NEUTRONS

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Sir James received the Nobel Prize in Physics in 1935,
for the discovery of the neutron.

NEUTRONS

1. Exposure Data

Exposure to neutrons can occur from the nuclear fission reactions usually associated with the production of nuclear energy, from cosmic radiation in the natural environment and from sources in which reactions in light target nuclei are used. The main exposures are related to occupation, medical irradiation and cosmic rays.

1.1 Occurrence

The occurrence and characteristics of neutrons are described in detail in the Overall introduction. Neutrons are uncharged particles that interact with the nuclei of atoms, whereas X- and γ-radiation interact primarily with orbital electrons. The spectrum of exposure to neutrons depends on their source, which is ultimately the atomic nucleus. The nuclear constituents are tightly bound, and several million electron volts are required to free a neutron from most nuclei.

Neutrons can be released in several ways, resulting in human exposure. In the interaction of high-energy cosmic radiation with the earth's atmosphere, neutrons are ejected at high energy from the nuclei of molecules in the air. In the fission or fusion of nuclei, nuclear energy is released and many neutrons are produced. Neutrons produced by fusion have more energy (~14 MeV) than those released upon nuclear fission. Fission neutrons (with energy up to several million electron volts) are themselves initiators of the fission event, but their energy must be reduced by collisions with a moderating medium (usually water or graphite) to allow a chain reaction to proceed. Neutrons in the environment of reactors therefore have very little energy. Neutrons produced by nuclear explosions and those that drive breeder reactors have more energy, but not as much as the neutrons resulting from interactions with cosmic radiation. A third way in which neutrons can be released is by collision of charged particles with a lithium or beryllium target, when part of the neutron binding energy in the nucleus of lithium or beryllium is converted into kinetic energy of 14–66 MeV. Radionuclides and ion accelerators that emit α-particles are used to initiate these reactions, and the neutrons emitted are used for radiography and radiotherapy.

The mean free path of neutrons in tissues varies with their energy from a fraction to several tens of centimeters. Since neutrons are uncharged, they do not interact directly with orbital electrons in tissues to produce the ions that initiate the chemical events

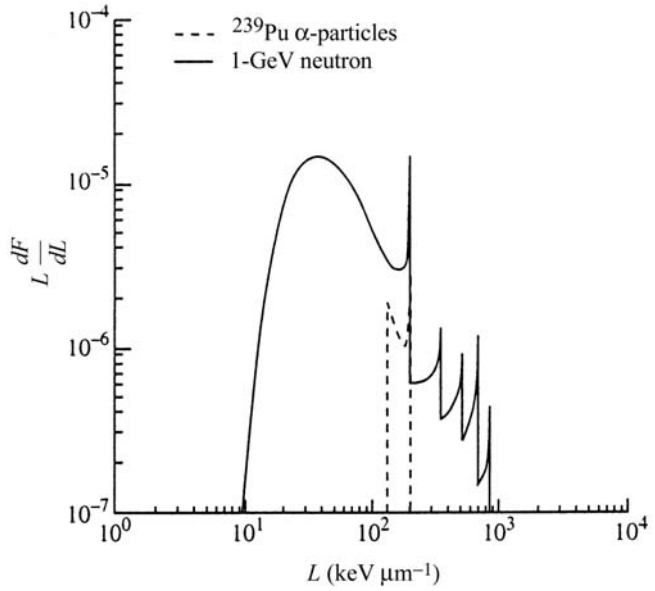
leading to cell injury. Rather, they induce ionizing events in tissues mainly by elastic collision with the hydrogen nuclei of the tissue molecules; the recoiling nucleus (charged proton) is the source of ionizing events. As about half of the neutron's energy is given to the proton on each collision, the low-energy neutrons provide an internal source of low-energy protons deep within body tissues. The low-energy protons form densely ionizing tracks (high linear energy transfer (LET)) which are efficient in producing biological injury. The ICRP (1991) therefore defined weighting factors for estimating the risks associated with exposure to neutrons which are larger than those for X- or γ-radiation. Neutrons with an energy of about 1 MeV are judged to be the most injurious (see Table 2 of the Overall introduction). After approximately 20–30 collisions with hydrogen, a 1-MeV neutron will come into equilibrium with ambient material and will continue to scatter, both losing and gaining energy in collision until nuclear absorption occurs, usually when hydrogen gives up 2.2-MeV of γ-radiation. Neutrons with > 50 MeV of energy interact mainly with large nuclei (e.g. C, N, O, Ca) in tissue in violent events, producing many low-energy charged particles with a broad distribution of LET (Figure 1; Wilson *et al*., 1995), and can produce secondaries such as α-particles, protons, deuterons and other neutrons. With increasing energy, the frequency of neutron-induced nuclear disintegration, which produces high-LET α-particles, increases. Exposure to high-energy neutrons is thus quite distinct from exposure to low-energy neutrons, in which only a single recoil proton with LET extending to 100 keV μm^{-1} is formed. The initial LET values of recoil protons are less than about 30 keV μm^{-1} and increase to about 100 keV μm^{-1} as the protons come to a stop. At 100 keV μm^{-1}, the spatial separation of the ionizing events is about 2 nm, comparable to the diameter of the DNA helix, therefore increasing the probability of double-strand breaks in DNA. All neutrons in the course of their interaction with matter generate γ-radiation.

1.2 Relative biological effectiveness

The difference in effectiveness between two radiation qualities, for example, neutrons and γ-radiation, is expressed as the relative biological effectiveness (RBE), which is defined as the ratio of the doses of the two types of radiation that are required to produce the same level of a specified effect. The ratio of the effect of neutrons per unit dose to that of reference low-LET radiation is greater than unity (ICRP, 1984). The reference radiation used has conventionally been X-radiation, but since many experimental and clinical data are derived from studies of the effects of γ-radiation, either X-radiation or γ-radiation can be used as the reference. The effects of X-radiation and γ-radiation at very low doses may, however, be significantly different. While this difference may be important in determining the RBE of stochastic events, it should not be of concern in the case of deterministic effects because of the higher doses required to induce most such effects.

A major disadvantage of RBEs is that they vary not only with radiation quality but also with dose, dose rate and dose fractionation, mainly because these factors affect the response to the reference radiation but only slightly, if at all, the response to neutrons.

Figure 1. Distribution of linear energy transfer produced by a 1-GeV neutron in tissue, and the spectrum of decay of α-particles from ^{239}Pu for comparison



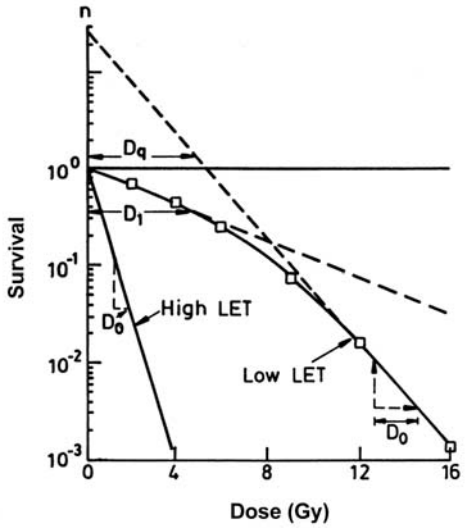
From Wilson *et al.* (1995)

The only singular RBE for any specific effect is the maximum RBE (RBE_M for stochastic effects and RBE_m for deterministic effects). In the case of stochastic effects, the RBE_M is defined as the ratio of the initial and linear slopes of the dose–response curves for the reference radiation and the radiation under study.

RBEs are based on the assumption that the effects of different types or qualities of radiation may differ quantitatively but not qualitatively. Since most deterministic effects depend on cell killing, the assumption that the nature of the induced effect is independent of radiation quality seems justified. In the case of heavy ions, the validity of this assumption has not been proven unequivocally.

The survival curves of cells exposed to neutrons *in vitro* appear to be linear on a semi-logarithmic plot, with little or no evidence of a shoulder and with a steeper curve, reflected in a lower D_0 value, than for low-LET radiations (Figure 2; see section 5.1 of the Overall introduction). The slope of the survival curve decreases and the RBE increases with decreasing neutron energy. The effectiveness of the neutrons is maximal at about 400 keV. The lack or the marked reduction of the shoulder of the survival curve reflects a greatly reduced or even completely absent ability to repair sublethal damage after exposure to neutrons (Barendsen, 1990). This lack of repair

Figure 2. Cell survival after exposure to radiation with low and high linear energy transfer (LET) as a function of dose



D_1, indicated here for low-LET radiation only, is the dose required to reduce the survival to 37%; *n* is the extrapolation number; and D_q is the 'quasi-threshold' dose, which, like *n*, is a measure of the shoulder on the low-LET survival curve. D_0 is the reciprocal of the slope of the linear portion of the curves. Note that the curve for high-LET radiation is steeper than that for low-LET radiation (D_0 is smaller) and that there is a shoulder on the low-LET curve.

results in little or no reduction in effectiveness when the neutron dose is fractionated or when the dose rate is reduced.

