada before age 21 and enrolled for treatment on the Children's Cancer Group therapeutic protocols between 1983 and 1995; followed until 1999 to determine the incidence of second neoplasms. Median age at diagnosis of ALL was 4.7 years, median follow-up time 15 years. Expected number of cases derived from the Surveillance, Epidemiology, and End Results Program.	Increased cancer risks for acute myeloid leukemia (SIR = 52.3; 95% CI = 28.6–87.7) non-Hodgkin's lymphoma (SIR = 8.3; 95% CI = 2.6–17.2), parotid gland tumors (SIR = 33.4; 95% CI = 9.1–85.6), thyroid cancer (SIR = 13.3; 95% CI = 3.6–34.1), brain tumors (SIR = 10.1; 95% CI = 5.9–16.2), soft tissue sarcoma (SIR = 9.1; 95% CI = 2.4–20.2). thyroid at exposure to 2,400 cGy (RR = 30.8; 95% CI = 1.2–62.9). Risk for leukemia was highest in the first 5 years after radiation treatment and declined thereafter. Risk of second cancer increased with radiation dose for all cancers, all solid and all hemato- and lymphoietic cancers. 75% of all solid cancers developed within the radiation fields.	Adjusted for age and sex.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Ng *et al.* 2002	All second malignan-cies after primary Hodgkin's disease	The radiation fields included total nodal irradiation in 13% of patients, mantle and paraaortic irradiation in 66%, mantle alone in 17%, and pelvic and paraaortic in 3%. The median dose to the mantle field was 36 Gy, with a boost to bulk disease to a median total dose of 40 Gy, daily fractions ranged from 1.5 Gy to 2 Gy, 5 days per week.	Among 1,319 patients diagnosed and treated with clinical stage I-IV Hodgkin's at Brigham and Women's Hospital in Boston from 1969–1997, 181 second and 18 third malignancies were observed. The median follow-up was 12 years (32% had more than 15 years, 17% more than 20 years of follow-up). Expected number of cases was based on rates from the SEER data.	Increased risk for all second malignancy RR = 4.6 (95% CI = 4.0–5.4). Relative risk of breast cancer dropped steadily according to age at diagnosis of Hodgin's disease: 111.8 (95% CI = 36.2–261.0) at age < 15 years, 32.0 (95% CI = 14.6–60.7) for ages 15–19 3.7 (95% CI = 1.0–9.5) for ages 30–35 no increased risk after age 40. Women with a high risk for breast cancer had received radiation therapy to the chest prior to the age of 30 years. Relative risk for all secondary malignancies dropped with age 10.7 (95% CI = 7.8–14.4) for < 20 years 4.9 (95% CI = 4.0–5.9) for 20–50 years 2.4 (95% CI = 1.6–3.4) for > 50 years. Solid tumors showed a clear increase in risk with time since radiation treatment and risk increased with increasing radiation field. Acute leukemias and lymphomas showed a bimodal distribution with time since treatment; the largest relative risks were observed in the first 5 to 10 years of follow-up and a second peak after 20 and more years of follow-up.	Adjusted for age and sex.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Recent cohort studies examining medical irradiation for non-cancer diseases					
Ron *et al*. 1999	Cancer incidence at all sites after treatment for infertility	Dose estimated using information about typical treatment techniques, medical records and phantom simulations. Mean estimated dose to the brain 0.8 Gy, to the colon 0.6 Gy, to the ovary 1 Gy and to the bone marrow 0.36 Gy.	Cohort of 968 Israeli women treated with radiotherapy for infertility at a mean age of 28 years between 1940–1972 (mostly during the 1950s). Most of these women received radiation to the ovaries and the pituitary gland.	More than 10 years after radiation treatment, 60 incident cancers observed compared with 74.5 expected: SIR = 0.8; 95% CI = 0.6–1.0 Deficit due to a low risk of breast cancer: SIR = 0.7; 95% CI = 0.4–1.1 Increased risks for cancers of the colon: SIR = 1.6; 95% CI = 0.6–3.3 the uterine corpus: SIR = 3.8; 95% CI = 1.2–8.8 Only 2 cases of leukemia observed (1.61 expected).	Age, calendar time (and sex) stratification for SIR calculations

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Lichter *et al.* 2000	Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) incidence after irradiation for benign diseases of the skin	Dose estimates derived from medical chart review of subjects (for 79% of those who reported receiving therapeutic ionizing radiation treatment in interviews). Doses of < 2–10 Gy per treatment/week (total > 30 Gy for most patients).	Skin cancers (BCC, n = 592) and squamous cell carcinoma (SCC, n = 289) identified in a population-based incidence survey conducted in New Hampshire 1993–1995 after therapeutic ionizing radiation mostly for benign diseases of the skin such as keloids, acne, tinea, fungus, and warts. Cases were matched to 536 population-controls selected through driver's license and Medicare records. Cases were histologically confirmed.	Increased risks for both BCC and SCC in relation to therapeutic ionizing radiation at the site of prior radiation exposure: BCC OR = 3.30; 95% CI = 1.60–6.81; SCC OR = 2.94; 95% CI = 1.30–6.67 Effects most pronounced for those cases previously irradiated for acne: BCC OR = 17.35; 95% CI = 2.30–130.80 (n = 18); SCC OR = 9.97; 95% CI = 1.15–86.40 (n = 5); risks increased somewhat with the frequency of radiation treatments. Larger risks observed for early age at first treatment (< 20 years) and for those treated 40 years or more before diagnosis. For SCC, association with radiotherapy was restricted to individuals whose skin was likely to burn with sun exposure, for BCC risks were comparable in size in both groups.	Controls from population lists and age- and sex-matched to cases. Confounder data available from interviews: SES, and lifestyle factors (smoking, sun exposure, tanning, sun burn and sensitivity).

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Modan *et al*. 2000	Cancer incidence at all sites after cardiac catheteri-zation	Dose to the skin estimated to range from 5 – 40 Gy; and a dose of 1.1 cGy to the active bone marrow during heart catheterization.	Cohort of 674 children who underwent diagnostic cardiac catheterization due to congenital anomalies between 1950–1970 in three major medical centers in Israel and were followed through the end of 1996. 28.6% of the participants underwent more than one procedure (mean age at treatment 8.9 years, mean follow-up time 28.6 years). Linkage with the Israeli National Cancer Registry to identify cancer cases.	All diagnosed cancers occurred in males (but only 56.2% of the catheterized children were males). Expected number of malignancies for all sites in males = 4.75, the observed number = 11; SIR = 2.3; 95% CI = 1.2–4.1 Of the 11 cancer cases: 4 were lymphomas, 0.63 expected, SIR = 6.3; 95% CI = 1.7–16.2; one was Hodgkin's disease three cases of melanoma as opposed to 0.62 expected, SIR = 4.9; 95% CI = 1.0–14.2	Demographic data and vital status from the Israeli National Registry and a review of the children's medical files in each hospital.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Yeh *et al.* 2001	Cancer incidence and/or mortality at all sites after nasopharyngeal radium treatment .	Dose estimates derived from length, number, kind of treatments used, and distance of radium tip to organs estimated from skull films of children at different ages. Dose estimates ranged from < 0.04 – 0.44 Gy for the thyroid, from 0.44 – 1.7 Gy for the pituitary gland, and from 0.09 – 0.26 Gy for the salivary gland	Cohort of 2,925 subjects with adenoid hypertrophy, 904 of whom received radium treatment of the nasopharynx in Washington County, Maryland, between 1943–1960. Controls who were not treated with radiation mostly received tonsillectomy or adenectomy. Subsequent neoplasms identified from the Washington county cancer registry, death certificates, and questionnaires mailed twice, in 1978 and in 1994-1995. Radium implants emitted approximately 70% gamma rays.	No general increase in total cancer observed Total of 41 cancer cases identified in 808 patients, 83 in 1,819 traced non-exposed persons, RR = 1.0; 95% CI = 0.7–1.5 No salivary gland cancers found in either group. Excess risk of thyroid cancer in irradiated group, RR = 4.2; 95% CI = 0.4–46.6; 2 exposed cases and one unexposed case Seven brain tumor cases (three malignant and four benign) identified in irradiated group versus none in non-irradiated group, malignant RR = 14.8; 95% CI = 0.8–286.3; benign RR = 30.9; 95% CI = 1.9–541.7. Irradiated group showed decreased risks of breast cancer, female genital cancers, and prostate cancer, RR = 0.4; 95% CI = 0.2–1.0.	Year started follow-up, age at start of follow-up, sex, race, socioeconomic (SES), lifestyle factors (smoking and oral contraceptive use and hormone replacement therapy), reproductive history, breast cancer, and family history.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Occupational Exposures					
Sont *et al*. 2001	Cancer incidence at all sites	Personal radiation monitoring devices (mostly film badges).	Cohort of 191,333 workers whose occupational records for ionizing radiation doses were reported to the National Dose Registry of Canada between 1951–1988. Canadian cancer data base used to identify incidence cancers and to calculate standardized incidence ratios for 1969–1988.	Excess relative risks with increasing radiation dose for males and females combined for the following cancers: rectum: ERR per Sv = 13.8; 95% CI = 3.7–33.6 leukemia: ERR per Sv = 5.4; 95% CI = 0.2–20.0 lung: ERR per Sv = 3.0; 95% CI = 0.5–6.8 all cancers combined: ERR per Sv = 2.5; 95% CI = 1.2–4.0 all cancers except lung: ERR per Sv = 2.3; 95% CI = 0.9–4.1 all cancers except leukemia: ERR per Sv = 2.3; 95% CI = 1.1–3.9 For males: cancers of the colon: ERR per Sv = 2.8; 95% CI = 0.0–8.0 pancreas: ERR per Sv = 9.2; 95% CI = 0.1–36.8 testis: ERR per Sv = 38.3; 95% CI = 1.4–147.9	Stratifying by age, sex, and calendar year.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Richardson and Wing 1999	Total cancer mortality	Personal radiation monitoring devices (mostly film badges).	Extended follow-up of 8,307 white male workers hired at Oak Ridge National Laboratory (ORNL) between 1943–1972 and monitored for whole body exposure to ionizing radiation. Vital status and cause of death ascertained through 1990.	Cancer mortality Cumulative radiation dose: 1.8% (SE = 0.9) increase all-cancer mortality per 10 mSv (10-year lag). Age effects Radiation doses at older ages (> 45 years of age at exposure): larger effects than doses received at younger ages (after age 45 a 5.9% [SE = 1.7] per 10 mSv). In older age range, dose-response associations consistent across periods of follow-up, periods of hire, and ages at risk.	Adjusted for SES (pay type). No information on lifestyle factors and non-occupational exposures.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Ritz *et al*. 1999a, Ritz *et al*. 1999b	Total cancer mortality, radiosensitive solid (ICD-9[a] 150, 151, 153, 162, 174, 188, 189, 192) lung, hemato- and lympho-poietic cancers (ICD-9 200-208 excluding CLL).	Personal radiation monitoring devices (mostly film badges).	4,563 nuclear workers monitored for external radiation exposure at Rocketdyne in Los Angeles between 1950–1993 (follow-up from 1950–1994, 258 total cancer deaths, average follow-up 26.1 years)	<u>Overall mortality rates</u> total cancers: ERR per 100 mSv = 1.22; 95% CI = 0.86–1.73 radiosensitive solid cancers: ERR per 100 mSv = 1.25; 95% CI = 0.80–1.94 rates increased monotonically with cumulative radiation dose. Mortality rates (exposed to 200 mSv) hemato- and lymphopoietic cancers: RR per 100 mSv = 1.99; 95% CI = 0.83–3.40 lung cancer: RR per 100 mSv = 1.52; 95% CI = 0.90–2.55 <u>After the age of 50 years</u> total cancers: RR per 100 mSv = 1.98; 95% CI = 0.63–6.26 radiosensitive solid cancer: RR per 100 mSv = 3.29; 95% CI = 1.10–9.89 lung cancer: RR per 100 mSv = 3.89; 95% CI = 1.23–12.3.	Adjusted for SES (pay type) and exposure to radionuclides and some chemicals. Some information on smoking and radiation exposures in previous jobs. No information on other lifestyle factors and non-occupational exposures.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Ritz 1999	Total cancer mortality, radiosensitive solid (ICD-9 150, 151, 153, 162, 174, 188, 189, 192), hemato- and lympho-poietic cancers (ICD-9 200-208 excluding CLL), lung, digestive tract, urinary tract cancers.	Personal radiation monitoring devices (mostly film badges).	Cancer mortality in a cohort of 4,014 uranium-processing workers at the Fernald facility.	Mortality rates total cancer: RR per 100 mSv external radiation = 1.92; 95% CI = 1.11–3.32 radiosensitive solid cancer: RR per 100 mSv = 2.00; 95% CI = 1.02–3.94 lung cancer: RR per 100 mSv = 2.77; 95% CI = 1.29–5.95 Effects were stronger when exposure had occurred at older ages (> 40 years of age).	Adjusted for internal doses from radionuclide exposures and for exposure to chemical carcinogens. Some information on smoking. No information on other lifestyle factors and non-occupational exposures.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Wing *et al.* 2000	Multiple myeloma mortality	Personal radiation monitoring devices (mostly film badges).	Multi-facility nested case-control study of nuclear workers at U.S. Department of Energy facilities at Hanford, Los Alamos National Laboratory, Oak Ridge National Laboratory, and the Savannah River site. 98 multiple myeloma identified from the combined roster of 115,143 workers hired before 1979 (followed for vital status through 1990; and 1986 for Hanford). 391 age-matched controls.	Age effects Lifetime cumulative whole body ionizing radiation dose: not associated with multiple myeloma At older age at exposure (> 45 years), positive association observed (> 45 with a 5-year lag): ERR = 6.90% per 10 mSv (SE = 2.90). Similar size but non-significant deficit for multiple myeloma at younger ages of exposure observed: increase per 10 mSv = 6.83 (SE = 6.11).	Demographic data, work history and occupational carcinogen exposure data abstracted from personnel, occupational medicine, industrial hygiene, and health physics records. Also controlled for exposures to radionuclides and exposures received prior to employment at the nuclear facilities, and to some extent for smoking.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Kreisheimer *et al*. 2000	Lung cancer mortality	Personal radiation monitoring devices (mostly film badges).	Two subcohorts of male Mayak workers who started employment between 1948–1958 and followed through 1995: 1) 1,669 workers (with 117 lung cancers) employed in the Mayak plutonium and reprocessing plants who received internal exposure from plutonium and, in addition, external gamma radiation; 2) 2,172 Mayak reactor workers (with 74 lung cancers) who were exposed only to external gamma rays.	Lung cancer mortality rate: consistent with linear dose dependence. ERR for lung cancer mortality = 0.20/Sv (95% CI = -0.04–0.69), exposure to gamma-rays at age 60. ERR for gamma radiation in workers not exposed to plutonium = 0.43 (SE = 0.31).	Unable to adjust for smoking in the analyses, but authors did not expect smoking behavior to be differentially distributed according to radiation dose.

Reference	Cancer outcome	Methods of determining radiation dose	Population	Effects	Comments
Gilbert *et al*. 2000	Liver cancer incidence	Personal radiation monitoring devices (mostly film badges).	11,000 Mayak production workers including female workers (with 60 liver cancers) initially employed between 1948–1958 and followed through 1995.	Excess risk for workers with external doses exceeding 1 Sv to the liver, and workers in the plutonium plant with detectable plutonium burdens. Compared to the Russian population, large standardized mortality ratios (SMRs) found for female workers: SMR 0–0.1 Sv = 0.5; 95% CI = 0.03–2.0 SMR 0.1–1 Sv = 1.3; 95% CI 0.3–3.4 SMR 1–3 Sv = 7.9; 95% CI = 4.1–13 SMR > 3 Sv = 9.2; 95% CI = 2–21 Due to the concomitant exposure to internal and external radiation, risk from external gamma dose alone could not be reliably evaluated.	Information about alcohol consumption available for cases only. No information on hepatitis infections.

[a]ICD-9- International Classification of Disease, 9th revision, originally published by the World Health Organization

C.1 Appendix C References

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Appendix D: Germ Cell Mutations in Experimental Animals

D.1 X radiation and gamma radiation

D.1.1 Dominant visible mutations

Dominant visible mutations are detected in the F_1 progeny of the irradiated generation. For skeletal abnormalities of all classes, single X-ray doses in mice induce a mutation rate of 1×10^{-5} per gamete per cGy for spermatogonial stem cells and 3×10^{-5} per gamete per cGy for post-spermatogonial stages (Ehling 1965, 1966). Similar frequencies (2.3×10^{-5} per gamete per cGy) were observed by Selby and Selby (1977) for mouse spermatogonial stem cells exposed to gamma rays (two fractions separated by 24 hours). No dose rate reduction factor has been reported.

The mutation rate for abnormalities of the lens was reported to be about 3 to 13 x 10^{-7} per gamete per cGy for X- or gamma-irradiated spermatogonia (Ehling 1985). Post-spermatogonial stages were about two- to five-fold more sensitive than spermatogonia. The lower overall sensitivity to mutations leading to abnormalities of the lens compared to the skeleton is quite likely due to the larger number of genes that can be mutated for the latter system.

A range of additional dominant mutations have been assessed for their induction by radiation. These include mutations that lead to changes in growth rate, coat color, limb and tail structure, eye and ear size, hair texture, and histocompatability. In summary, for female mice the induced frequency was 5 to 10×10^{-7} per gamete per cGy of acute X-ray exposure and about 1×10^{-7} per gamete per cGy for protracted gamma ray exposure of spermatogonia (Batchelor *et al.* 1966, Lyon *et al.* 1979). No dose rate reduction factors can reasonably be calculated given the differences between the experimental designs. There appear to be no increases in mutation frequency for histocompatability with irradiated sperm or spermatogonia (Kohn and Melvold 1976, Dunn and Kohn 1981).

D.1.2 Dominant lethal mutations

Dominant lethal mutations are most frequently assessed as a reduction in F_1 offspring of an irradiated parent. These losses can occur either as preimplantation or post-implantation events. A great majority of dominant lethal mutations are the consequence of loss of genetic material via the induction of chromosomal alterations (largely deletions). Dominant lethal mutation rates are on the order of 1×10^{-3} per gamete per cGy for post-spermatogonial cells and about 1×10^{-4} per gamete per cGy for spermatogonial cells following acute exposures (Kirk and Lyon 1984). The mutation rates are about one-half these values for more chronic exposures (Grahn *et al.* 1979).

D.1.3 Recessive autosomal lethal mutations

Recessive autosomal lethal mutations have not been the subject of much study in mammals. (Lüning and Eiche 1975) reported a mutation rate of about 1×10^{-4} per gamete per cGy. Very similar mutation rates were obtained for X irradiation of mature or maturing mouse oocytes (Lüning and Eiche 1982).

D.1.4 Recessive visible mutations

Recessive visible mutations are measured by one version or another of a specific locus test (reviewed by Favor 1989). The method relies on the tester stock that is homozygous recessive for the loci that define the visible phenotypes to be assessed. Thus, any induced mutation at these specific loci in exposed wild-type individuals will be recovered as a visible phenotype, since it will be in a homozygous recessive state. The mutation rates induced by low-LET radiations in mouse spermatogonia are about 2×10^{-7} per locus per cGy for acute exposures and 7×10^{-8} per locus per cGy for chronic exposures (Russell and Kelly 1982a). Post-spermatogonial cells were more sensitive to the induction of specific locus mutations with a mutation rate of about 7×10^{-7} per locus per cGy following an X-ray dose of 3 Gy given at a low dose rate (Russell and Kelly 1982b).

For females, exposure of mature oocytes to high dose-rate X rays resulted in a mutation rate of 4×10^{-7} per locus per cGy. At lower dose rates, a mutation rate of 1 to 3×10^{-8} per locus per cGy was reported (Russell 1977, Lyon *et al.* 1979).

The mutation rates for male and female postgonial staged are quite similar for acute exposures. However, the dose-rate reduction factor for males is about 3 and that for females is about 10. This has potential implications for heritable risk assessments.

D.1.5 Reciprocal translocations

Reciprocal translocations have been assessed in several species following exposure to X rays. The majority of data has been collected for reciprocal translocations assayed in primary spermatocytes derived from exposed spermatogonial stem cells. The range of sensitivities on a per cell basis is not that large with the mouse being the least sensitive with 1 to 2×10^{-4} reciprocal translocations per cell, and the marmoset being the most sensitive with 7×10^{-4} per cell (Brewen and Preston 1973, Brewen *et al.* 1975, van Buul *et al.* 1986). A study of a small set of data for human males reported a reciprocal translocation frequency of 3×10^{-4} per cell (Brewen *et al.* 1975). The relative sensitivities of spermatogonial stem cells to acute low-LET exposure is quite similar to those published for peripheral lymphocytes following exposure *in vivo* or *in vitro* (Brewen and Preston 1973). The frequency of reciprocal translocations induced by low-dose gamma rays is about 1 to 2×10^{-5} per cell, giving a dose-reduction factor of about 10 (Brewen *et al.* 1979).

There are few data for reciprocal translocation induced in females, and these are limited to the mouse. Brewen *et al.* (1976) reported a reciprocal translocation rate of 2.6×10^{-4} per cell for acute X-ray exposures for mature oocytes. Based on these data, the sensitivity of males and females to the reduction of reciprocal translocations, assessed cytogenetically, are quite similar.

For genetic risk assessment, it is necessary to have available an estimate of the proportion of induced reciprocal translocations that are recovered in the F_1 generation, i.e., the heritable component. Again, only limited information is available, but it seems reasonable to conclude that about one-half of the frequency of reciprocal translocations observed in primary spermatocytes is recovered in F_1 (Ford *et al.* 1969). The reasons for

this reduction are not known, but almost certainly relate to the segregation of the chromosomal quadrivalent formed as a consequence of the translocation.

D.1.6 Minisatellite mutations

Minisatellite mutations studied in exposed humans were discussed in Sections 5.1.1.1 and 5.1.1.2. The phenotypic consequences of such mutations, when induced in germ cells, is unknown or there are none. Low-LET radiations can induce alterations in the size of minisatellite sequences in mouse germ cells at very high frequencies, of the order of several percent (Fan *et al.* 1995). Acute and chronic exposures of gamma rays were reported to be equally effective at inducing minisatellite alterations in mouse germ cells (Dubrova *et al.* 2000). In addition, it was reported that exposure of mouse spermatogonial cells, including stem cells, resulted in an approximately linear increase in minisatellite mutations, whereas there appeared to be no induction in post-meiotic male germ cells (Dubrova *et al.* 1998). There is a need to extend these studies and to attempt to establish how relevant they are for heritable risk assessment (UNSCEAR 2001).

D.2 Neutrons

D.2.1 Dominant visible mutations

Dominant visible mutations were induced at a rate of 2.6×10^{-6} per gamete per Gy in spermatogonia by fission neutrons (mean energy, 0.7 MeV) (Batchelor *et al.* 1966). The background rate is approximately 8×10^{-6} per gamete per generation.

D.2.2 Dominant lethal mutations

Dominant lethal mutations were measured in male mice for germ cells exposed to fission neutrons at the post-spermatogonial stage. The mutation rate was approximately 2×10^{-1} per gamete per Gy (Grahn *et al.* 1979). For cells similarly irradiated but at the spermatogonial stem cell stage, the dominant lethal mutations rate was 4×10^{-2} per gamete per Gy (Grahn *et al.* 1979). There was no effect of dose rate comparing exposures that were single or weekly. Maturing and immature oocytes were exposed to recoil protons from 0.43 MeV neutrons. This neutron quality was used so that damage to the plasma membrane was minimized, thereby mitigating much of the cell death. Dose-response relationships were obtained for chromosomal alterations and dominant lethal mutations induced in maturing and immature oocytes. The two stages were equally sensitive (Straume *et al.* 1991).

A direct comparison between the effectiveness of gamma rays and fission neutrons at inducing dominant lethal mutations could be made from a series of experiments by Grahn *et al.* (1984, 1986). Male mice were exposed to once-a-week doses of fission neutrons or ^{60}Co gamma rays for up to one year. The effect of the different radiation scenarios was assessed by dominant lethal mutation induction. The neutron doses were 0.0013-0.027 Gy per week and the gamma ray doses were 0.05-0.32 Gy per week. Direct comparison for pre- and post-implantation deaths could be made with the same data for males exposed to single doses of neutrons or gamma rays. This comparison showed that weekly neutron doses were much more effective than single doses for inducing post-implantation loss, whereas single doses of gamma rays were more effective than the weekly fractions.

The RBE for neutrons was about 5 for single doses and about 12 for weekly doses. Pre-implantation losses were not a sensitive measure of genetic injury at the low doses used.

A similar type of experiment was reported by Grahn *et al.* (1984) for single whole-body doses of fission neutrons or gamma rays. Dominant lethal responses were assessed in detail for neutron doses of 0.01 to 0.4 Gy and for gamma ray doses of 0.23 to 1.45 Gy. Significant effects were seen at 0.02 and 0.025 Gy of neutrons. The RBE value for post-implantation loss and total dominant lethal rates were about 6 at doses greater than 0.1 Gy and about 12 at doses less than 0.1 Gy. The values for pre-implantation loss were between 15 and 25 at doses greater than 0.1 Gy, and possibly higher at doses less than 0.1 Gy. A number of confounders could account for the high values at low doses (Grahn *et al.* 1984).

D.2.3 Recessive visible mutations

Recessive visible mutations are measured by specific locus tests and are induced in male mouse post-spermatogonial stages with doses of neutrons up to 1 Gy; however, specific locus mutations induced by neutrons have only been studied in a very limited fashion. The mutation rate was 1 to 1.5×10^{-4} per locus per Gy. There was no effect of dose rate (Russell 1965). The general conclusion is that neutron radiation is more effective than gamma radiation at inducing recessive visible mutations in mouse spermatogonia (Batchelor *et al.* 1966).