The dose–effect relationship of early-responding tissues can be predicted from the responses of the relevant clonogenic cells. There is no apparent difference in the ability of tissues to repopulate after exposure to neutrons, apart from a greater reduction in the number of proliferative cells per unit dose of neutrons than with low-LET radiation. The RBE increases with increasing LET and reaches a maximum, in the case of cell killing and mutagenesis, at LET values of about 100–200 keV μm^{-1}. At higher LET values, the effectiveness decreases. In 1990, a revision of the relationship between the radiation quality factor, which is based on RBEs, and the LET for stochastic effects was introduced which took into account the decrease in effectiveness of radiations with a very high LET. The relationship between RBE and LET for deterministic effects has not been codified explicitly, and the use of quality factors is restricted to stochastic effects. For deterministic effects, the influence of radiation quality is taken into account by using RBEs to adjust the absorbed doses (ICRP, 1990).

RBEs for deterministic effects are derived from the ratios of the threshold doses for neutron and reference radiation or of the doses required to induce a selected level of effect. Since deterministic effects have thresholds by definition, use of the ratio of the threshold doses seems a reasonable approach for determining RBEs. In 1990, however, an ICRP task group introduced the concept of RBE_m, which is comparable to the RBE_M for stochastic effects. The group suggested that singular RBE values for neutrons and other high-LET radiations could be obtained from the linear–quadratic model used to describe the survival curves of the cells responsible for the maintenance of tissues. In the case of deterministic effects, the threshold dose lies on the curved portion of the dose–response curve. Since, in general, deterministic effects result from the killing of a critical number of cells and assuming that the dose–response curve for cell killing can be described by a linear–quadratic model, it is theoretically possible to derive the initial slope of the response. Thus, a RBE_m can be obtained for specific endpoints in specific tissues for which there are adequate data on dose–response relationships for different α:β ratios (see ICRP, 1990, for the method of deriving RBE_m). This approach is, of course, totally dependent on the validity of the linear–quadratic model at low doses at which effects cannot be measured.

The clinical importance of the difference between the effects of neutrons and low-LET radiations on normal tissues was revealed by the high incidence of tissue damage during the early use of neutrons to treat cancer. The effectiveness of fractionated neutrons is underestimated if it is based on the effects of single doses and if the difference in the repair of slowly dividing tissues is not taken into account.

1.3 Exposure

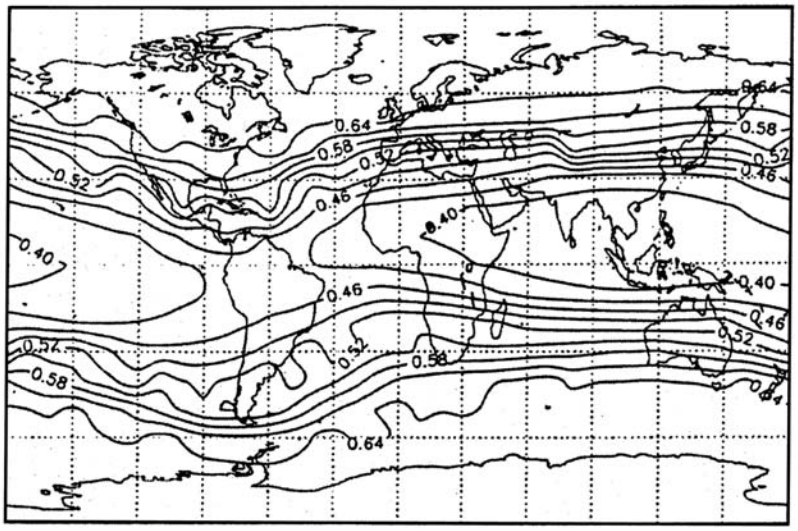
1.3.1 *Natural sources*

The effective dose equivalent rates of cosmic rays are discussed in the Overall introduction (section 4.4.1), in which the rates were evaluated on the basis of measurements with neutron spectrometers, tissue equivalent ion chambers and nuclear emulsion detectors augmented by Monte Carlo calculations. Dose equivalence is derived by summing dose contributions and weighting by LET-dependent quality factors. The ratio of the estimated neutron dose equivalent rate to the total dose equivalent rate according to the parametric atmospheric radiation model is shown for various altitudes in Figure 3. It can be seen that 40–65% of the dose equivalent at ordinary aircraft altitudes is due to neutrons, depending on the latitude and longitude of the flight trajectory. The fraction of neutrons depends on altitude, being nearly negligible at sea level and contributing over half of the exposure at aircraft flight altitudes. The fraction varies little over most of the altitudes at which aircraft operate. Since most commercial flights are at relatively high latitudes, approximately 60% of the dose equivalent is due to neutrons.

Although consistent measurements were made over most geomagnetic latitudes and altitudes during solar cycle 20 which started in October 1964, many of the individual

Figure 3. Fraction of dose equivalent due to neutrons at various altitudes, at minimum solar energy (1965)

$H_{neutron}/H$ at 40 000 ft [~12 000 m]



$H_{neutron}/H$ at 50 000 ft [~15 000 m]

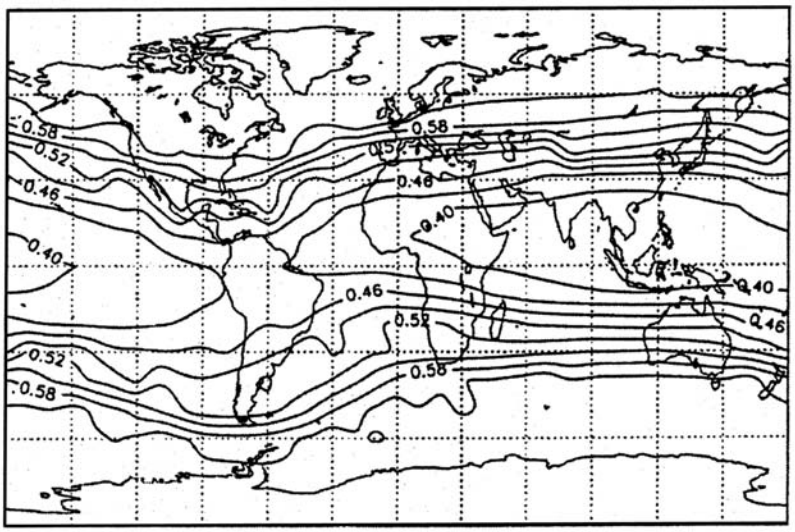
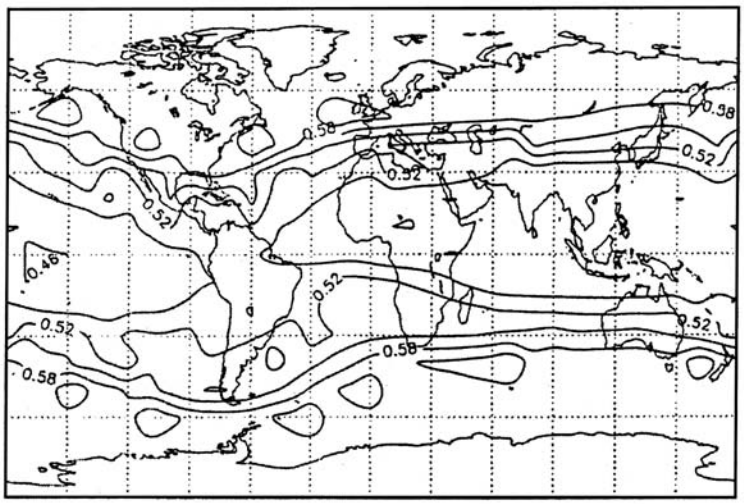
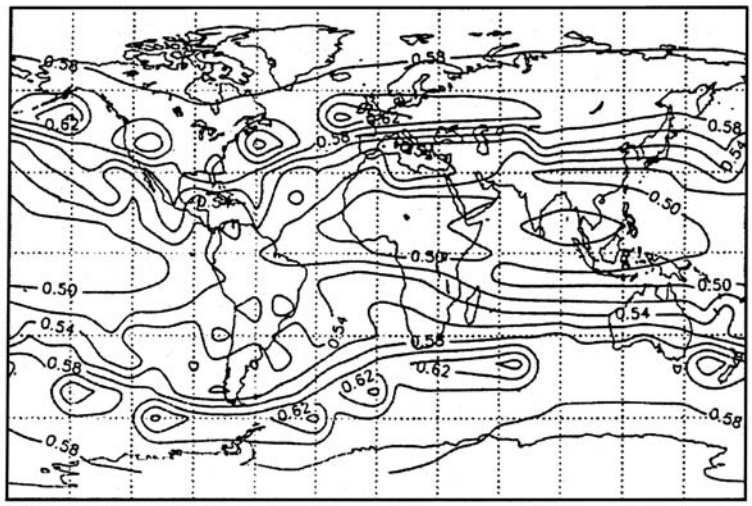


Figure 3 (contd)

$H_{neutron}/H$ at 65 000 ft [~20 000 m]