A small amount of data shows that mutations also are induced in mature and maturing oocytes by neutrons at low dose rates (Russell 1967, Batchelor et al. 1969). Similar mutation rates (1.5×10^{-4} per locus per Gy) were reported for recessive visible mutations induced in female mice with fission neutron doses of 0.3, 0.6, and 1.2 Gy (Russell 1972).

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Appendix E: DNA Repair

E.1 Single-strand breaks

In general, repair of single-strand breaks is by simple religation of the broken ends with modification of the broken ends being necessary to produce a 3' OH and a 5' phosphate group. Loss of a nucleotide during this broken end modification process presents no great problem to the cell because a presumably undamaged base is present on the complementary strand allowing "fill in" repair. This process of repair is predicted to be largely error-free and quite rapid. In fact, the majority of single-strand breaks are repaired in a matter of a very few minutes even when doses of ten's of Gy have been delivered (Van der Schans *et al.* 1983). There is little or no evidence to suggest that the misrepair of single-strand breaks is involved in the production of mutations, and since their repair is rapid, there is little expectation that they would be involved to any great extent with mutation induction by errors of DNA replication. It has been shown by Natarajan and Obe (1978) that if X ray-induced single-strand breaks are converted into double-strand breaks by means of an introduced Neurospora single-strand endonuclease, then an increase in chromosome aberrations results. However, this shows that double-strand breaks are of importance in the formation of chromosome alterations, rather than implicating the conversion of single-strand breaks into double-strand breaks as a normal cellular phenomenon.

E.2 Double-strand breaks

More information is available on the repair of double-strand breaks, to a large extent because such lesions can be introduced in a defined manner by restriction endonucleases and because many of the genes involved in double-strand breaks repair have been identified (Jackson 2002). Much of the information still remains pertinent only for high exposures (several Gy) since the frequencies of double-strand breaks are low even at these high exposures. It is important to note also that radiation-induced double-strand breaks are much more variable with regard to cut end structure and DNA sequence location than those produced by restriction endonucleases.

There are two major pathways involved in the repair of DNA double-strand breaks, homologous recombination and non-homologous end-joining (reviewed by Jackson 2002). Homologous recombination is the major repair pathway in yeast, whereas non-homologous end-joining is the major repair pathway in mammalian cells.

E.3 Non-homologous end-joining repair

A double-strand break can be repaired by ligation of the cut ends, usually with some modification. It is unlikely that radiation-induced double-strand breaks will be equivalent to the blunt-end type of lesion produced by some restriction endonucleases but rather more similar to the cohesive or overlapping-end type, bearing in mind that there is unlikely to be a specific DNA sequence among radiation-induced double-strand breaks in contrast to restriction enzyme induced double-strand breaks. This consideration will clearly be of importance when considering the process of misrepair or misjoining. The various modes of religation of restriction enzyme-induced double-strand breaks have been discussed by Pfeiffer and Vielmetter (1988) and Goedecke *et al.* (1994). Cohesive ends will be the major kind of double-strand breaks induced by radiation and these are repaired by the non-homologous end-joining process. It should further be noted that a

double-strand break can be formed when two single-strand breaks occur within a region of five to seven base pairs (Van der Schans 1969).

It is of significance to the integrity of the genome that repair of double-strand breaks be accurate and restore the original DNA molecule. On the other hand, for considerations of genetic alterations it is of importance to consider the consequences of inaccurate repair (reviewed by Rothkamm and Lobrich 2002).

The probability of misrepair during non-homologous end-joining of double-strand breaks would be expected to be influenced by the frequency of double-strand breaks, the length of time that double-strand breaks remain unrepaired, the cellular distribution of the double-strand breaks, the fidelity of the ligation process, the relative proportion of the repair of double-strand breaks that occurs via this process, and, hence also, the nature of induced double-strand breaks (related largely to radiation quality). Failure to repair a double-strand break by non-homologous end-joining can lead to a mutation, most likely a deletion (but possibly a point mutation as the consequence of a replication error at the site of the unrepaired double-strand breaks).

On the assumption that the chromatin of a cell is not static, it would be expected that the longer a double-strand break remains unrepaired, the greater the chance that it will be in contact with an adjacent DNA molecule and subsequently produce a misjoining event. While the analysis of the repair of double-strand breaks has not distinguished among the various modes of repair, there is no indication that overall repair of double-strand breaks differs significantly among species. Again of note, there are a number of reports of two types of double-strand breaks, one that is rapidly repaired and one that is slowly repaired (Fox and McNally 1988, Metzger and Iliakis 1991). From the above, it would be predicted that the slowly repaired double-strand breaks would be more likely to be involved in misjoining events leading to genomic alterations.

It was proposed by Goodhead (1994) that clustered damage that might typically include multiple double-strand breaks along with other damages is resistant to repair and, perhaps, especially when induced by high-LET radiations, irreparable.

E.4 Recombination repair

It has also been established that double-strand breaks can be repaired by a recombination process (Szostak *et al.* 1983). This has been most clearly demonstrated for the process of mitotic recombination or homologous recombination where an initiating event is an endonuclease produced double-strand break. Although it should be noted that this process is not for the repair of double-strand breaks *per se*, but rather they are repaired as a necessary component of the recombination. The evidence for recombination repair of radiation-induced double-strand breaks in mammalian cells is becoming more clearly defined (Thompson and Schild 2002). Recent data have indicated that a specific gene (XRCC2) is involved in recombination repair of radiation-induced double-strand breaks (Johnson *et al.* 1999), and the essential role of Nbs1 recently has been described (Tauchi *et al.* 2002).

It should be emphasized that recombinational repair can involve intrachromosomal or interchromosomal (homologous sequences on non-homologous chromosomes) recombination. The former can lead to no mutations or a variety of point mutations, deletions and rearrangements depending upon the fidelity of the process. The latter (interchromosomal) can result in translocations, deletions and point mutations as a consequence of the process itself, where non-homologous chromosomes are involved, and also as a consequence of the fidelity of the process. There is insufficient information to determine whether or not there are interspecific differences in the extent, fidelity or nature of the recombinational repair process.

It would be of importance to determine whether a double-strand break will be repaired by non-homologous end-joining or a recombinational event. Although this is not clearly defined at the moment, there is some information that addresses the question. For example, it has been shown that double-strand breaks in transcriptionally active genes are preferentially repaired in both human and rodent cells, and that this repair is via a recombination process (Frankenberg-Schwager *et al.* 1994). The constraints that chromatin structure might impose on recombination could determine the pathway chosen for repair. Lopez and Coppey (1987) showed that the end-structure at the break site can be a determining factor as to which method of repair a cell will utilize. For example, dephosphorylation of the break ends prevents the ligation pathway but does not affect the recombination pathway; blunt or 5' protruding ends can be repaired by recombination, whereas 3' overhanging ends cannot. This could be a consequence of the polarity of the exonuclease and recombinase activities associated with the repair process. Short stretches of perfect homology rather than long stretches of partial homology were found to govern the efficiency of recombination. Wahls *et al.* (1990) found that when a double-strand break was introduced into hypervariable minisatellite sequences, recombination was stimulated over and above that caused by double-strand breaks in other sites in the plasmid used. Efficient recombination in mammalian cells can be maintained with a homologous stretch of DNA as short as 165 to 320 bp and with a low level of recombination measurable for 29 bp of homology (Lopez *et al.* 1992). This would allow for the recombination to occur between non-homologous chromosomes that contain relatively short sequences of homologous DNA, such as minisatellites or other repetitive DNA segments. The consequence would be chromosomal rearrangements and/or deletions. Determining the balance between non-homologous end-joining and recombination repair is of importance when considering the mechanistic basis for radiosensitivity.

E.5 Base damage repair

It is generally agreed that the major pathways by which DNA base damages are repaired are (1) via a glycosylase leading to an apurinic or apyrimidinic site that can be filled by the appropriate base, or can be removed by an excision repair pathway; or (2) removal of the damaged base is directly by an excision repair pathway (Demple and Harrison 1994). Satoh *et al.* (1993) showed that some specific base damages, produced by gamma-radiation and H_20_2 are inefficiently removed by XP-A cells that are defective in the nucleotide excision repair pathway. This suggests that perhaps more than one excision

repair pathway is involved in the repair of DNA base damages. This might not be surprising given the variety of types that are induced.

Because of the difficulty of assessing the repair of the myriad of base damages induced by ionizing radiations, little information on their repair has been forthcoming. The development of antibodies to DNA base damage has provided some information on the kinetics of repair and consequences of replication on a template containing a specific base damage (Demple and Harrison 1994). A recent report by Lee *et al.* (1998) described an ultrasensitive method for measuring base damage that used immunochemical recognition coupled with capillary electrophoresis and laser-induced fluorescence detection. In human carcinoma cells, glycols induced by 0.25 Gy could be detected. In addition, an inducible repair process for radiation-induced damage to DNA bases was reported.

E.6 Characterization of genes (enzymes) involved in DNA repair

The intention of this section is to provide a general description of the types of enzymes so far characterized. This section is not meant to provide a detailed description of the isolation and characterization of DNA repair genes nor the details of the genes and putative enzymes themselves.

It is only in the past five or six years that progress has been made in identifying genes involved in the recognition and repair of ionizing radiation-induced DNA damage. With this improved understanding, a much better handle on the mechanisms behind ionizing radiation sensitivity has been obtained (Jackson 2002).

There has been recent progress in understanding the role of a DNA-dependent protein kinase in DNA repair via non-homologous end-joining (Gao *et al.* 1998). This enzyme consists of two parts, a catalytic component (DNA-PK_{cs}) and a DNA binding component. This latter component has been named Ku protein, and is a heterodimer of Ku70 and Ku80 (more accurately designated as Ku86 based on molecular weight). A significant feature of Ku is that it binds to DNA ends, and recruits the DNA Pk_{cs} protein to form a complex. Substrates for the kinase activity of the complex include p53, Sp1 a transcription factor, and RNA polymerase II (Dvir *et al.* 1992, Gottlieb and Jackson 1993, 1994). The precise role of the kinase in DNA repair has not yet been established, but several intriguing observations have been made. A series of rodent cell lines that are X-ray sensitive and deficient in repair of double-strand breaks, have parallel deficiencies in the V(D)J recombination of lymphocyte antigen receptor genes. One of these, Xrs6, is complemented by XRCC5 that encodes the p86 subunit of Ku (Taccioli *et al.* 1994). Xrs6 cells are not only defective in Ku but also in the DNA-dependent protein kinase activity (Finnie *et al.* 1995). These observations describe a link between Ku DNA-dependent activities with the repair of double-strand breaks and V(D)J recombination. This association was further extended by the studies of Blunt *et al.* (1995) who showed that X ray-sensitive V3 mutant hamster cells and homozygous severe combined immunodeficiency (scid) mice both retained Ku activity but were defective in the DNA-PK_{cs} catalytic component of the DNA-dependent protein kinase. Thus, DNA damage in the form of double-strand breaks can be recognized by Ku which then recruits DNA-PK_{cs} to form a kinase complex that can initiate DNA repair through phosphorylation of

additional repair proteins, that might include components of the V(D)J recombination process (Jackson 2002).

Details of the homologous recombination DNA repair pathway also are being uncovered. This effort has been facilitated by the finding that several human radiosensitivity disorders are the result of defects in double-strand repair via homologous recombination (Thompson and Schild 2002) (See Section 6.7.4 and Appendix E.4). For example, the Nbsl gene that is mutated in Nijmegen breakage syndrome, has been shown to be essential for homologous recombination double-strand breaks repair (Tauchi *et al.* 2002). The gene is part of a complex of proteins, Mre11-Rad50-Nbsl, and it appears quite feasible that this complex as a whole is involved in double-strand breaks repair. It is of additional interest that BRCA-1 and BRCA-2, genes that when mutated are involved in susceptibility to breast cancer, are strongly implicated in homologous recombination repair of double-strand breaks (Thompson and Schild 2002).

A further significant advance in our understanding of cellular responses to ionizing radiation was the cloning of the ATM gene (see Section 6.7.3 and Appendix E.3). The gene that when mutated is responsible for ataxia telangiectasia (ATM, mutated in AT) has been cloned (Savitsky *et al.* 1995). Despite the fact that four complementation groups had been identified as possibly representing different genes, the ATM gene is mutated in all of these. The potential role of AT heterozygosity in an increase in tumor incidence remains to be determined. It appears that ATM regulates multiple cell cycle checkpoints as well as regulating DNA repair and apoptosis. Thus, it is a central regulator of responses to DNA double-strand breaks (reviewed in Khanna *et al.* 2001). The isolation and characterization of additional DNA repair genes will help to clarity how a cell recognizes DNA damage and removes it with fidelity or in an error-prone manner.