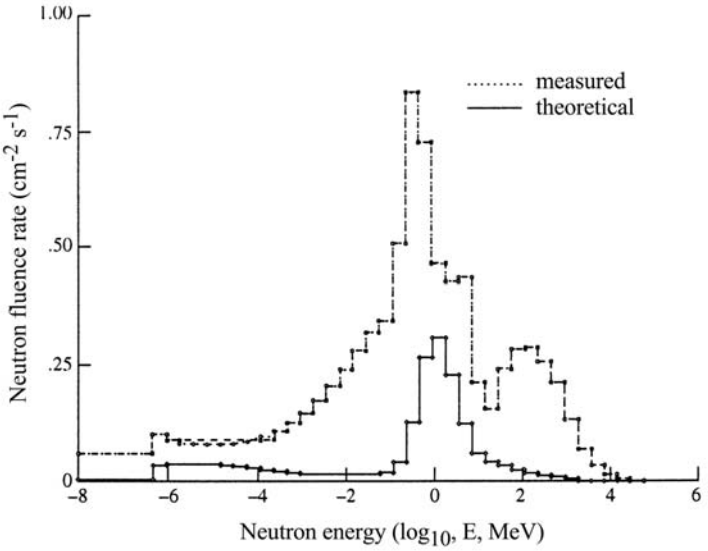
$H_{neutron}/H$ at 73 000 ft [~22 000 m]



From Wilson *et al.* (1995); H, equivalent dose

components were not resolved because of instrumental limitations at that time. Most of the neutron spectrum therefore depends on theoretical calculations of proton interactions with the atmosphere (Hajnal & Wilson, 1992; National Council on Radiation Protection and Measurements, 1995). Early measurements of the atmospheric neutron spectrum are shown in Figure 4. Hess *et al*. (1961) measured the neutron spectrum in a bismuth fission chamber with a boron fluoride counter, supplemented by a model spectrum. Korff *et al*. (1979) used a liquid scintillator spectrometer (see section 2.1.1 in Overall introduction) sensitive mainly to 1–10-MeV neutrons with analysis assuming a simple power law spectrum. [The Working Group noted that the data of Korff *et al*. (1979) are for a higher altitude than those of Hess *et al.* (1961).] Hewitt *et al*. (1980) used a Bonner sphere set-up (see section 2.1.1 in Overall introduction) at subsonic flight altitudes and analysed the data after assuming a simplified spectral analysis. Their results confirm the importance of high-energy neutrons, although the exact nature of the spectrum remains uncertain owing to limitations of the analytical methods. Nakamura *et al*. (1987) used a Bonner sphere set-up at much lower latitudes and multiplied their results by three for a comparison of spectral shape. Incomplete knowledge of the neutron spectrum thus makes the present estimates uncertain (National Council on Radiation Protection and Measurements, 1995).

Figure 4. Neutron spectra measured at 17.46° N at 23.5 km by Hajnal and Wilson (1992) and that derived theoretically by Hess *et al.* (1961)



Estimates of dose equivalent rates for exposure to radiation from natural sources are available in a number of publications, but only a few give separate values for the contributions of neutrons. Bagshaw *et al*. (1996) reported that the average rate on long-haul flights from London to Tokyo was 3 μSv h^{-1} for neutrons; an additional 3 μSv h^{-1} for other components gave a total of 6 μSv h^{-1}. Table 1 shows the dose equivalent rates derived with a high-pressure ion chamber and a simplified form of a Bonner sphere, in relation to altitude and latitude (Akatov, 1993). Although the quality of the ionizing dose is not given, it can be seen that the neutron dose equivalent rate represents half or more of the exposure.

Table 1. Atmospheric dose equivalent rates measured on board a Tupolev-144 aeroplane during March–June 1977 (near solar minimum)

Altitude (km)	Latitudes (° N)					
	40–45		46–58		65–72	
	Ionizing (μGy h^{-1})	Neutrons (μSv h^{-1})	Ionizing (μGy h^{-1})	Neutrons (μSv h^{-1})	Ionizing (μGy h^{-1})	Neutrons (μSv h^{-1})
13	2.3	2.6	2.9	4.2	3.5	5.0
14	2.6	3.0	3.2	5.0	4.1	5.9
15	2.8	3.0	3.4	5.4	4.7	6.7
16	2.9	3.2	3.5	5.8	5.2	7.6
17	3.0	3.5	3.7	6.1	–	–
18	3.1	3.4	3.8	5.5	–	–

From Akatov (1993)

In estimating the collective dose equivalent, UNSCEAR (1993) assumed 3×10^9 passenger hours in flight during 1985 and an annual average rate of 2.8 μSv h^{-1} (~1.6 μSv h^{-1} of neutrons) resulting in a collective dose equivalent of 8400 person–Sv (5040 person–Sv of neutrons). By 1997, air travel had grown to 4.3×10^9 passenger hours in flight (ICAO, 1999) leading to a collective dose equivalent of 12 000 person–Sv (7200 person–Sv of neutrons).

1.3.2 *Medical uses*

The medical use of neutrons is limited, as no therapeutic benefit has been noted when compared with conventional radiotherapy; however, neutrons are used to a limited extent in external beam therapy and boron neutron capture therapy.

1.3.3 *Nuclear explosions*

In the reassessment of the radiation dosimetry associated with the atomic bombings of Hiroshima and Nagasaki, Japan (see Overall introduction, section 4.1.1; Fry & Sinclair, 1987), the estimated dose of neutrons was reduced in both cities, particularly in Hiroshima, where the new value was only 10% of the previously estimated level. The neutron doses were now so small (only 1–2% of the total dose in Hiroshima and less in Nagasaki) that direct estimates of the risk for cancer associated with exposure to neutrons were no longer reliable. The neutron dosimetry is once again under review and may be revised (National Council on Radiation Protection and Measurements, 1997; Rühm *et al*., 1998).

1.3.4 *Occupational exposure*

Occupational exposure to neutrons occurs mainly in the nuclear industry. Compilations have been made of the exposure of nuclear workers in the United Kingdom for the years 1946–88 (Carpenter *et al.*, 1994) and of those in the USA for the years 1970–80 (Environmental Protection Agency, 1984). In the United Kingdom compilation, the upper limit of the neutron component was estimated to be 3% of the total exposure (Table 2). The estimates are uncertain because neutron dosimetry was implemented in fuel processing plants only in 1960, a few workers worked at reactors where there was a significant energetic neutron component for which the dosimetry is inadequate, and there were systematic under- and over-recordings when the dosimetry read-outs were below threshold of detection or the dosimeter was in some way inoperative. The average annual dose equivalent for all workers in the United Kingdom was reduced from 12.5 mSv year^{-1} (neutrons, < 0.4 mSv year^{-1}) in the early 1950s to < 2.5 mSv year^{-1} (neutrons, < 0.1 mSv year^{-1}) in 1985. The average cumulative doses

Table 2. Upper limits of estimated cumulative exposure to neutrons of radiation workers, by last site of employment, United Kingdom, 1946–88

Employer	No. of exposed individuals	Cumulative whole-body dose equivalent (mSv)	Collective dose equivalent (person–Sv)
Atomic Energy Authority	21 344	1.2	26
Atomic Weapons Establishment	9 389	0.3	3.1
British Nuclear Fuels, Sellafield	10 028	4.0	40
Total	40 761	1.7	69.1

From Carpenter *et al*. (1994)

were highest at the Sellafield nuclear fuel processing plant, where 22 workers had single annual doses > 250 mSv (neutrons, > 7.5 mSv year^{-1}), seven of whom had doses > 500 mSv year^{-1} (neutrons, > 15 mSv year^{-1}).

Occupational exposure to neutrons in the USA in 1980 based on data for 1977–84 are shown in Table 3. It was estimated that such exposure had decreased by a factor of two between 1970 and 1980 due to improved protection (Klement *et al.*, 1972; Environmental Protection Agency, 1984).

Table 3. Estimated exposure to neutrons of radiation workers in the United States, 1980

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
US Navy	12 000	0.24	2.9
Total	38 100	1.8	67.5

From National Council for Radiation Protection and Measurements (1987)
[a] Total number of workers

Staff involved in radiotherapy with neutrons are exposed mainly to γ- and β-rays due to activation of the room and equipment. The dose rates are well below 1 μGy h^{-1} and are not detectable by personal dosimetry (Smathers *et al.*, 1978; Finch & Bonnett, 1992; Howard & Yanch, 1995).

Neutron sources are used to chart progress in the search for gas and oil resources. The exposure of oil-well loggers has been monitored with film (Fujimoto *et al.*, 1985) and nuclear track detectors (Inskip *et al.*, 1991). Canadian workers were exposed to 1–2 mSv year^{-1} (Fujimoto *et al.*, 1985), whereas Chinese workers monitored for three months had very low doses of neutrons, only seven of the 1344 workers having doses above the threshold of detection (0.02 mGy) (Inskip *et al.*, 1991).

The exposure of commercial aircraft crews to neutrons depends not only on the flight route (see section 1.3.1) but also on the number of flight hours, which may be as many as 1000 per year. Hughes and O'Riordan (1993) estimated that long-haul crews are airborne for 600 h year^{-1}, while short-haul crews log only 400 h year^{-1}; they therefore used an average value of 500 h year^{-1}. Bagshaw *et al.* (1996) estimated that crews who fly both ultra-long-haul and long-haul flights fly for 600 h year^{-1}, while those who fly only ultra-long-haul flights fly for up to 900 h year^{-1}. Oksanen (1998) found that the annual average number of flight hours of cabin crews was 673 h, while that of the technical crew was 578 h, with a range of 293–906 h year^{-1}. Air crews have

additional exposure during off-duty flights in returning to a home base, which are estimated to account for 20% of the actual flight hours logged.

Hughes and O'Riordan (1993) estimated an average dose equivalent of 3 mSv year^{-1} (neutrons, ~1.8 mSv year^{-1}) for crews on United Kingdom airlines and 6 mSv year^{-1} (neutrons, ~3.6 mSv year^{-1}) for near-polar flights. Montagne *et al*. (1993) estimated that the average exposure of Air France long-haul pilots was 2–3 mSv year^{-1} (neutrons, ~1.2–1.8 mSv year^{-1}). Wilson *et al*. (1994) estimated that the exposure of domestic crews in Australia in 1982–83 was 1–1.8 mSv year^{-1} (neutrons, ~0.6–1.1 mSv year^{-1}), while crews of international flights received 3.8 mSv year^{-1} (neutrons, ~2.3 mSv year^{-1}). Preston (1985) proposed an average dose equivalent of 9.2 μSv h^{-1} (neutrons, ~5.5 μSv h^{-1}) in British Airways operation of the Concorde in 1979, with a maximum observed rate of 38.1 μSv h^{-1} (neutrons, ~23 μSv h^{-1}). The average exposure of the technical crew was 2.8 mSv year^{-1} (neutrons, ~1.7 mSv year^{-1}) and that of the cabin crew was 2.2 mSv year^{-1} (neutrons, ~1.3 mSv year^{-1}). Similar differences (20–30%) between the exposures of personnel on the flight deck and in the cabin were observed by Wilson *et al*. (1994). Differences of up to 20% between aircraft type were also observed.

1.4 Summary

The average effective dose of neutrons received by the world population per year was estimated to be 80 μSv by UNSCEAR (1993). Assuming a 75-year life span, the average lifetime dose would be 6.0 mSv. The highest average lifetime effective dose of neutrons (67.5 mSv) is found in the high-altitude city (3900 m) of La Paz, Bolivia. Table 4 gives the individual and collective lifetime doses for a number of populations. The atomic bombings of Hiroshima and Nagasaki are estimated to have contributed not more than 2% of the total exposure of the survivors, as estimated from the total exposure of 24 000 person–Sv of 86 752 persons and the total exposure of 4 Sv of the 'worst-case' survivors. Insufficient information was available to estimate the individual average exposure of nuclear workers over a working lifetime. The maximal known lifetime exposure of contractors of the Department of Energy in the USA was estimated on the basis of a 50-year career. The collective dose of the world's nuclear workers is based on the assumption that workers in the United Kingdom and the USA represent 20% of such workers. UNSCEAR (1993) estimated that the average total exposure of the world population from air travel was 2 μSv year^{-1}, of which 60% is to neutrons, although the maximal individual exposure due to air travel depends mainly on flight duration. The collective dose for crew members is based on the assumption that there are five crew members for every 100 passengers.

Table 4. Exposure to neutrons of major exposed human populations

Population	Exposure path	Individual lifetime[a] dose (mSv)		Collective dose (person–Sv per year)	Variation
		Average	Maximum		
World (5800 million)	Natural sources (cosmic radiation)	6.0	67.5	4.64×10^5	Large
Tumour therapy	Collateral irradiation of healthy tissue				Highly skewed distribution
Survivors of atomic bombs	Fission neutrons	< 5.5	< 80.0	< 480	Relatively more important at lower exposures (?)
Nuclear workers[b]	Civilian and military nuclear fuel cycle	44.4	130[c]	350[d]	
Aircrews, courriers[e]	Flying at high altitude, cosmic secondary neutrons	30	46	320	Higher on flights over earth poles
Airline passengers	Flying at high altitude, cosmic fusion neutrons	0.09	–	7200	Higher on flights over earth poles

From UNSCEAR (1993)
[a] 75 years
[b] 50-year career
[c] Department of Energy contractors in the USA
[d] Workers in the United Kingdom and the USA assumed to represent 20% of all nuclear workers
[e] 30 years

2. Studies of Cancer in Humans

Until the system for estimating the doses received by the survivors of the atomic bombings in Japan was revised in 1986 (DS86), it was reported consistently that the incidence of cancers after exposure to similar doses was higher among the survivors in Hiroshima than among those in Nagasaki (Kato & Schull, 1982). The bomb dropped on Hiroshima was composed of uranium and that dropped on Nagasaki of plutonium, but it was believed that the design of the two weapons had resulted in greater exposure to neutrons in Hiroshima. For many years, differences in cancer rates and in the frequency of chromosomal aberrations in circulating lymphocytes were attributed to differences in the quality of radiation, and attempts to separate the effects of neutrons and γ-rays were made by comparing the rates in Hiroshima with those in Nagasaki (Committee on the Biological Effects of Ionizing Radiations (BEIR I), 1972). On the basis of these calculations, neutrons were estimated to be about 20 times more carcinogenic than γ-radiation, although it was recognized that a wide range of values was possible.

During the early 1980s, the dosimetry of the radiation from the atomic bombs was reassessed (Fry & Sinclair, 1987; Roesch, 1987a,b). The estimated neutron doses delivered to both cities were now considered to be so small (only 1–2% of the total dose in Hiroshima and less in Nagasaki) that estimates of the risks for cancer associated with exposure to neutrons were not reliable (Jablon, 1993; Little, 1997). The change in the estimates of doses to the Japanese atomic bomb survivors thus meant that there was no longer a useful database of human exposures for estimating the carcinogenic risks of exposure to neutrons.

Some workers in the nuclear industry are occasionally exposed to neutrons, but the number of such workers is too small and the doses are generally too low for any meaningful estimate of risk. In addition, these workers were also exposed to higher doses of γ-radiation. In studies of patients treated with neutrons (Catterall *et al.*, 1975, 1977; Hübener *et al.*, 1989; Richard *et al.*, 1989; Kolker *et al.*, 1990; MacDougall *et al.*, 1990; Silbergeld *et al.*, 1991; Stelzer *et al.*, 1991; Laramore *et al.*, 1993; Russell *et al.*, 1993), the numbers of survivors and those developing second cancers are small, and the dosimetry is very complex (Geraci *et al.*, 1982). Other complicating factors include the killing of cells at the high doses used, scattering of low doses and contaminating exposures to γ-rays. High-energy linear accelerators for medical use produce low levels of neutrons through the photonuclear effect, and a dose of the order of 1 cGy is possible (Hall *et al.*, 1995); however, the dose is again too low—in contrast to the dose used for tumour treatment, of the order of 6000 cGy—to allow quantification of the risk for second cancers attributable to neutrons. Epidemiological studies of air crew, pilots and flight attendants have been initiated because of their exposure to neutrons from cosmic rays during frequent high-altitude flights (Blettner *et al.*, 1998; Boice *et al.*, 1999). The annual exposure of air crew is about 1–2 mSv, which, even after a career of 30 years, is still too low a dose to allow detection, much

less quantification, of a cancer excess by epidemiological means. In addition, the dosimetry is complex and this population is also exposed directly to ionizing radiation, making it difficult to evaluate the effects of neutrons.

3. Studies of Cancer in Experimental Animals

Neutrons have been studied in order to compare their carcinogenicity with that of low-LET radiations such as X-radiation and γ-radiation, not only to improve understanding of the risks of exposure to neutrons but also to test biophysical models and their applicability to radiation-induced cancer. This section does not give a comprehensive presentation of all studies in animals. The studies in mice summarized below are those which have provided data on dose–response relationships and on the effects of fractionation and dose rate at low doses of neutrons. The results of experiments in other species provide evidence that the results in mice are not unique.