E.7 DNA repair and cell cycle progression

DNA damages induced in G_0, G_1, or G_2 can be converted into chromosome alterations and point mutations as a result of errors in the repair process. This misrepair can be a consequence of joining incorrect DNA ends together during ligation, recombination, or excision repair or from the insertion of an incorrect base during these various repair processes. There is very little that the cell can do about this, given that repair of ionizing radiation-induced DNA damage is a potentially error-prone process and that there is a cellular need to repair DNA damage. On the other hand, there is something that the cell can do about the control of entry into the two critical phases of the cell cycle (S and mitotic/meiotic division) with the genome as intact as possible. This is achieved through the development of the so-called “cell cycle checkpoints” that arrest cells prior to entry into the S phase or prior to the commitment in G_2 to contract and segregate mitotic chromosomes. The signature gene for describing the G_2 checkpoint is p53, a tumor suppressor gene, with several linked functions that contribute to the general phenotype. It appears that wild-type p53 protein can inhibit cell cycle progression by binding to the TATA binding protein that is a component of the transcription complex, thereby inhibiting transcription of genes that are necessary components of cell cycle progression (Seto *et al.* 1992). It also can activate the transcription of genes that have a p53-responsive element (Kern *et al.* 1991), and act as a regulator by binding to the replication

protein RPA (Dutta *et al.* 1993). Further, p53 induces the expression of p21 Cipl, a cell cycle inhibitor of cyclin-dependent protein kinases, thereby preventing the induction of DNA replication (Waga *et al.* 1994). Any one or a combination of these features of p53 could result in cell cycle arrest.

Perhaps the most important characteristic of p53, however, is that its expression is induced by DNA damage. This, then, provides a way in which its checkpoint function can be available as needed. Coupled to the checkpoint function, evidence is accumulating that wild-type p53 can bind to single-stranded DNA ends, at the sites of single- or double-strand breaks and, in bacteria, can catalyze DNA renaturation and strand transfer (Bakalkin *et al.* 1994). This would suggest that p53 can play a direct role in the repair of DNA breaks. Recent evidence supports this contention. It has been suggested that not only does p53 stimulate the synthesis of p21, it also upregulates the expression of Gadd45 (Smith *et al.* 1994). However, the role of this up regulation of Gadd45 in nucleotide excision repair is a matter of debate (Kazantsev and Sancar 1995). Both Gadd45 and p21 can complex with PCNA; p21 seems to prevent PCNA from conducting replication of long stretches of DNA, but not the short stretches involved in repair (Li *et al.* 1994). The cell cycle is checked and the DNA damage repaired through the coordinated activity of p53. Thus, p53 can be considered as being involved in preventing the cell from progressing into the DNA replication phase, and at the same time using this checking time for the repair of induced (endogenously or exogenously) DNA damage. The way in which wild-type p53 can carry out these functions, and why the various mutant p53s are unable to do so is elegantly demonstrated by the crystal structure of the tumor suppressor-DNA complex (Cho *et al.* 1994). The majority of recovered mutations, as might be suspected, occur in the core domain that contains the sequence-specific DNA binding activity of the p53 protein.

A role for p53 in G_2 prior to mitosis has not been established, although it has been reported that there is either no p53-induced cell cycle checkpoint in response to DNA damage in G_2, or that there can be in certain circumstances (Bunz *et al.* 1998). However, it is quite feasible that there is a p53-mediated DNA repair function that is active in all stages of the cell cycle (Donner and Preston 1996). It could be that this repair involves p53 binding at DNA strand breaks as a signal for repair enzymes, much as the stalled RNA polymerase transcription-coupled repair factor can serve in this capacity for the nucleotide excision repair system in *E. coli* (Selby and Sancar 1993) or the TFIIH transcription complex and associated proteins in eukaryotes (Sancar 1994). There also is what might be described as a “bail out” process, namely apoptosis, or so-called programmed cell death, that involves p53 protein expression. In simple terms, if a cell contains so much DNA damage that check and repair would be ineffective, the cell enters the apoptotic pathway. Cell death in this case would be preferable to high probability of mutation. It also should be noted that in lymphoma cells or activated T cells, apoptosis can be induced following genotoxic exposures of p53 -/- mice (Strasser *et al.* 1994). Clearly, the processes of cell cycle checking and/or apoptosis in response to induced DNA damage are complex and can require more than the activities of p53. However, p53's mode of action serves as an example of how cellular gene expression can influence the sensitivity to radiation-induced genetic alterations. This includes how the relative

sensitivities of different cell types within a species, or among different species, could be influenced by the specific genotype for a whole gamut of regulating genes.

E.8 Genetic susceptibility to ionizing radiations

The preceding section on ionizing radiation-induced DNA damage repair and the variation of DNA repair kinetics in the different stages of the cell cycle make it clear that mutations in the genes involved in these cellular processes can strongly influence susceptibility to radiation-induced genetic alterations.

Individuals who are homozygous recessive for the AT gene (ATM) are X-ray sensitive to cell killing and have an increased susceptibility to develop leukemias and lymphomas (IARC 2000) (see Section 6.7.3 and Appendix E.3). It is important to note that genetic predisposition to cancer and sensitivity to radiation-induced cancer has been largely restricted to discrete genetic subgroups because of the ease of their detection (Chakraborty and Sankaranarayanan 1995). Any effects of radiation exposure even in these groups is likely to be detectable only in high therapeutic dose groups (ICRP 1999). In the context of population effects, it is perhaps the more subtle, genetically controlled increase in radiation sensitivity (i.e., genetic polymorphisms, for example) that will be of greater impact (ICRP 1999). These types of subtle changes should be the subject of enhanced study.

Sanford *et al.* (1989) reported that increased sensitivity to X-ray-induced chromatid breaks and gaps in G_2 is an identifier of cancer-prone individuals. The aberration sensitivity would be a measure of DNA-repair deficiency. A particular concern is that for all the cancers, and other syndromes assessed, there was a similar enhancement in sensitivity. This is unlikely, and in this regard Scott *et al.* (1996) have been unable to repeat the Sanford *et al.* (1989) assay except for AT homo and heterozygotes. This type of assessment requires further study. It is expected that alterations in DNA repair would be involved in sensitivity to tumor formation for some tumors, and that in some cases this would involve repair deficiencies for ionizing radiation.

The subject of the impact of predisposition to radiation-induced cancer has been addressed by an ICRP Task Group (ICRP 1999). This Report shows that the impact is quite low at the population level, although it can be very significant at the individual level. The role of genetic susceptibility in cancer induction will remain a rapidly growing field as our understanding of the molecular basis of cancer continues to expand.

E.9 Appendix E References

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Appendix F: Cellular Responses To Radiation Damage And The Radiation Sensitive Disorders

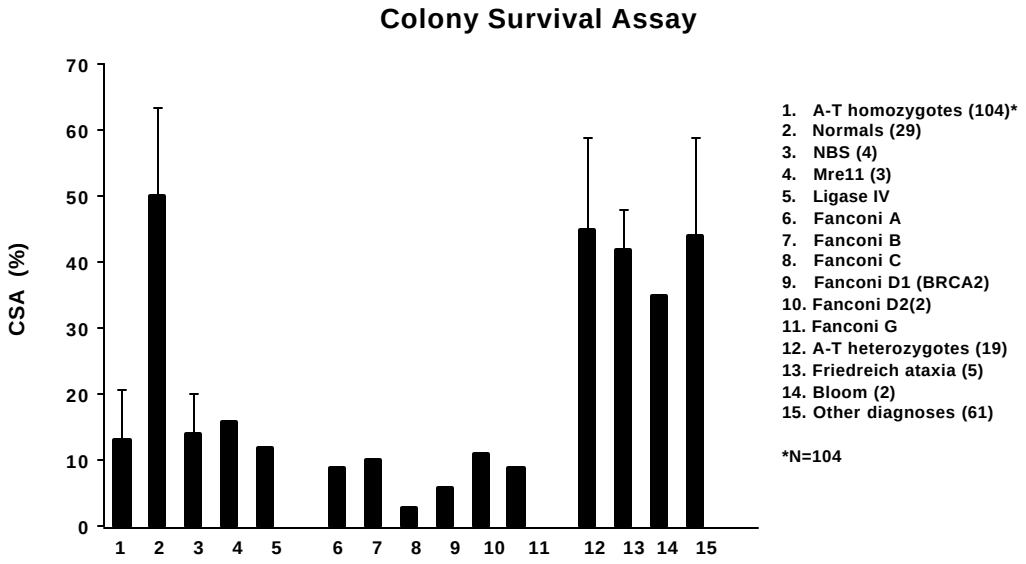
F.1 Assays for radiation sensitive disorders

Several laboratory assays have been established for characterizing cellular responses to ionizing radiation, but two have prevailed: colony survival (Taylor *et al.* 1975, Paterson *et al.* 1985) and radioresistant DNA synthesis (RDS) (Young and Painter 1989). Virtually all patients with ataxia-telangiectasia (A-T) were radiosensitive in both assays (Painter 1983).

By the early nineties, RDS testing was no longer available as an aid to diagnosis of A-T patients in the United States. This prompted development of the colony survival assay (CSA) by Huo *et al.* (1994), which measured the survival fractions of lymphoblastoid cell lines (LCLs) following exposure to 1 Gy of gamma radiation. This test is > 99% sensitive (103 of 104 bona fide A-T patients) and > 93% specific (Sun *et al.* 2002) for diagnosing A-T. When combined with immunoblotting for ATM protein, the specificity also exceeds 99% (Becker-Catania *et al.* 2000). Most other chromosomal instability syndromes also are radiosensitive by CSA, including: Nijmegen Breakage Syndrome (NBS), Mre11 deficiency (aka ATLD), ligase IV (LIG4) deficiency, Fanconi anemia, and several immunodeficiencies (Sun *et al.* 2002), but not Bloom syndrome (Figure C-1). The other form of recessive early-onset ataxia, Friedreich's ataxia, is not radiosensitive by CSA.

From 1981 to 1995, an international effort was made to identify the gene responsible for A-T (ATM) (Gatti *et al.* 1988, Lange *et al.* 1995, Savitsky *et al.* 1995) since this gene appeared to play a pivotal role in both cancer geneteics and radiation biology. The ATM protein was found to be a high molecular weight PI-3 kinase, phosphorylating serine or threonine residues in many target substrates (see details below) that are important in cell cycle control, DNA repair, and responses to oxidative stress (Jongmans and Hall 1999, Shiloh and Kastan 2001).

Today, new pathways are still being identified, based on yeast mutations of homologous and nonhomologous end joining repair (reviewed by Khanna and Jackson 2001). They provide candidate proteins for the discovery of other human RSDs. These "Experiments of Nature" are helping to unravel the cellular response to radiation damage. The cell signaling pathways of DNA repair have great potential for improving the treatment of cancer patients undergoing radiation therapy.



Source: Sun *et al*. 2002.

Figure F-1. Colony survival assay demonstrating radiosensitivity of RSDs.

Of particular interest to this report, clinical correlates of *in vitro* radiosensitivity have recently been reported for NBS and LIG4 deficiency (Riballo *et al.* 1999, Bakhshi *et al.* 2003), further extending the observations of Gotoff *et al.* (1967). Knockout mouse models for the RSD genes result mostly in embryonic lethals, i.e., the embryo does not live to full term, with the notable exceptions of the *atm* (Barlow *et al.* 1996) and *H2AX* (Celeste *et al.* 2002) knockout mouse models.

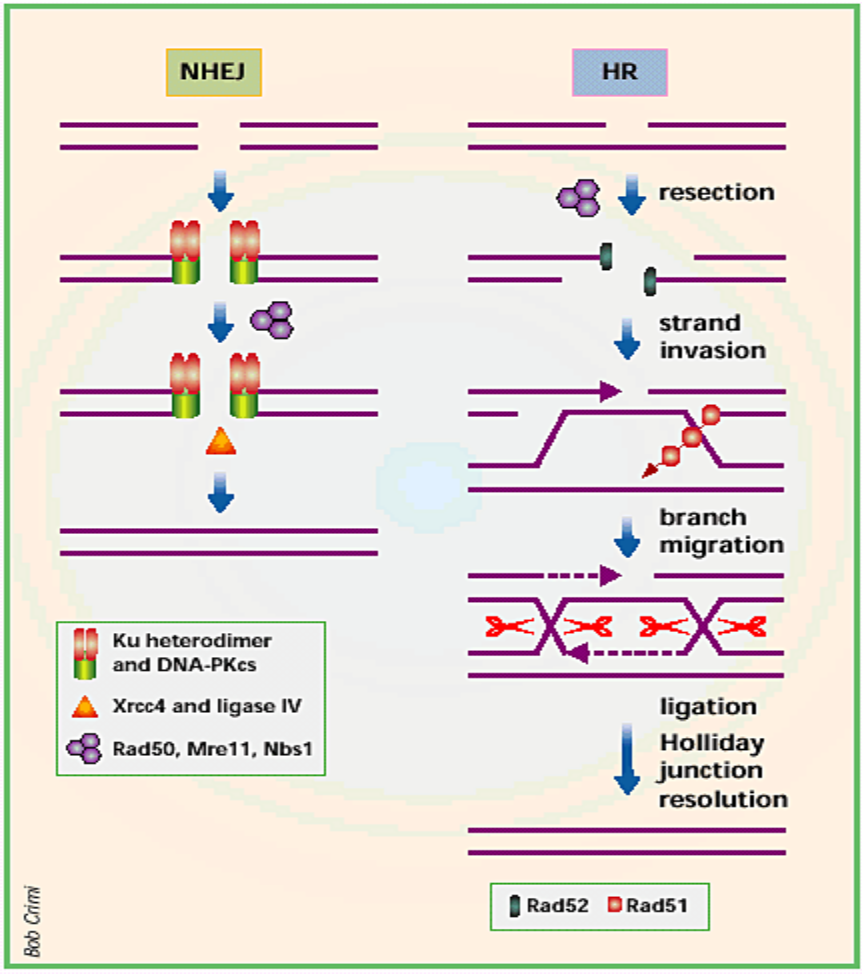
F.2 General concepts linking RS with cancer and immunodeficiency

Ionizing radiation (IR) is a common experimental tool in DNA repair research because it creates a lesion, the double strand break, that is not repaired efficiently in most chromosomal instability disorders. Bleomycin and neocarzinostatin create similar lesions by chemically cross-linking DNA and are sometimes used instead of irradiation. It also is worth noting that although DNA 'repair' implies *abnormal* damage to DNA, the breaks that occur ubiquitously in cells are seldom ‘abnormal’. Physiologically, DNA breaks occur during the process of replication, in meiosis and mitosis, when nucleotide errors must first be corrected. For example, at S phase of each cell division, whenever a replication fork meets a gap in the template DNA or single strand break, a double strand break arises. Physiological double strand breaks also are created by oxidative free radicals, which result from normal metabolic processes, such as when food is metabolized. In addition, DNA has to be broken and rejoined during gene rearrangement and class switch recombination as part of normal lymphoid development and immunoglobulin production. It is even possible that DNA repair mechanisms and the

immune system evolved in tandem, a concept that implies that species with more sophisticated immune responses would be more sensitive to ionizing radiation, for which there is some phylogenetic evidence.