3.1 Adult animals

3.1.1 *Mouse*

Groups of 21–114 male and 31–197 female non-inbred RF/Un mice, 10 weeks of age, were exposed to 0–9.3 Gy of whole-body irradiation with 1-MeV or 5-MeV neutrons [source and γ-radiation component not specified] at dose rates of 0.04–114 mGy day^{-1} and 0.00003–850 mGy min^{-1}. The animals were allowed to die naturally or were killed when moribund, at which time all animals were necropsied. Only selected lesions were examined histopathologically, as needed, to confirm diagnosis. In the control group of 301 unirradiated females and 115 unirradiated males, neoplasms occurred in about 64% of females and 47% of males. The incidence of myeloid leukaemia was markedly increased by acute exposure, passing through a maximum at 2 Gy and declining at higher doses (Table 5). Chronic irradiation at up to 5.7 Gy also enhanced the incidence, but this declined after exposure to 9.3 Gy. The incidence of reticulum-cell neoplasms, in contrast to those of myeloid leukaemia and thymic lymphoma, decreased with the increased doses delivered at a high rate. Of the unirradiated control mice, 11–14% had pulmonary tumours (adenomas); in treated mice, however, the incidences decreased with increasing dose. The incidence of ovarian tumours (granulosa-cell tumours, luteomas, tubular adenomas and haemangiomas) was statistically significantly increased ($p < 0.05$) only at the lowest dose of 16 mGy at a rate of 0.00003 mGy min^{-1} [statistical method not specified]. The incidences of solid tumours other than of the lung and ovary were increased in the irradiated animals, but the numbers were reported to be insufficient to establish a quantitative dose–effect relationship. The relative biological effectiveness (RBE; see section 1.2) for the induction of myeloid leukaemia was 16 with daily and chronic exposure as

Table 5. Time to death and incidences of tumours in various organs of RF/Un mice exposed to fast neutrons

Mean accumulated dose (Gy)	Average dose rate (mGy min^{-1})	No. of mice	Mean age at death (days)	Myeloid leukaemia (%)	Thymic lymphoma (%)	Ovarian tumours (%)	Pulmonary tumours (%)	Other solid tumours (%)
Females								
0	–	301	582	3	12	2	11	3
0.016	0.00003	111	584	5	11	19	15	4
0.12	0.00021	97	549	8	11	2	10	1
0.15	0.0015	79	558	6	9	4	13	2
0.16	0.00022	99	566	7	7	4	11	3
0.16	0.0007	117	558	3	8	5	14	4
0.27	0.0004	129	549	4	12	1	12	2
0.28	0.033	50	533	4	8	0	12	2
0.30	0.0062	100	544	8	11	4	15	4
0.31	0.0012	148	578	4	11	2	17	4
0.33	0.0043	90	522	8	19	4	4	3
0.68	0.0037	60	523	7	10	9	17	0
0.75	0.0034	120	471	9	21	2	12	7
0.94	0.0062	197	464	12	19	3	14	4
0.96	0.0099	49	509	5	27	14	5	5
0.98	0.033	85	464	15	20	5	7	4
1.69	0.0033	123	489	14	12	6	17	4
2.10	0.0185	50	451	15	41	4	7	2
2.11	0.0099	49	370	8	39	2	8	0
2.39	0.0275	58	324	20	25	2	4	0
2.91	0.0171	49	431	9	40	9	13	4
3.90	0.0098	120	398	17	35	1	10	3
4.61	0.0207	186	301	12	45	2	6	2
5.70	0.0185	50	363	23	25	0	16	0
9.30	0.083	50	189	2	20	0	2	0

Table 5 (contd)

Mean accumulated dose (Gy)	Average dose rate (mGy min^{-1})	No. of mice	Mean age at death (days)	Myeloid leukaemia (%)	Thymic lymphoma (%)	Ovarian tumours (%)	Pulmonary tumours (%)	Other solid tumours (%)
Females (contd)								
2.03	850	31	382	20	23	10	0	0
2.60	850	60	304	16	10	7	4	0
3.60	850	98	360	10	23	8	4	2
4.43	850	82	342	9	16	7	2	1
Males								
0	–	115	548	3	1		14	2
0.17	0.0012	77	561	8	1		17	1
0.29	0.0029	69	482	17	1		17	1
1.20	0.0243	21	502	29	5		19	5
1.30	850	27	460	33	7		11	0
1.72	850	48	436	34	11		11	2
2.22	850	114	428	38	4		7	1
2.70	850	103	413	30	5		8	3
3.32	850	79	408	23	9		13	4

From Upton *et al*. (1970)

compared with acute exposure (Upton *et al.*, 1970). [The Working Group noted that the tumour incidences were not analysed for competing causes of death. Since a large fraction of the irradiated mice died early from myeloid leukaemia, such an analysis for solid tumours is essential.]

A total of 3265 female RFM/Un mice, 12 weeks of age, received whole-body irradiation with neutrons at doses of 0.048, 0.096, 0.192, 0.24, 0.47, 0.94 or 1.88 Gy at rates of 50 or 250 mGy min^{-1} or 10 mGy day^{-1}. A reactor was used to deliver the high dose rate, and the low dose rate was produced from a 1.1-mg ^{252}Cf source surrounded by a depleted ^{238}U sphere (Storer *et al.*, 1979). The ratios of neutrons:γ-rays were 7:1 for the reactor and 3:1 for the ^{252}Cf source. A control group of 648 mice was available. The animals were followed for life, and tumours were diagnosed histologically. A positive dose–response relationship for thymic lymphoma was observed at all doses up to 1.0 Gy at both dose rates; at the highest dose, the low dose rate was more effective (Table 6). At low doses, a weak dependence on rate was observed. Increased incidences of thymic lymphoma, lung adenoma and endocrine tumours were seen at doses as low as 0.24 Gy. The highest dose of radiation at the low rate (10 mGy day^{-1}) appeared to induce thymic lymphomas more efficiently than irradiation at the high dose rate (250 mGy min^{-1}). The incidence of ovarian tumours was lower at all doses given at the low rate than at the high rate. After exposure to doses of 0.24–0.47 Gy, the RBE for thymic lymphoma was 3–4 in relation to acute exposure to ^{137}Cs γ-rays, and the induction of mammary tumours also appeared to be more sensitive to neutrons; however, no apparent effect of dose or dose rate was reported over the dose range used. Because of the relatively large carcinogenic effect, the authors concluded that the γ-radiation component had little or no effect on the dose–response relationship observed (Ullrich *et al.*, 1976).

The dose–response relationships for the induction of lung tumours were studied in 592 female RFM/Un mice, 10–12 weeks of age, given thoracic exposure to 0.05–1.5 Gy of fission neutrons at a rate of 50–250 mGy min^{-1} and compared with 88 controls. When the mice were killed nine months after irradiation, the relationship between the number of lung tumours per mouse and doses up to 0.25 Gy was linear, or a threshold model with a linear response above the threshold was reported. The RBE increased with decreasing dose from 25 at 0.25 Gy to 40 at 0.10 Gy in relation to acute exposure to X-rays (Ullrich *et al.*, 1979). In another study (Ullrich, 1980), mice of the same strain were irradiated with 0, 0.1, 0.15, 0.2, 0.5, 1.0 or 1.5 Gy as either single doses or two equal doses separated by 24-h or 30-day intervals. The animals were observed until nine months of age. Dose fractionation had no effect on lung tumour induction at any dose.

In a study with female BALB/c/AnNBd mice, 296 control and 3258 irradiated mice, 12 weeks of age, received whole-body exposure to fission spectrum neutrons at doses of 0, 0.048, 0.096, 0.192, 0.24, 0.47, 0. 94 or 1.88 Gy at a dose rate of 50 or 250 mGy min^{-1} or 10 mGy day^{-1}. The animals were observed for life, and the induced tumours were examined histologically. The tumours that were most sensitive to induction by neutrons

Table 6. Incidences of neoplasms in female RFM/Un mice after neutron irradiation at various doses and rates

Dose rate	Type of neoplasm	Incidence (%)				
		0	0.048 Gy	0.096 Gy	0.192 Gy	0.47 Gy
50 mGy min^{-1}	Thymic lymphoma	7.3	11	11	20	33
	Lung adenoma	24	10	24	30	46
	Endocrine tumours	7.0	5.2	11	50	54
		0	0.24 Gy	0.47 Gy	0.94 Gy	1.88 Gy
250 mGy min^{-1}	Thymic lymphoma	4.6	24	30	40	39
	Reticulum-cell sarcoma	62	53	53	52	31
	Myeloid leukaemia	0	0.56	5.3	1.3	0.62
	Other leukaemias	6.4	6.9	7.5	7.7	9.6
	Lung adenoma	31	42	45	53	16
	Ovarian tumours	0	20	25	52	39
	Pituitary tumours	3.9	7.9	29	21	16
	Harderian gland tumours	0	13	28	35	6.3
	Uterine tumours	1.3	11	25	27	19
	Mammary tumours	2.6	8.0	8.4	10	3.9
	Other solid tumours	3.9	14	24	20	26
10 mGy day^{-1}	Thymic lymphoma	–	18	25	43	63
	Reticulum-cell sarcoma	–	64	58	48	45
	Myeloid leukaemia	–	0	2.4	0.27	0.26
	Other leukaemias	–	4.4	2.9	3.1	4.2
	Lung adenoma	–	48	48	53	32
	Ovarian tumours	–	2.3	8.7	22	24
	Pituitary tumours	–	11	11	19	2.5
	Harderian gland tumours	–	11	20	25	4.5
	Uterine tumours	–	4.8	17	21	18
	Mammary tumours	–	7.3	7.6	5.4	8.9
	Other solid tumours	–	21	14	21	12

From Ullrich *et al.* (1976)

were malignant lung adenocarcinomas, mammary adenocarcinomas and ovarian tumours, and increases in the incidences of these three types of tumours were observed after exposure to doses of neutrons as low as 50–100 mGy at a high dose rate (Table 7; Ullrich *et al*., 1977).

Groups of 140–182 female BALB/c/AnNBd mice, 12 weeks of age, received a single whole-body exposure to 0.025, 0.05, 0.10, 0.20, 0.50 or 2.0 Gy of fission neutrons at a dose rate of 50–250 mGy min^{-1}. The animals were studied for life, and tumours were examined histologically. A group of 263 controls was available. The ovary was very sensitive to the induction of tumours (granulosa-cell tumours, luteomas

Table 7. Incidences of leukaemias and solid tumours in neutron-irradiated female BALB/c mice

Dose rate	Dose (Gy)	Thymic lymphoma (%)	Reticulum-cell sarcoma (%)	Lung adenoma (%)	Lung adenocarcinoma (%)	Mammary tumours (%)	Ovarian tumours (%)
Control	0	1.1 ± 0.6	41 ± 4.1	26 ± 4.5	13 ± 3.4	7 ± 1.6	6 ± 2.1
50 mGy min^{-1}	0.048	1.0 ± 0.9	39 ± 6.6	11 ± 5.4	27 ± 4.8	7 ± 2.6	7 ± 4.1
	0.096	2.1 ± 1.4	32 ± 6.5	13 ± 6.2	39 ± 5.1	25 ± 4.5	11 ± 4.6
	0.192	2.2 ± 1.6	30 ± 5.8	17 ± 4.9	19 ± 5.1	18 ± 5.0	20 ± 4.9
	0.47	2.8 ± 1.7	27 ± 4.6	28 ± 4.6	22 ± 4.7	17 ± 5.6	49 ± 4.0
250 mGy min^{-1}	0.24	1.8 ± 0.8	29 ± 4.6	25 ± 4.5	19 ± 4.8	17 ± 2.4	37 ± 4.6
	0.47	2.4 ± 0.7	32 ± 6.4	27 ± 5.4	23 ± 5.1	19 ± 3.7	57 ± 5.4
	0.94	4.1 ± 1.3	26 ± 5.4	30 ± 4.9	19 ± 5.7	17 ± 3.9	62 ± 3.5
	1.88	4.5 ± 1.2	21 ± 3.6	23 ± 3.3	13 ± 5.2	15 ± 5.4	39 ± 5.5
10 mGy day^{-1}	0.24	2.1 ± 1.2	38 ± 4.5	28 ± 5.1	13 ± 4.6	14 ± 2.9	7 ± 2.9
	0.47	2.3 ± 0.9	36 ± 4.8	23 ± 5.1	27 ± 5.7	17 ± 3.7	10 ± 3.7
	0.94	2.9 ± 1.0	36 ± 4.1	22 ± 4.0	32 ± 5.5	19 ± 3.8	19 ± 4.2
	1.88	6.1 ± 1.6	28 ± 6.2	13 ± 2.6	43 ± 5.7	45 ± 5.3	21 ± 5.1

From Ullrich *et al*. (1977); incidences are means ± SE.

and tubular adenomas), the incidence increasing from 2% in controls to 76% after exposure to 0.50 Gy; at 2.0 Gy, the incidence was 56%. For mammary adenocarcinomas, a linear dose–response relationship was reported up to a dose of 0.50 Gy, from 8% in controls to 25%. For lung adenocarcinomas, a convex upward curve was seen over the dose range 0–0.50 Gy. In the dose range 0.1–0.2 Gy, the dose–response curve for the induction of lung and mammary tumours appeared to 'bend over'. The percentage incidences of lung and mammary adenocarcinomas and ovarian tumours are given in Table 8 (Ullrich, 1983).

Table 8. Incidences of solid tumours in female BALB/c mice after fission neutron irradiation

Dose (Gy)	No. of animals	Lung adenocarcinoma (%)	Mammary adenocarcinoma (%)	Ovarian tumours (%)
0	263	15 ± 2.4	8 ± 1.7	2 ± 1.0
0.025	140	17 ± 3.7	11 ± 2.9	3 ± 1.4
0.05	160	21 ± 4.3	17 ± 3.8	7 ± 2.1
0.10	160	18 ± 4.0	18 ± 4.2	10 ± 2.6
0.20	167	30. ± 6.1	20 ± 4.7	16 ± 3.7
0.50	182	37 ± 6.9	25 ± 5.5	76 ± 3.0
2.0	182	27 ± 6.1	8 ± 3.2	56 ± 3.8

From Ullrich (1983); incidences are means ± SE.

In the same model, the effects of dose rate and of dose fractionation on the carcinogenic effects of fission spectrum neutrons were examined for doses of 0, 0.025, 0.05, 0.10, 0.20 or 0.50 Gy in 263 controls and 140–191 animals in the various irradiated groups. Whole-body irradiation was given as a single dose or split at 24-h or 30-day intervals at dose rates of 10–250 mGy min^{-1}, depending on the total dose. The incidence of ovarian tumours was not altered by fractionation, but lowering the dose rate reduced the incidence of ovarian tumours and enhanced the frequency of mammary tumours at doses as low as 0.025 Gy (Ullrich, 1984).

A total of 1814 male RFM/Un mice, 10 weeks of age, were exposed by whole-body irradiation to 0.05, 0.1, 0.2, 0.4 or 0.8 Gy of fission neutrons at a rate of 0.25 Gy min^{-1}. The radiation facility was the same as that used in previous studies. A group of 602 controls was available. The lifetime incidence of myeloid leukaemia was increased in a dose-related manner from 0.8 ± 0.4 in controls to 2.1 ± 0.5 at 0.05 Gy, 2.6 ± 0.7 at 0.1 Gy, 4.8 ± 1.3 at 0.2 Gy, 7.5 ± 2.2 at 0.4 Gy and 14.9 ± 3.8% at 0.8 Gy. In comparison with acute ^{137}Cs γ-radiation, the RBE for myeloid leukaemia was 2.8 (Ullrich & Preston, 1987).

Radiation-induced late somatic effects and the shapes of the dose–response curves after graded doses of 1.5-MeV fission neutrons at 0.17, 0.36, 0.71, 1.07, 1.43, 1.79 or

2.14 Gy were reported in 360 male $BC3F_1$ [(C57BL/Cne × C3H/HeCne) F_1] mice, three months of age, after whole-body irradiation. The γ-ray component represented about 12.5% of the total dose. A control group of 561 male mice was available. A significant decrease in the mean life span was observed at 0.36 Gy and with increasing doses from 1.07 to 2.14 Gy ($p < 0.001$, Student's *t* test). Myeloid leukaemia, malignant lymphoma and solid tumours including cancers of the lung, liver and soft tissues were observed. A significant increase in the incidence of myeloid leukaemia was reported at doses of 0.71 to 1.79 Gy ($p < 0.001$, χ^2 test) when compared with controls (0%). A significant decrease in the incidence of malignant lymphoma was observed after exposure to 1.43–2.14 Gy. The incidences of solid tumours were significantly ($p < 0.05$) increased even at doses of 0.36–1.79 Gy when compared with controls (31%). The incidence of myeloid leukaemia fit a curvilinear model, and the RBE at the lowest dose of 0.17 Gy was about 4 with reference to an acute dose of 250-kVp X-rays (Covelli *et al*., 1989).

The thoraxes of 474 male and 464 female SAS/4 albino outbred mice, three months of age, were exposed locally to 0.10, 0.25, 0.5, 0.75, 1, 2, 3 or 4 Gy of fast neutrons (mean energy, 7.5 MeV, with 3% γ-rays, beryllium target) at a rate of 1.06 Gy min^{-1}; the rest of the body was shielded. At the time of irradiation, the mice were anaesthetized with 57 mg $(kg\ bw)^{-1}$ sodium pentobarbitone. A group of 219 male and 210 female controls was available. After 12 months of irradiation, the animals were necropsied. Histologically, the lung tumours appeared to be a mixture of benign encapsulated adenomas and malignant invasive adenocarcinomas. The dose–response curve for animals of each sex was 'bell shaped' and steeply linear up to 1 Gy, peaked between 1 and 3 Gy and sharply declined at 4 Gy. In females, the incidences of lung tumours were 9% at 0 Gy (control) and 17.5, 24.1, 25.5, 27.9, 30.5, 33.9, 29.5 and 15.5% at the respective doses; in males, the percentage incidences were 16.5 (controls), 28.3, 32.7, 27.6, 29.1, 41.5, 42.2, 44.9 and 20.0%, respectively. The RBE for doses < 1 Gy of neutrons in comparison with < 3 Gy of 200-kVp acute X-ray exposure was 7.1 for females and 4.5 for males (Coggle, 1988).

Groups of 60 female (C57BL/6N × C3H/He) F_1 ($B6C3F_1$) mice, seven to eight weeks of age, were exposed by whole-body irradiation to a dose of 0.27 Gy at 0.059 mGy min^{-1} or 2.7 Gy at 0.53 mGy min^{-1} from ^{252}Cf fission neutrons (mean energy, 2.13 MeV; 35% γ-ray contamination). A group of 60 age-matched females was used as controls. The carcinogenic effects were examined 750 days after irradiation by gross observation and histopathologically. Both doses induced significantly higher incidences of neoplasms in the ovary, pituitary gland, Harderian gland, liver, mammary gland and reticulum cells (at 2.7 Gy only) and of lipoma (at 0.27 Gy only) (χ^2 test). No RBE was reported. There was no significant increase in the incidences of tumours in the lung, uterus and vagina, adrenal gland, soft tissue, bone, pancreas, stomach or thyroid gland, or of haemangiosarcoma or leukaemia after exposure to 0.27 or 2.7 Gy. More frequent development of multiple tumours was reported in the

neutron-irradiated animals in comparison with animals exposed to γ-rays (^{60}Co, ^{137}Cs) (Seyama *et al*., 1991).