The inherited radiosensitive disorders represent primarily defects of homologous repair (HR), rather than non-homologous end joining (NHEJ) repair (Figure C-2). HR is efficient and error free in its repair, but depends upon the presence of a homologous sister chromatid; it is not the predominant mechanism of double strand break repair in mammalian cells. If this template is not available, the non-homologous end joining (NHEJ) repair pathway must be used. NHEJ is the major mammalian repair mechanism and involves primarily five genes: DNA-PK, Ku70, Ku80, XRCC4, and Ligase IV (LIG4). The R/M/N complex and BRCA1 involve both HR and NHEJ pathways, and BRCA1 interacts with the Fanconi protein cascade as well. Ligase IV deficient cells are radiosensitive but do not display cell cycle checkpoint defects, in contrast to cells deficient in ATM, nibrin, Mre11, BRCA1 or FANC proteins.

Thus, there is much crosstalk (binding, and complexing) between proteins that provide DNA repair and those that control cell cycle checkpoints. The ATM protein, once activated by double strand breaks, coordinates DNA repair and cell cycle checkpoint pathways by phosphorylating serine or threonine residues in an ever-expanding list of substrates; thus, it must be considered as the hierarchical kinase coordinating both cell cycle checkpoints and DNA repair. Since p53 deficient cells do not display radiosensitivity, it would appear that sensitivity to ionizing radiation arises primarily from defects in sensing or repairing double strand breaks, and not from checkpoint defects *per se*.



Source: Khanna and Jackson 2001.

Figure F-2: NHEJ and HR Pathways of DSB repair.

NHEJ rejoins the two broken ends directly and generally leads to small DNA sequence deletions. It requires the DNA-end-binding protein Ku, which binds free DNA ends and recruits DNA-PKcs. Xrcc4 is then recruited along with DNA ligase IV. The Rad50-Mre11-Nbs1 complex, which contains helicase and exonuclease activities, also may function in NHEJ, particularly if the DNA ends require processing before ligation. HR requires Rad52, a DNA-end-binding protein, and Rad51, which forms filaments along the unwound DNA strand to facilitate strand invasion. The resected 3' end invades a homologous DNA duplex and is extended by DNA polymerase. In meiotic cells, the ends are ligated by DNA ligase I and the interwound DNA strands (Holliday junctions) are resolved resulting in either crossover or non-crossover gene conversion products. Only one of the many recombination products is shown here. This model may not apply to DSB repair in mitotic cells, as recent data indicate that mitotic recombination is not usually associated with crossing over; rather, it may be coupled intimately with replication (not shown).

F.3 Ataxia-telangiectasia, a prototype for radiosensitivity and cancer susceptibility

F.3.1 Immunodeficiency

The most consistent immune defects of A-T patients are those of IgA, IgE, IgG2, or IgG4 deficiencies (Ammann *et al.* 1969, Oxelius *et al.* 1982, Rivat-Peran *et al.* 1981, Gatti *et al.* 1982). About one-third of A-T patients do not manifest any obvious immunodeficiency, nor do they have increased infections. Antigenic challenge of patients with polyvalent pneumococcal polysaccharide vaccine reveals a poor IgG response (Sanal *et al.* 1999). A more consistent finding is the inappropriate rejoining at VDJ regions in A-T cells (Yuille *et al.* 1998).

When infections are seen, they are usually sinopulmonary and are almost always due to conventional infections, unlike that of other immunodeficiency disorders. Almost half of A-T patients die with pulmonary failure and an associated pneumonia. In this regard, ATM protein is quite prominent in the bronchial epithelial cells of normal tissue; perhaps the absence of this protein in the lungs of A-T patients plays an important role in the development of the chronic cough and poor oxygenation that precede the irreversible pulmonary failure of so many A-T patients. This late-stage syndrome is initially responsive to steroids although eventually even these become ineffective. Poor swallowing coordination and excessive drooling in some patients also can lead to frequent aspiration pneumonia.

The T cell system is abnormal, usually in subtle ways. T cell responses to viral antigens and to histocompatibility antigens are often impaired to various degrees (Yarchoan *et al.* 1985, Regueiro *et al.* 2000). T cell responses to various mitogens are often subnormal, although this is not a consistent finding and should not influence the diagnosis of new patients (Gatti *et al.* 1982). CD45-RA (naïve) memory cells are below normal levels in the peripheral blood of most A-T patients (Paganelli *et al.* 1992). Very high NK cell levels have been observed in many A-T patients, although many others seem to have normal levels (Regueiro *et al.* 2000). Patients are often anergic when skin tested. Because these findings are not common to all A-T patients, they are assumed to be secondary effects, perhaps of inappropriate cell signaling.

Only one immune parameter is consistently abnormal in A-T – the thymus is dystrophic, with poor corticomedullary differentiation, and no Hassall's corpuscles (Peterson *et al.* 1964, Amromin *et al.* 1979). This probably reflects a perturbation in the maturation of T cells, as they try to rearrange the T cell receptor (TCR) genes, a form of nonhomologous recombination. A similar situation probably arises during the differentiation of B cells. Interestingly, the most severe humoral deficiencies observed in A-T patients (IgE and IgG4 are deficient in over 80% of patients) correlate with the physical distance between the heavy chain *variable* genes in the VDJ region and those in the heavy chain *constant* regions (Gatti 1983). On the other hand, other mechanisms also might influence the immune development and function of A-T cells. For example, ATM protein appears in the nucleus of replicating cells but in the cytoplasm of differentiating cells. ATM protein plays a role in apoptosis which could be pivotal to negative selection in the thymus (and in the central nervous system). Lim *et al.* (1998) suggested that b-adaptins and movement

of vesicles may be abnormal in A-T cells and might play a role in the secretion of immunoglobulin molecules. Studies by Rivero-Carmena *et al.* (2000) suggest that membrane function of T cells from A-T patients is intact. Reguiero *et al.* (2000) exhaustively reviewed the immunological literature of A-T.

F.3.2 ATM mutations

As noted in Section 6.7.3.3, over 400 unique mutations in the ATM gene have been described in A-T patients worldwide (www.vmresearch.org/atm.htm). Many founder mutations have been identified in international studies: Costa Rica, England, Norway, Sardinia, Turkey, Poland, Brazil, Spain, the United States, and in ethnic isolates within those countries, such as Amish, Mennonite, American-Hispanic (Vorechovsky *et al.* 1996c, Concannon and Gatti 1997, Telatar *et al.* 1998a, Telatar *et al.* 1998b, Stankovic *et al.* 1998, Laake *et al.* 2000, Li and Swift 2000, Campbell *et al.* 2003, Mitsui *et al.* 2003) (Table C-1).

Various types of mutations have been described: nonsense (including premature coding terminations and frameshifts) [50%], splicing defects (including intron/exon junctions, splicing enhancer sequences, and pseudoexon formation) [30%], large intragenic deletions [2%], and missense mutations [15%]. Given the large size of the ATM gene and the low frequency of recurring mutations, mutation detection is not an efficient way to establish a clinical diagnosis at the present writing. Phenotype/genotype correlations are only recently becoming evident, so that mutations still have little clinical significance. Structure/function studies also are just getting underway, due to initial difficulties in expressing sufficient amounts of purified ATM protein. One interesting result of having defined the wide mutation spectrum in A-T patients has been that it differs substantially from that of cancer patients (discussed below). By identifying recurring haplotypes in ethnic populations, mutation detection of low-frequency mutations is becoming more efficient in that haplotyping can help to stratify mutation array testing (Campbell *et al.* 2003). One haplotype, common to the English Midlands region, has been associated with intermediate radiosensitivity (Taylor *et al.* 1994).

Table F-1. Most common ATM mutations in ethnic populations

ethnicity	mutation	frequency (%)
Costa Rica	5908C>T	56
	7449G>A(del70)	12
	4507C>T	12
	IVS63del17kb	7
Poland	IVS53-2A>C(del159)	9
	6095G>A(del89)	7
	7010delGT	5
	5932G>T(del88)	5
	5546gelT	5
Italy	7517del4	20
	3576G>A	7
	3894insT	Sardinia (>95%)
Japan	7883del5	25
	IVS33+2T>C	25
Norway	3245ATC>TGAT	55
Turkey	3576G>A	4
	5554insC	5
	1563delAG	5
	IVS21+1G>A	5
Iran	4852C>T	9
	381delA	9
	IVS21+3insT	5
	8201del11/ins6	5
Brazil	IVS28+1711del3450	24
	7913G>A	12
	3802delG	9
	8264delATAAG	9
Spain	8977C>T	18
	9010del28	15
	IVS21+1G>A	9
	8264delATAAG	5
	2413C>T	5
Hisp-Amer	103C>T (and Moroccan Jews)	6
	1348delG	6
	IVS20-579delAAGT	6
	5644C>T	5
Amish	1563delAG	>99
Utah Mormon	IVS32-12A>G	-
	8494C>T	-
	IVS62+1G>A	-
African-Amer	IVS16-10T>G	-
	2810insCTAG	-
	7327C>T	-
	7926A>C	-
Ashk-Jew	1027delGAAA	-
	3511C>T	-
	6100C>T	-
	IVS45+1G>A	-

F.3.3 Cancer risk for ATM heterozygotes

While the cancer risk for ATM homozygotes has been discussed in Section 6.7.3.4, the risk for ATM heterozygotes also has been investigated. UNSCEAR (2001) reviewed the extensive data on breast cancer risk in A-T carriers. When members of A-T families were

studied for breast cancer incidence, a 3-fold to 8-fold increase was found in almost every study (Swift *et al.* 1987, Swift *et al.* 1991, Easton *et al.* 1997, Athma *et al.* 1996, Olsen *et al.* 2001). Conversely, when large breast cancer cohorts have been screened for ATM mutations, the incidence of ATM mutations has seldom been higher than that in the control populations (Chen *et al.* 1998, Bay *et al.* 1998, Vorechovsky *et al.* 1996b, Stoppa-Lyonnet *et al.* 1998, FitzGerald *et al.* 1997, Broeks *et al.* 2000). Rare allelic variants are seen in certain populations. For example, Vorechovsky *et al.* (1997) screened 81 breast cancer patients and found 3 mutations and 5 rare variants. When FitzGerald *et al.* (1997) used the protein truncation test (PTT) to screen 401 late onset breast cancer patients and 200 controls, only 2 ATM mutations were found in each group, with no significant difference.

Since PTT does not detect point mutations, attention has focused on the relevance of rare variants and missense mutations. As noted above, only 15% of mutations in A-T patients are of the missense type, whereas >80% of ATM mutations associated with cancer have been of this type. This prompted Gatti *et al.* (1999) to propose that perhaps the phenotypes are different for nonsense and missense ATM mutations, i.e., perhaps certain missense changes have dominant negative effects that do not necessarily cause the A-T syndrome. Most enzymes have two complementary functions: they *bind* specifically, and then they *catalyze* generically. If a mutation interferes with one function but not the other, the defective molecule can actually create additional harm by keeping its natural substrates 'in complex' indefinitely. In this way, a single defective copy of a gene that normally functions in a recessive way for null alleles (those not producing any protein), would now function in a dominant way. This would further suggest that defective proteins should be immediately targeted for destruction. Table C-2 summarizes the expected phenotypes that might arise from having two types of A-T carriers in the general population -- $ATM^{nonsense}$ and $ATM^{missense}$. This concept also would necessitate a reanalysis of cancer risks based on epidemiological studies, using different frequencies for each type of heterozygote. Current evidence suggests that the frequency of $ATM^{missense}$ mutations in the general population may be as high as 5-8%, and these do not generally appear to cause A-T. Accumulating data to date suggest that missense mutations may play a more significant role in some cancers, such as breast cancer, than in others, such as childhood leukemia, in which the mutation spectrum seems to resemble that of A-T patients (Vorechovsky *et al.* 1997).