In a study of the influences of strain and sex on the development of tumours, 190 male and 151 female B6C3F$_1$ hybrid (C57BL × C3H), 65 male and 60 female C3B6F$_1$, 117 male and 112 female C57BL/6N and 156 male and 139 female C3H/HeN mice, six weeks of age, were exposed by whole-body irradiation to 0 (control), 0.125, 0.5 or 2 Gy of ^{252}Cf neutrons at a rate of 6–8 mGy min^{-1} (mean energy, 2.13 MeV; γ-ray component, 35%) and were observed up to 13 months of age. Tumours were identified histopathologically. The total tumour incidence was high in C3H/HeN, moderate in B6C3F$_1$ and C3B6F$_1$ and low in C57BL/6N mice (Table 9) because of high frequencies of liver tumours in males and ovarian tumours in females. A dose-dependent increase in liver tumours was reported in both males and females of all strains but the increase was greater in males than in females. Ovarian tumours were more frequent in C3H/HeN mice, followed by B6C3F$_1$, C3B6F$_1$ and C57BL/6N. Of the strains and hybrids, B6C3F$_1$, C57BL/6N and C3H/HeN were the most sensitive to low doses around 0.50 Gy (Ito *et al*., 1992; Takahashi *et al*., 1992).

In a series of experiments during the period 1971–86, thousands of male and female B6C3F$_1$ mice were exposed by whole-body irradiation to single or fractionated doses of fission neutrons. The effects on survival were reported by Ainsworth *et al*. (1975), Thomson *et al*. (1985a,b, 1986) and Thomson and Grahn (1988). In a report on tumour induction, several thousand male and female B6CF$_1$ (C57BL/6 × BALB/c) mice, 110 ± 7 days of age, were exposed to 0–2.4 Gy of fission neutrons, as single doses, 24 equal doses once weekly or 60 equal doses once weekly. The mean energy was 0.85 MeV; 2.5% of the dose was due to γ-radiation and 0.1% was thermal neutrons. A total of 901 age-matched males and 1199 age-matched females were used as controls. All the mice were followed for life, and the tumours were identified histopathologically. Most of those found in both control and irradiated mice were lymphoreticular, vascular and pulmonary tumours. About 85% of the irradiated mice died with or from one or more neoplasms. Dose-dependent increases in the incidence of lymphoreticular, lung, liver, Harderian gland and ovarian tumours were observed. The connective tissues showed less sensitivity to radiation-induced cancers than epithelial tissues, and the latter showed RBE values of 75 or greater with reference to chronic exposure to γ-rays (Grahn *et al*., 1992).

A total of 742 male BC3F$_1$ mice, three months of age, were exposed to five equal daily fractions of fission neutrons with a mean neutron energy of 4 MeV and a 12% γ-ray component, to yield cumulative doses of 0.025, 0.05, 0.1, 0.17, 0.25, 0.36, 0.535 and 0.71 Gy, given at a rate of 4 mGy min^{-1}. A group of 193 controls was available. The animals were kept for life, and tumours were examined grossly and histopathologically. The incidence of myeloid leukaemia showed a significant positive trend (Peto's test) at doses of 0–0.17 Gy and up to 0.36 Gy. The incidence of epithelial tumours was increased significantly ($p < 0.001$) at doses from 0.17 Gy, those of liver and lung tumours at doses from 0.025 Gy, that of skin tumours from 0.36 Gy and that

Table 9. Strain and sex differences in the incidence of ^{252}Cf neutron-induced tumours in mice

Reference	Dose (Gy)	Strain and sex	Effective no. of mice	Survival rate (%)	Liver tumours (%)	Lymphoma (%)	Adrenal tumours (%)	Ovarian tumours (%)
Ito *et al*. (1992)		C57BL/6N Male						
	0		23	82	0	0	0	
	0.125		32	100	6.3	9.4	0	
	0.50		31	97	3.2	3.2	0	
	2.0		31	91	9.7	16	3.2	
		Female						
	0		25	89	0	16	0	12
	0.125		30	94	3.3	13	0	20
	0.50		31	97	0	19	3.2	9.7
	2.0		26	81	3.8	15	12	0
		C3H/HeN Male						
	0		43	78	40	2.3	2.3	
	0.125		28	88	61	0	3.6	
	0.50		37	95	70	14	19	
	2.0		48	79	71	6.3	4.2	
		Female						
	0		35	100	11	2.9	0	66
	0.125		29	91	0	0	0	35
	0.50		40	100	18	13	0	94
	2.0		35	79	31	2.9	15	85

Table 9 (contd)

Reference	Dose (Gy)	Strain and sex	Effective no. of mice	Survival rate (%)	Liver tumours (%)	Lymphoma (%)	Adrenal tumours (%)	Ovarian tumours (%)
Ito *et al*. (1992)		$C3B6F_1$ Male						
(contd)	0		34	100	12	0	0	
	2.0		31	97	55	3.2	0	
		$C3B6F_1$ Female						
	0		33	97	0	0	0	6.1
	2.0		27	84	19	3.7	30	0
Takahashi *et al*. (1992)		$B6C3F_1$ Male						
	0		53	96	3.8	Not studied	0	
	0.03		24	100	13		0	
	0.06		24	100	21		0	
	0.125		30	94	37		3.3	
	0.50		30	94	43		0	
	2.0		29	91	62		0	
		Female						
	0		63	95	3.2		0	4.8
	0.125		29	91	3.4		3.4	28
	0.50		30	94	6.7		6.7	80
	2.0		29	91	28		21	62

of soft-tissue tumours only at the highest dose, 0.71 Gy. The total numbers of solid tumours in the lung, liver, gastrointestinal tract, adrenal gland, kidney, soft tissues, mammary gland, urinary bladder, vascular system, bone, Harderian gland, skin and salivary gland were 33, 41, 25, 28, 24, 24, 26, 20 and 27 at the respective doses. There were no differences in survival or tumour incidence between this study at 4 mGy min^{-1} (Di Majo *et al*., 1994) and a previous report (Di Majo *et al*., 1990) in which dose rates of 50 and 250 mGy min^{-1} were used. In a subsequent study, it was shown that male CBA/Cne mice were more susceptible to tumour induction than females (Di Majo *et al*., 1996).

A total of 4689 male and female hybrid $B6CF_1$ (C57BL/6 Bd × BALB/c Bd) mice, 16 weeks of age, were exposed to fission neutrons at doses of 0.06, 0.12, 0.24 or 0.48 Gy in 24 weekly fractions of 0.0025 Gy, 12 fractions of 0.01 Gy every two weeks, six fractions of 0.04 Gy every four weeks or three fractions of 0.16 Gy every eight weeks. A group of 398 male and 396 female controls was available. The animals were observed for life, and tumours were identified histopathologically. The survival and the incidences of most neoplasms increased with dose in the low-dose range (Table 10). Fractionation of the neutron dose did not affect the magnitude of the response at equal total doses (Storer & Fry, 1995).

3.1.2 *Rat*

Most of the studies of the carcinogenicity of neutrons in rats have addressed the effects on the mammary gland (Table 11). The tumour incidence was shown to be influenced by strain and hormonal status (Clifton *et al*., 1975, 1976a,b; Shellabarger *et al*., 1978; Jacrot *et al*., 1979; Shellabarger *et al*., 1982, 1983). The most comprehensive studies are summarized below.

A total of 312 adult female Sprague-Dawley/ANL rats, two to three months of age, were exposed by whole-body irradiation to single doses of 0 (control), 0.05, 0.10–0.12, 0.18–0.22, 0.35, 0.5, 1.5 or 2.5 Gy of fission neutrons (10–15% γ-ray contamination, see Vogel, 1969). The animals were observed for life, and mammary tumours were examined histologically. At the end of the study, the percentages of rats with mammary tumour were 48, 78, 85, 73, 80, 84, 87 and 76% at the different doses, respectively. Of the 126 mammary tumours in 223 rats irradiated with 0.05–2.5 Gy, 66% were benign (67 fibroadenomas, four fibromas, one fibrolipoma, eight adenofibromas and three cystadenomas), and 34% were malignant (13 sarcomas and 30 carcinomas). The RBE in relation to an acute dose of 250-kVp X-rays was 20–60. In a comparison of partial and whole–body exposures to a dose of 0.35 Gy of neutrons, 28 animals received irradiation of one mammary gland at a mean energy of 540 ± 50 keV and 15 animals were exposed to 0.35 Gy of fission neutrons with a mean energy of about 1 MeV. Palpable mammary tumours (mostly fibroadenomas) developed in 75% of those receiving partial irradiation and 80% of those given whole-body exposure (Vogel & Zaldivar, 1972).