Table F-2. Possible phenotype/genotype relationships for ATM mutations

Genotype	Phenotype
$ATM^{wt/wt}$	normal
$ATM^{trunc/trunc}$	ataxia-telangiectasia/ cancer susceptibility
$ATM^{mis/mis}$	ataxia-telangiectasia / cancer susceptibility
$ATM^{mis/trunc}$	Ataxia-telangiectasia/cancer susceptibility
$ATM^{trunc/wt}$	cancer susceptibility?
$ATM^{mis/wt}$	cancer susceptibility?/neurological symptoms?

Two large ongoing NIH-funded epidemiologically studies are addressing the above issues (although both are being encumbered by ever-more-stringent Institutional Review Board restrictions). The IMECAT (International Molecular Epidemiological of A-T) study is evaluating the cancer risk of A-T relatives in six countries, encompassing 600 families; independent of cancer information gathering, the ATM mutation carriers in each family are being distinguished from non-carriers by haplotyping. The types of ATM mutations in each family also are being determined for assessing their cancer-causing ability. A second independent study, WECARE, is using denaturing High Performance Liquid Chromatography (dHPLC) to identify the frequency and types of ATM mutations in over 2500 breast cancer patients from Europe, Australia, and the U.S (Bernstein *et al.* 2003). In addition, studies are underway to test the functional status of DNA changes (rare variants) in the ATM gene so that they can be characterized as mutations or polymorphisms (e.g., 2546delSRI, S707P, P1054R, IVS10-6T>G and 7271T>G). The delSRI +/- mouse knockin is discussed above (Spring *et al.* 2002).

F.3.4 Molecular studies of ATM function

Efforts to understand A-T have turned towards unraveling the function of ATM, the protein that is absent or non-functional in all A-T patients. The complexity of the role of ATM in cells parallels the multi-faceted phenotype of the disorder. What also is becoming clear is that this otherwise basic research on a rare disorder is unraveling new therapeutic strategies for cancer patients and for individuals exposed to ionizing radiation.

ATM is involved in sensing DNA double-stranded breaks (DSB) that are caused by metabolic/cellular events. Once damage has occurred, ATM is activated and proceeds to activate numerous proteins involved in different signaling pathways participating in cell cycle checkpoints, DNA damage repair, and stress activated apoptosis (Figure C-3). ATM is involved in initiating the mechanisms necessary to maintain the cell's genomic integrity, making it a crucial component in the immediate response to potentially damaging events.

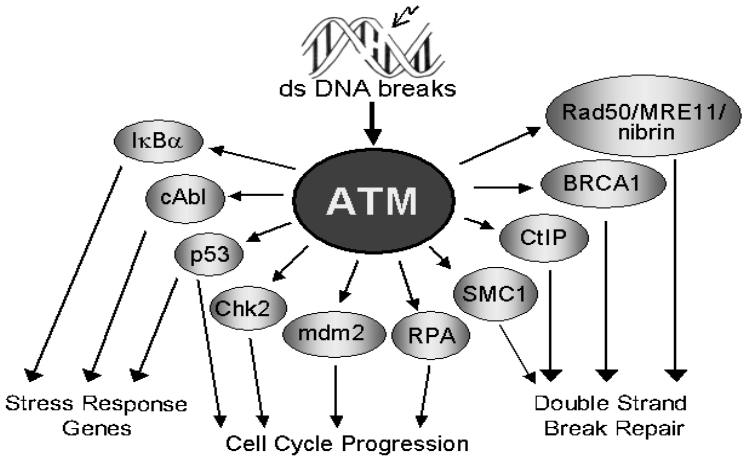


Figure F-3. Some phosphorylation targets of ATM protein and downstream effects.

The disease was linked to chromosome 11q23.1 and this eventually led to the cloning of a single ATM gene (Gatti *et al.* 1988, Lange *et al.* 1995, Savitsky *et al.* 1995). The gene spans 150 kb of genomic DNA, containing 66 exons. It produces a 13 kb major transcript and a 370 kDa protein. Recent analyses suggest that various isoforms also may be produced by alternative splicing (unpublished). Based on protein sequence homology, ATM is a member of a family of high molecular weight kinases that share the phosphoinositide-3 kinase (PI3K) domain in the C-terminal end (Figure C-3). Other proteins in this group of PI3K-like kinases (PIKK) include Mec1p (*S. cerevisiae*), Tel1p (*S. cerevisiae*), Rad3 (*S. pombe*), Tel1 (*S. pombe*), Mei-41 (*D. melanogaster*), DNA-PKcs, mTOR, ATX, and ATR. Although PIKK proteins share the PI3K domain commonly found in lipid kinases, the PIKK kinases possess only protein kinase activity. The proteins in this family control cell cycle checkpoints and DNA damage repair. ATM is primarily localized in the nucleus; however, there also are reports of cytoplasmic ATM (Brown *et al.* 1997, Watters *et al.* 1997). The binding site for p53 and other substrates has been identified at the N-terminal end. Other homologies involve a putative leucine zipper, sarc-homology region (SH3), and a Rad3-like domain (FAT).

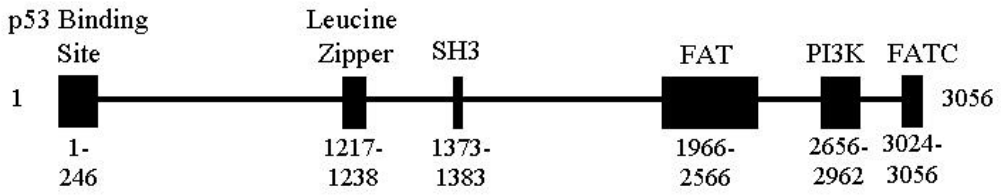


Figure F-4. Domain structure of ATM gene.

ATM, DNA-PKcs and ATR, recognize Serine/Glutamine (SQ) or Threonine/Glutamic acid (TE) target motifs for phosphorylation. This kinase activity is inhibited by wortmannin. The PIKK proteins share a low degree of functional redundancy, but for the most part, are specific in their activation signals and response to genomic damage (reviewed by Durocher and Jackson 2001). ATM-deficient cells are sensitive to ionizing radiation (IR) and radiomimetic agents and unaffected by UV exposure, although some investigators report defective UV-mediated pathways in A-T cells (Hannan *et al.* 2002). DNA-PKcs is the catalytic subunit in the DNA-PK repair enzyme. Subunits, Ku70 and Ku80, bind to DNA ends as a heterodimer and recruit DNA-PKcs to the damage site, coming together to form DNA-PK. Ku80 also stabilizes the R/M/N complex (see below) and may regulate Mre11 nuclease activity (Paull and Gellert 1999). Although the mechanisms are still unclear, DNA-PK is involved with signaling DNA damage and promoting rejoining of DNA in DSB repair. ATR is essential for cell proliferation and viability; ATR-deficient cells are embryonic lethal. In contrast to A-T, cells expressing ATR-kinase dead protein are sensitive to UV light and DNA replication inhibitors. ATR appears to play a somewhat redundant role to ATM, using most of the same downstream pathways; however, it is activated more slowly. To date, no human mutations have been reported for DNA-PK or ATR.

ATM functions in the early stages of the DNA DSB response, whether the breaks are produced through 'damage' or programmed pathways. The upstream activator of ATM remains unknown, although recent reports suggest that the R/M/N complex and Ku80 may be involved. Activated ATM initiates several signaling cascades that participate first in controlling the cell cycle, then in DNA repair, and also in apoptosis. Thus, A-T cells have both abnormal DNA repair signaling and they exhibit dysfunctional G1/S, S, and G2/M checkpoints when exposed to IR (Beamish *et al.* 1996). Both checkpoint activation and DNA repair are reviewed in the following sections.

F.3.5 ATM-dependent cell signaling in response to radiation damage

G1/S checkpoint

ATM influences p53 function and stability in at least four ways: 1) direct phosphorylation of p53 and, indirectly, through 2) MDM2, 3) CHEK2 (discussed later), and 4) 53BP1. p53, a tumor suppressor and transcription factor is responsible for

initiating cell cycle G1 arrest and upregulating p53-responsive genes, such as p21, MDM2, and GADD45 (Wu and Levine 1997). p53 function allows time for repair of DNA before damage is permanently integrated into the genome. The cell cycle is resumed once repair is completed; apoptosis is triggered if the repair seems irreversible. The IR-induced G1/S checkpoint defect in A-T cells is due to abnormal p53 stability. A-T cells required hours longer to reach maximal levels of p53, normally reached within minutes after IR damage in normal cells (Kastan *et al.* 1992, Khanna and Lavin 1993, Canman *et al.* 1994). Canman *et al.* (1998) and Banin *et al.* (1998) independently showed that ATM directly phosphorylates p53 at Serine15 and this activates p53 (Dumaz and Meek 1999, Gao *et al.* 1999). It is this particular event that occurs slowly in A-T cells. p53 Ser15 phosphorylation is virtually absent at 1 hour in A-T cells but eventually takes place due to ATR kinase activity (Canman *et al.* 1998, Tibbetts *et al.* 1999). Thus, ATM appears to be important for the immediate phosphorylation of p53 Serine15, whereas ATR maintains the activation signal, prolonging p53 function.

MDM2 is an E3 ubiquitin protein ligase that targets p53 for degradation. However, MDM2 expression is p53-activated, thereby establishing a negative feedback loop. In the nucleus, MDM2 binds to p53 and shuttles the complex out to the cytoplasm where p53 undergoes degradation (Roth *et al.* 1998, Lain *et al.* 1999). MDM2 translocates back into the nucleus, unless sequestered to the nucleolus by p19/ARF thereby blocking p53 translocation and presenting another means of stabilizing p53 levels (Tao and Levine 1999). ATM rapidly phosphorylates MDM2 at Serine395 after IR treatment (Khosravi *et al.* 1999, Maya *et al.* 2001). This interferes with MDM2 ability to export p53 to the cytoplasm and allows p53 to remain longer in the nucleus (Maya *et al.* 2001). p53 binding protein 1 (53BP1) enhances p53-dependent transcription. 53BP1 forms discrete foci after IR or UV radiation but the appearance of these foci is reduced in A-T cells (Rappold *et al.* 2001). As expected, ATM phosphorylation of 53BP1 *in vitro* and *in vivo* is inhibited by wortmannin. Recent evidence with the 'ataxia mutant mouse' (not to be confused with the *atm* mouse) links neurologic damage to the absence of Usp14, an E3 ubiquitin specific protease that is similar to MDM2 (Wilson *et al.* 2002, Ehlers 2003). Perhaps Usp14 will prove to be yet another substrate for ATM phosphorylation and provide a link between ATM-dependent cell signaling and neurological degeneration.

S phase checkpoint

In response to double strand break damage, BRCA1 undergoes phosphorylation in S phase by ATM (Cortez *et al.* 1999, Gatei *et al.* 2000). BRCA1 interacts directly with RAD51 in DNA synthesis and homologous recombination DNA repair and, in a separate pathway, with RAD50 in non-homologous end-joining DNA repair (Zhong *et al.* 1999). ATM-dependent phosphorylation is not necessary for BRCA1 recruitment to sites of broken DNA because both A-T and wildtype cells exhibit BRCA1 focus formation after IR (Cortez *et al.* 1999). Phosphorylation may regulate interactions with the other proteins in BRCA1 complex formations. Similar to p53, phosphorylated BRCA1 was observed in A-T cells 2 hours after IR exposure, again suggesting a redundancy of function by another kinase (Cortez *et al.* 1999). A large BRCA1 associated complex was identified after immunoprecipiation of BRCA1 from HeLa nuclear extracts (Wang *et al.* 2000). The megaDalton-sized structure, called BASC, is made up of multiple DNA damage recognition and repair proteins including ATM, NBS1, Mre11, Rad50, MSH6, MSH2,

MLH1, and BARD1. Oddly enough, BRCA2 and RAD51, known to complex with BRCA1, were not found in the complex. BRCA2 was recently shown to be identical to the Fanconi anemia protein, FANCD1 (Howlett *et al.* 2002)

FANCD2, a downstream member of the Fanconi anemia protein pathway, was recently identified as another ATM phosphorylation target. ATM phosphorylation of FANCD2 at Serine222 is necessary for S phase checkpoint activation (Taniguchi *et al.* 2002). Activated FANCD2 co-localizes with BRCA1 foci formation after IR, connecting the Fanconi anemia proteins to the DNA repair pathways (Garcia-Higuera *et al.* 2001).

ATM also phosphorylates CHEK2, indirectly stabilizing Cdc25a, the phosphatase responsible for Cdk2 activation and DNA synthesis initiation (Falck *et al.* 2001, Matsuoka *et al.* 1998). Inhibition of Cdk2 inhibits the S phase checkpoint and results in radioresistant DNA synthesis in A-T cells (Painter 1993). Double strand break damage in A-T cells fails to activate CHEK2, downregulating Cdc25a and inhibiting Cdk2, rendering it unable to protect the cell from undergoing premature DNA synthesis. ATM-mediated CHEK2 phosphorylation is discussed further under *G2/M checkpoint*.

G2/M checkpoint

A-T cells enter mitosis prematurely and then accumulate at the G2/M checkpoint. They subsequently undergo apoptosis, thus displaying defective signaling. The G2/M checkpoint is controlled mainly by cdc2 inhibition. In normal cells, phosphorylation of Cdc25c interferes with activation of Cdc2 and blocks entry into mitosis. Chk1 and CHEK2/Cds1 phosphorylate Cdc25c Serine216, resulting in the cytoplasmic sequestration and inactivation of Cdc25c and G2 arrest (Peng *et al.* 1997). CHEK2 is phosphorylated and activated following IR, allowing it to phosphorylate Cdc25c; this response is delayed in A-T cells (Matsuoka *et al.* 1998, Chaturvedi *et al.* 1999, Tominaga *et al.* 1999). Matsuoka *et al.* (2000) identified ATM as the kinase responsible for IR-induced phosphorylation of CHEK2-Threonine68, a modification required for CHEK2 activation. CHEK2 also phosphorylates p53 at Serine20 (Hirao *et al.* 2000). Serine20, like Serine15, is located within the MDM2 binding domain of p53; thus, phosphorylation at these sites is expected to interfere with formation of the MDM2-p53 ubiquitin degradation complex (Shieh *et al.* 1999). Thus, it is not surprising that mutations in both CHEK2 and p53 are found in Li-Fraumeni syndrome, i.e., families with multiple types of cancer, including breast cancer (Wu *et al.* 2001, Vahteristo *et al.* 2002).

Only CHEK2 and BLM (Bloom protein) are phosphorylated by ATM via threonine residues; all other target substrates use serine residues. A cdc2 independent, but ATM-dependent, pathway also occurs via the phosphorylation of Pin2/TRF1-Serine 219 (Kishi and Lu 2002) and is discussed further below. Inhibition of Pin2/TRF1 function complements (i.e., corrects) radiosensitivity. The BLM protein, a RecQ DNA helicase, probably acts as a DNA damage sensor.

hRad17, sharing homology to yeast replication factor c (RPC), is responsible for localizing the Rad9-Rad1-Hus1 (911) damage complex to sites of DNA damage. Bao *et al.* (2001) reported that IR exposure resulted in ATM-dependent phosphorylation of hRad17; however, Post *et al.* (2001) demonstrated that ATR, not ATM, is responsible for

hRad17 phosphorylation. hRad17 phosphorylation is important for G2/M checkpoint function and essential for interaction with the 911 damage complex.

F.3.6 Apoptosis

ATM induction of apoptosis is presumed to occur when DNA damage is too severe to repair. Stress-activated protein kinase (SAPK) activity is defective in A-T cells when induced by IR, whereas UV- and anisomycin-treated A-T cells exhibit a normal SAPK response (Shafman *et al.* 1995). ATM phosphorylation and activation of cAbl at Serine465 link ATM to the SAPK apoptotic pathway (Baskaran *et al.* 1997). ATM association with c-Abl is constituitive but activation of c-Abl is dependent on DNA damage (Baskaran *et al.* 1997, Shafman *et al.* 1997). In addition to apoptosis, ATM-dependent c-Abl activation has a function in G1 arrest (Shafman *et al.* 1997).

ATM is required for NF-κB activation in that A-T cells exhibit abnormal NF-κB functions in response to double strand breaks (Jung *et al.* 1995, Lee *et al.* 1998). Although the precise mechanisms for this association are unclear, it appears that I-κB kinase (IKK) function is mediated by ATM (Li *et al.* 2001). IKK is responsible for phosphorylation of I-κBα, the inhibitory protein of NF-κB. I-κBα phosphorylation results in its dissociation from NF-κB and allows for translocation of NF-κB into the nucleus; this transcriptionally upregulates apoptotic genes (Mercurio *et al.* 1997).

F.3.7 Telomere and chromosome maintenance

ATM participation in the maintenance of telomere structure occurs through its influence on telomeres rather than of telomerase activity. Telomeres are DNA repeats (TTAGGG)n placed tandomly at the ends of chromosomes to protect important DNA sequences from loss or damage produced by exonucleolytic activity, breakage of chromosome ends, or incomplete replication (reviewed by Zakian 1995, Pandita 2002). Incorrectly maintained telomeres are exposed and resemble the ends of double strand DNA breaks. A-T cells have shortened telomeres and telomere fusions, characteristic of telomere instability. Accelerated telomere shortening occurs in A-T cells (Metcalfe *et al.* 1996).

Induction of telomere fusions in the presence of a dominant-negative ATM fragment suggested that telomere fusion is a consequence of ATM malfunction. ATM associates directly to chromatin as seen in immunofluoresence studies (Gately *et al.* 1998). Pin2/TRF1, a telomerase specific binding protein that participates in regulating telomere length and maintenance, binds to telomere repeats, inhibiting telomere elongation and entrance into abortive mitosis, which would result in apoptosis. ATM phosphorylates Pin2/TRF1 at Serine219 as a DNA damage-induced response, suppressing Pin2/TRF1 activity, thereby initiating G2 arrest in a Cdc2-independent pathway (Kishi and Lu 2002). TRF2, another telomere repeat binding protein, plays a role in telomere integrity rather than telomere length. Inhibition of TRF2 binding induces chromosome end fusions and rapid ATM--p53 dependent apoptosis (Karlseder *et al.* 1999).

Proteins involved in structural maintenance of chromosomes (SMC) dimerize to form sister chromatid cohesin complexes, chromosome condensation, DNA replication and double strand break repair. SMC1, one member of this protein family, is phosphorylated

by ATM following IR treatment (Kim *et al.* 2002, Yazdi *et al.* 2002). SMC1, important for cell cycle progression, assists in linking together sister chromatids during S phase. The (7;14) translocations found in A-T lymphocytes may reflect the ATM-dependent phosphorylation of SCM1.

H2AX is a member of the histone protein family; it assembles the nucleosome core around which genomic DNA wraps for packaging into chromosomes. Within one minute following exposure to IR, H2AX is phosphorylated (i.e., gamma-H2AX), reaching maximum levels at 10 minutes (Rogakou *et al.* 1998). ATM phosphorylates H2AX at Serine139 in response to double strand breaks (Burma *et al.* 2001). The presence of gamma-H2AX is related to the decondensation of chromatin and the accessibility of DNA for repair proteins. This interaction with H2AX links ATM to chromosomal modifications, a necessary event in the initiation of the DNA repair process. Gamma-H2AX co-localizes in nuclear foci with the R/M/N complex as well as with BRCA1, 53BP1, and ATM foci (Paull *et al.* 2000, Schultz *et al.* 2000, Rappold *et al.* 2001). AID is required for R/M/N–gamma-H2AX focus formation at sites of immunoglobulin class switching (Petersen *et al.* 2001).

Class switching recombination is a DNA recombination mechanism that exchanges heavy chain constant region genes downstream of a single already-rearranged VDJ region, producing different classes of mature immunoglobulin molecules with unchanged antigen specificity. Many of the same ATM-dependent DNA damage response proteins, such as the R/M/N complex and gamma-H2AX, participate in DNA recombination during class switching, suggesting a functional role for ATM in CSR (Petersen *et al.* 2001, Pan-Hammarstrom *et al.* 2003).

One of the hallmarks of A-T chromosomes has been the translocations that typically involve the six sites where gene rearrangements occur physiologically: 14q11, the T cell receptor alpha/delta chains; 14q32, the immunoglobulin heavy chain; 7q14, the T cell gamma chain; 7q35, the T cell beta chain; 2p11, the immunoglobulin kappa light chain; and 22q11, the immunoglobulin lambda light chain. These reciprocal translocation breakpoint sites are non-random in lymphocytes, in contrast to A-T fibroblasts where translocations occur in an increased but random pattern (Kojis *et al.* 1989, Kojis *et al.* 1991). The thymic lymphomas of atm-/- mice also manifest such rearrangements (Liyanage *et al.* 2000). The ATM-dependent mechanism underlying this finding remains unclear; however, because NBS1 and Mre11 deficient cells share a similar non-random pattern of translocations, the R/M/N complex also must be involved. Whether Rad50-deficient lymphocytes would display (7;14) translocations remains unknown.

F.3.8 Other ATM-dependent phosphorylation pathways

Some A-T patients show insulin-resistant and glucose intolerant diabetes, indicating a possible ATM-related defect in insulin-signaling pathway. The relationship between insulin and ATM is not clear; however, Yang *et al.* (2000) reported ATM-dependent phosphorylation of 4E-BP1. 4E-BP1/PHAS-1 is an insulin-responsive regulator of translation. The hypo-phosphorylated form of 4E-BP1 complexes with and inhibits eIF-4F, a translation initiation factor. *In vivo*, insulin and other growth factors mediate

phosphorylation of 4E-BP1, releasing eIF-4E and allowing for initiation of translation. ATM phosphorylation of 4E-BP1 may be the first step in a series of events that result in dissociation of the inhibitory complex. Recent studies also suggest that transcription factor AP1, and the RNA surveillance protein, hUPF1/RENT1 (smg2 in C. *elegans*), are substrate targets for ATM phosphorylation (unpublished data, Gatti and coworkers).

Thus, ATM plays a complex role in many different aspects of the cellular response to radiation damage. ATM's primary focus most likely involves the repair of regularly broken DNA strands that must be rapidly rejoined. It plays a major role in the chromatin remodeling that is necessary for transcription. Judging from the severe ataxia that occurs in A-T patients, ATM also must be important in neurogenesis. In neuronal cells, in which ATM has a predominantly cytoplasmic localization, progress has been slow due to the lack of good neurodegeneration models and stem cell research restrictions (Soares *et al.* 1998, Barlow *et al.* 2000). Perhaps the role of ubiquitin in synaptic function will provide the key to unraveling how Atm protects neuronal integrity (Wilson *et al.* 2002, Ehlers 2003).

It should be stressed that one of the driving forces for private support of A-T research has been that of aiding children afflicted with A-T, an orphan disease. Despite the recent advances in understanding A-T and ATM function, an effective treatment for these children has not yet been achieved. Successful therapy for these children also is likely to help cancer patients, and the principles of radiation biology gleaned from this "Experiment of Nature" should shed valuable light on treating nuclear accidents and protecting future space travelers from DNA damage.

F.4 Nijmegen Breakage Syndrome (A-T variants 1 and 2)

NBS was first considered a variant of A-T because the patients were immunodeficient, cancer prone, t(7;14) translocations were noted during karyotype analyses, and the cells were radiosensitive (Weemaes *et al.* 1981, Jaspers *et al.* 1988, Sun *et al.* 2002). However, Sendai virus-fused fibroblasts from A-T and NBS patients corrected ('complemented') the radiosensitivity of both, suggesting that two distinct genes were involved (Jaspers *et al.* 1988). This later proved true (Stumm *et al.* 1995, Saar *et al.* 1997, Cerosaletti *et al.* 1998, Varon *et al.* 1998). Despite the above similarities, A-T and NBS are clinically distinct in that A-T does not usually include microcephaly or mental retardation, while NBS does not include ataxia nor telangiectasia. Serum AFP levels remain normal in NBS patients. Further, NBS females manifest marked ovarian failure, accompanied by lack of menarche and breast development, suggesting an important role for nibrin that does not involve ATM. The immunodeficiency of NBS patients (>100 have been analyzed to date) is often more severe than that of A-T patients (Chrzanowska *et al.* 1995). These patients also suffer more frequent sinopulmonary infections. However, the spectrum of immunodeficiences is variable, as in A-T.

Molecular studies have corroborated the close phenotypic relationships between A-T and NBS; nibrin, the protein lacking in NBS patients, is responsible for the nuclear localization of the R/M/N complex. Nibrin is phosphorylated by ATM at Serine 343 (Lim *et al.* 2000, Gatei *et al.* 2000, Wu *et al.* 2000, Zhao *et al.* 2000). Limited but distinct homology was noted between nibrin and yeast XRS2, a member of the

Mre11/Rad50/XRS2 DNA repair complex. Once inside the nucleus, the complex binds to broken ends of chromosomes and stalled replication forks, as described in detail above, and cleaves hairpin structures. Nuclear foci are formed that can be visualized by fluorescent antibodies to any of the components of this complex. Nelms *et al.* (1998) further showed that if chromosomes are damaged by soft X rays delivered through a grid with open slits, so that the damage is non-randomly distributed across a nucleus, the Rad50/MRE11/nibrin complexes only form in the regions where the chromosomes were exposed through the slits. Thus, the R/M/N complex actually migrates to these areas of DNA damage and may sense DNA damage. It may serve to activate ATM in a feedback loop although the upstream mechanisms for activating ATM still remain unclear.

As discussed further below, Mre11 also may be a phosphorylation target of ATM. Mre11 deficient cells also are radiosensitive, and progressive ataxia is seen in Mre11 deficiency. What is unclear is why NBS patients do not manifest ataxia; however, they do have mild to moderate mental impairment, immunodeficiency, and a >40% incidence of cancer, usually lymphoid. Of the solid tumors observed by the various NBS Registries, medulloblastoma appears to be the most common. One patient died from sequelae of radiation therapy for medulloblastoma, a cerebellar tumor (Bakhshi *et al.* 2003). NBS1 heterozygotes also have an increased cancer risk (Wegner *et al.* 1988, Hiel *et al.* 2000, Sperling *et al.* 2002).

Neither MRE11 nor Rad50 levels are altered in call lysates from patients with NBS. Rad50/Mre11/ Xrs2 complex yeast mutants have shortened telomeres, slow growth, and are radiosensitive (Ajimura *et al.* 1993, Petrini *et al.* 1997, Bressan *et al.* 1998). It is possible that one function of this complex is as a kinase that stabilizes telomere integrity. Tel-1 mutants show markedly decreased levels of XRS2. Tel-1, a close homolog of ATM, may open the DNA to insert telomere sequences (or telomerase) and exert its effects on telomere length via the phosphorylation of the Rad50/MRE11/XRS2 complex, perhaps by first loading the cdc13 'cap' protein (Haber 1998).

Almost all NBS patients of eastern European origin have the 657del5 mutation in the NBS1 gene and are the products of consanguinity (Varon *et al.* 1998, Resnick *et al.* 2002). Nine other mutations have been identified; all produce premature termination codons, which interfere with the translation of the protein. In all cases, however, the mutations occur between exons 6 and 10 (of 16 total), suggesting that the N-terminal FHA and BRCT domains must be preserved for viability. FHA (forkhead homology-associated) domains mediate the transmission of DNA damage signals involving protein-phosphoprotein interactions (Wu *et al.* 2000, 2001). Patients with mutations further downstream may have milder phenotypes that have not yet been recognized clinically.

Another related syndrome, Berlin Breakage Syndrome or A-T Variant 2, included anal stenosis and syndactyly (Wegner *et al.* 1988); however, these patients also have the 657del5 NBS1 mutation and, therefore, BBS is no longer thought of as a distinct syndrome or disorder, but simply as a subset or variant of NBS.

F.5 A-T_{Fresno}

This phenotype describes a small subset of patients with symptoms of both A-T and of NBS (Curry *et al.* 1989). Unlike most A-T patients, these children are microcephalic, growth retarded, and often mentally retarded. Serum alphafetoprotein level is elevated. CSA shows a radiosensitivity similar to that of classical A-T (Huo *et al.* 1994). These patients carry mutations in the ATM gene, which vary from site to site, and appear to progress clinically as typical A-T patients (Gilad *et al.* 1998, Becker-Catania *et al.* 2000). Considering that nibrin is a phosphorylation target of ATM, it is not surprising to see both A-T and NBS symptoms in these patients. It is possible that some A-T_{Fresno} patients may exist who have mutations in NBS1 rather than ATM.

F.6 MRE11 deficiency (Aka ATLD= AT-Like Disorder)

During the positional cloning of the ATM gene, a family with AT-like symptoms in two siblings did not link to chromosome 11q22.3-23.1 (Hernandez *et al.* 1993, Stewart *et al.* 1999). Subsequently, it was found that these patients had normal amounts of ATM protein but lacked the Mre11 protein of the Rad50/Mre11/nibrin complex. Progression of the neurological symptoms was somewhat slower than in A-T. Telangiectasiae were not present; the AFP remained normal.

Mre11 maps to chromosome 21.3, and plays an important role in stabilizing the R/M/N complex during non-homologous end joining of DNA. Cell lysates show decreased levels of nibrin and Rad50, as well as Mre11. In S. *cerevisiae*, Mre11 mutants are defective in double strand break repair and are radiosensitive. It has been suggested that since knockout Mre11-/- are embryonic lethals, Mre11 deficiency may only occur in patients with hypomorphic (i.e., mild) mutations, rather than null mutations. Interestingly, in a second Mre11 deficient family (consanguineous; Pakistani), the second allele could not be identified until RNA was recovered from cells grown in anisomycin, an antibiotic that blocks nonsense mediated decay and RNA surveillance (Pitts *et al.* 2001). A third family was recently reported (Pitts *et al.* 2001). Mre11 may act as the upstream sensor of DNA damage for activation of ATM.

Because the onset of ataxia in Mre11 patients occurs early in childhood, similar to that of A-T, and CSA shows a similar degree of radiosensitivity (Sun *et al.* 2002)(Table 6-1), 40 non-AT patients with a variety of AT-like symptoms were screened for ATLD. Western blots on all patients had normal amounts of Mre11, nibrin, and Rad50. When the Mre11 gene was screened for mutations by two methods, SSCP and dHPLC; no mutations were found. Thus, Mre11 deficiency comprises <1% of early onset ataxias, and not 6%, as originally predicted (Stewart *et al.* 1999).

F.7 Ligase IV deficiency

Ligase IV forms a complex with XRCC4 as the final step in the pathway of non-homologous end joining. LIG4 deficiency was first observed in a 14-year-old patient (180BR) with leukemia who dramatically over-responded to radiation therapy (Riballo *et al.* 1999). Homozygous for a missense mutation, which impaired *ligase* activity but not *expression*, this patient was quite different from four additional patients with LIG4 mutations (O'Driscoll *et al.* 2001). Although all five patients were radiosensitive, the first

patient had none of the clinical manifestations of the subsequent four, who resembled NBS or Seckel syndrome patients with prominent mid-facies, microcephaly, growth retardation, and immunodeficiency. Pancytopenia also was noted. Ligase IV expression also was decreased in three of the four patients. Thus, screening western blots for ligase IV deficiency may not be a definitive test for identifying all such patients. Ligase IV activity must be tested, or mutations must be sought in the gene. The CSA generically identifies such patients as radiosensitive (Figure C-1).

The most striking finding in LIG4 patients has been that cell cycle checkpoints are normal, suggesting that sensitivity to ionizing radiation in mammalian cells arises primarily from problems in the sensing or repair of double strand breaks and not from cell cycle checkpoint defects. The downstream, distal, position of this protein in the NHEJ pathway suggests that mutations in this gene may be more compatible with life than those of the upstream genes.

F.8 BRCA1 and BRCA2

The BRCA1 and BRCA2 genes were identified by positional cloning, tracking and analyzing genetic linkages in families with multiple affected breast cancers (Miki *et al.* 1994, Wooster *et al.* 1995). Mutations in BRCA1 also predispose to ovarian cancer. After several false starts, it was established that the BRCA1 gene plays a major role in maintaining genome stability. It functions in homologous repair of double strand breaks (Scully *et al.* 1997a, 1997b, Chen *et al.* 1999), via its interactions with Rad51 (the homolog of bacterial RecA), in non-homologous end joining (see below), and also in transcription-coupled repair (Gowen *et al.* 1998, Le Page *et al.* 2000). BRCA1 is drawn to damaged sites by the ATM-dependent phosphorylation of H2AX, which allows for remodeling of DNA prior to the repair. BRCA1 and BRCA2 are preferentially expressed during late G1-early S phase of the cell cycle and co-localize with RAD51 in the nucleus in mitotic cells. The BRCA1/Rad51 interaction is mediated by BRCA2. (BRCA2 is discussed under Fanconi anemia below.) BRCA1 associates with Rad50, of the R/M/N complex, in response to IR-induced DNA damage, and co-precipitates with many other proteins to form the megaDalton BASC complex, which also includes MSH2, MSH6, MLH1, ATM, BLM, and RFC (Wang *et al.* 2000). BRCA1 also is phosphorylated by ATM in response to double strand break damage (Cortez *et al.* 1999).

Founder mutations in BRCA1 have been identified in various ethnic groups, including Ashkenazi Jews, Canadian isolates, Iceland, and Hungary; the 185delAG mutation, found among Ashkenazi Jews, may have descended through 46 generations (Arver *et al.* 2000). Approximately 10% of familial breast cancer can now be related to mutations in BRCA1, BRCA2 and ATM, linking breast cancer to DNA repair (Venkitaraman 1999).

Mouse embryos carrying a BRCA1 null mutation are hypersensitive to gamma irradiation. BRCA1-deficient embryonic stem cells also are hypersensitive to both ionizing radiation and to hydrogen peroxide (Gowen *et al.* 1998). These cells are unable to perform transcription-coupled repair, a process in which damage is repaired more rapidly in the transcriptionally-active DNA strand. Targeted mutations in BRCA1 and BRCA2 genes result in embryonic lethals (Hakem *et al.* 1997, Ludwig *et al.* 1997). Despite much evidence implicating BRCA1/2 in radiation sensitivity, when Leong *et al.*

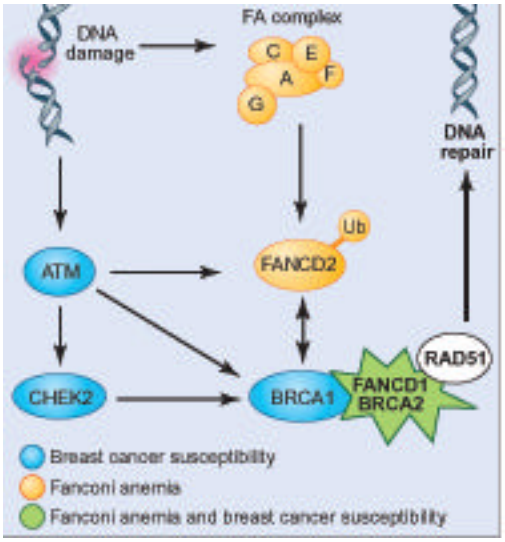
(2000) analyzed these genes in 22 cancer patients who had experienced severe normal tissue reactions after radiation therapy, no mutations were found. Perhaps future genetic studies of such patients also will screen the NBS1 and LIGIV genes.

F.9 Fanconi anemia

Fanconi anemia (FA) has typically been viewed as a childhood disorder, with bone marrow failure and cancer manifesting within the first decade of life. The cancers include acute myeloid leukemia and squamous cell carcinoma of the head and neck. These children also have growth retardation, skeletal defects, such as microcephaly and absent thumbs or radial bones, and abnormal skin pigmentation. The clinical diagnosis often requires laboratory confirmation by cellular hypersensitivity to the DNA cross-linking agents, Mitomycin C and diepoxybutane. The prognosis of adult patients with myelodyplasia also is more serious when found in association with Mitomycin C sensitivity (i.e., FA carriers).

FA is an autosomal recessive disorder that can result from mutations in one of at least eight distinct complementation groups or FANC genes, the products of which function as a complex. All but one of the FANC genes have been cloned (FANC B is the exception); however, because these genes lacked homologies with any other genes or even domains, the function of the Fanconi complex remained an enigma until 2001. Then, in a series of remarkable discoveries made by the D'Andrea lab and collaborators, FANC D2 was linked to the BRCA1 protein, and its DNA repair function, in an ATM-dependent interaction (Taniguchi *et al.* 2002, Garcia-Higuera *et al.* 2001). In the following year, the elusive FANC D1 gene was found to be identical with BRCA2 (Howlett *et al.* 2002), provoking many new insights and questions. [Insight: the finding that male breast cancer is associated with BRCA2 mutations now becomes an adult manifestation of Fanconi anemia.] Mutations in this gene account for virtually all inherited male breast cancers in Iceland. The 999del5 mutation accounts for > 75% of multiple affected breast cancer families in Iceland. [Question: Are FA heterozygotes generally at an increased risk of breast cancer, as are the parents of A-T children? Such an association was suggested by Swift and co-workers over twenty years ago (Swift *et al.* 1980).]

In response to IR-induced DNA damage, the FANC D1/BRCA2 protein associates with BRCA1 and binds to RAD51 (Figure C-5). Sun *et al.* (2002) reported that LCLs from FA patients belonging to at least six complementation groups are as sensitive to ionizing radiation as are those from patients with A-T and NBS (Figure 6-1). This is not surprising in view of earlier reports that BRCA2 -/- mouse embryos are DNA repair deficient and hypersensitive to X-ray irradation (Sharan *et al.* 1997, Xia *et al.* 2001). [Question: do FA patients manifest clinical radiosensitivity? This would have significant impact upon ablation protocols preceding bone marrow transplantion for this disorder.] In summary, the FA "experiments of nature" have brought together the pathways and functions for homologous repair of DNA, the hierarchical phosphorylation of multiple targets by ATM, hypersensitivity to ionizing radiation, and cancer susceptibility.



Source: Witt and Ashworth 2002.

Figure F-5. DNA repair proteins implicated in Fanconi anemia and breast cancer susceptibility.

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