Table 10. Survival and incidences of tumours in various organs of BCF_1 mice exposed to single or fractionated doses of fission neutrons

Dose (Gy)	No. of animals	Mean survival (days)	Incidence (%)									
			Lung carci-noma	Reticulum-cell carcinoma	Other lym-phoma and leukaemia	Fibro-sarcoma	Vascular tissue	Liver tumour	Breast carcinoma	Osteo-sarcoma	Ovarian tumour	Other epithelial
Single doses												
Males												
0	398	913	32	17	2.8	29	6.8	8.3	–	–	–	
0.025	396	888	31	22	2.8	32	6.4	9.7	–	–	–	
0.05	393	875	28	23	3.2	36	4.9	11	–	–	–	
0.1	397	870	36	25	2.5	32	5.8	12	–	–	–	
0.2	398	848	39	24	3.5	49	6.8	13	–	–	–	
Females												
0	396	938	13	35	12	6.6	6.1	0.51	9.1	0.76	1.3	
0.025	386	943	12	32	9.3	5.7	6.2	1.6	9.0	0.52	2.6	
0.05	389	926	12	39	14	8.3	8.8	1.5	7.4	0.60	2.4	
0.1	391	895	15	41	12	7.6	9.4	2.9	10	0.94	5.7	
0.2	390	866	21	46	13	5.5	7.5	2.8	9.6	1.7	11	
Fractionated doses												
Males												
0	398	913	32	17	2.8	29	6.8	8.3	–	–	–	1.3
$24 \times 0.0025 = 0.06$	193	875	36	20	3.0	36	2.7	5.9	–	–	–	3.6
$12 \times 0.01 = 0.12$	191	825	41	26	11	47	4.5	9.2	–	–	–	4.5
$4 \times 0.06 = 0.24$	196	825	43	3.1	2.8	49	6.0	11	–	–	–	11
$3 \times 0.16 = 0.48$	199	777	60	43	7.5	67	8.5	15	–	–	–	17
Females												
0	396	938	13	35	12	6.6	6.1	0.51	9.1	0.76	1.3	
$24 \times 0.0025 = 0.06$	194	926	17	42	10	7.1	4.4	–	4.9	0.65	1.9	
$12 \times 0.01 = 0.12$	190	894	13	48	15	11	4.8	2.6	8.4	1.4	3.2	
$4 \times 0.06 = 0.24$	192	841	23	56	20	16	14	12	12	0.93	5.0	
$3 \times 0.16 = 0.48$	194	800	33	68	22	11	3.3	11	12	3.3	11	

From Storer & Fry (1995); –, no tumours

Table 11. Mammary tumours in rats and mice after exposure to neutrons

Species and strain	Dose (Gy)	Mean energy (MeV)	No. of animals	No. of animals with tumours	Incidence of tumours (%)	Reference
Sprague-Dawley/ ANL rat	0	1	89	43	48	Vogel & Zaldivar (1972)
	0.05		27	21	78	
	0.10–0.12		34	29	86	
	0.18–0.22		41	30	73	
	0.35		25	20	80	
	0.50		31	26	84	
	1.50		31	27	87	
	2.50		34	26	76	
Fischer rat	0	Not reported	24	2	8	Clifton *et al.* (1976a)
	0.50		24	17	71	
Sprague-Dawley rat	0	0.43	167	20	12	Shellabarger (1976)
	0.01		182	28	15	
	0.04		89	16	18	
	0.16		68	21	31	
	0.64		45	26	58	
Sprague-Dawley rat	0	14.5	31	2	6.5	Montour *et al*. (1977)
	0.25		30	0	0	
	0.5		30	6	20	
	0.10		25	6	24	
	0.20		25	6	40	
	0.40		25	17	68	
Sprague-Dawley rat	0	1.2	62	2	3	Vogel (1978)
	0.5 + 0.5		40	6	15	
	0.10		38	10	26	
	0.10 + 0.10		29	15	52	
	0.20		29	10	34	
	0.35 + 0.35		35	22	63	
	0.70		37	20	54	
Sprague-Dawley rat	0	14.8	60	1	1.7	Jacrot *et al*. (1979)
	0.6		38	4	11	
Wistar/ Furth rat	0	2.0	18	0	0	Yokoro *et al*. (1980)
	0.48		16	1	6.3	
	0.089		16	0	0	
	0.195		16	0	0	

Table 11 (contd)

Species and strain	Dose (Gy)	Mean energy (MeV)	No. of animals	No. of animals with tumours	Incidence of tumours (%)	Reference
Sprague-Dawley rat	0	0.5	40	Not reported	30	Broerse *et al.* (1987)
	0.02		40		15	
	0.08		40		53	
	0.32		40		63	
	0	15	40		30	
	0.05		40		40	
	0.15		40		65	
	0.50		40		90	
WAG/Rij rat	0	0.5	40		27	
	0.05		40		20	
	0.2		40		33	
	0.8		40		53	
	0	15	40		27	
	0.15		40		35	
	0.50		40		58	
	1.5		40		56	
BN/Bi rat	0	0.5	40		8	
	0.05		40		11	
	0.2		40		19	
	0.8		40		44	
	0	15	40		8	
	0.15		40		22	
	0.5		40		56	
	1.5		40		78	
BALB/c mouse	0	1	263	Not reported	7.9	Ullrich (1983, 1984)
	0.25		140		11	
	0.5		160		17	
	0.10		160		18	
	0.20		167		20	
	0.50		182		25	
	2.00		182		8.4	

Groups of 110 female Sprague-Dawley rats, two months of age, were exposed to single doses of 0.1, 0.2 or 0.7 Gy or to split doses of 0.05 + 0.05, 0.1 + 0.1 and 0.35 + 0.35 Gy at 24-h intervals; 62 rats served as unirradiated controls. The radiation was ^{235}U fission neutrons with a mean energy of 1.2 MeV and a neutron:γ-ray ratio of approximately 7:1. Induction of mammary tumours was examined 11 or 12 months after irradiation [mode of examination not given]. Mammary tumours were reported in 2/62 controls, 10/38 at the single dose of 0.1 Gy and 6/40 given split exposure, in 10/29

at the single dose of 0.2 Gy and 15/29 given split exposure, in 20/37 at the single dose of 0.7 Gy and 22/35 given split exposure. No significant difference was seen in the incidence of mammary tumours with the single and the paired neutron doses (Vogel, 1978).

Groups of 15 and 34 female Long-Evans/Simonsen, 14 and 36 female Sprague-Dawley/Harlan, 15 and 34 female Buffalo/Simonsen, 14 and 36 female Fischer 344/Simonsen and 14 and 36 female Wistar-Lewis/Simonsen rats, two months of age, received whole-body irradiation with a single dose of 0 (control) or 0.5 Gy of fission neutrons (see Vogel, 1969). One year after irradiation, mammary tumours were identified histopathologically. The Long-Evans and Sprague-Dawley strains were the most sensitive, Buffalo and Fischer rats were moderately sensitive, and Wistar-Lewis rats were quite resistant to radiation-induced mammary tumours, the incidences being 56, 56, 29, 26 and 5.5% in exposed rats of the five strains, respectively (Table 12). This result strongly suggested a genetic predisposition in neutron-induced mammary tumorigenesis in rats (Vogel & Turner, 1982).

Groups of 20 (intermediate and high dose) or 40 (control and low dose) female WAG/Rij, BN/BiRij and Sprague-Dawley rats, eight weeks of age, were exposed by whole-body irradiation to single or fractionated doses of monoenergetic neutrons of 0.5, 4 or 15 MeV. In subsequent experiments, the numbers of animals in these groups were increased to 40 and 60, respectively. The animals were observed for life, and tumours were identified by gross and histopathological observation. The three strains developed different types of tumours and showed marked differences in susceptibility for mammary tumorigenesis. The RBE of the 0.5-MeV energy neutrons in relation to acute exposure to 300-kVp X-rays was 15 for the induction of adenocarcinomas and 13 for fibroadenomas in WAG/Rij rats and 7 for the induction of fibroadenomas in Sprague-Dawley rats (Broerse *et al*., 1986, 1987). [The Working Group noted that the numbers of animals in each group were not clearly stated.]

A total of 135 female Sprague-Dawley rats, 35–40 days of age, were exposed to doses of 0.025, 0.05, 0.1, 0.2 or 0.4 Gy of 14.5-MeV energy neutrons produced by a 35-MeV deuteron beam. A group of 31 controls was available. Mammary tumours were identified histopathologically as adenocarcinoma, fibroadenoma (including adenofibroma) and fibrosarcoma. By 11 months after exposure, 2/31 unirradiated rats had developed single fibroadenomas, whereas 42 mammary tumours were reported in 39/135 irradiated rats. The incidence increased with dose, from 0/30 to 6/30, 6/25, 6/25 and 17/25. Six of the rats that died within 11 months after irradiation had mammary tumours. Three rats died with neoplasms at other sites: lymphocytic type lymphosarcoma (0.4 Gy at seven months), osteogenic sarcoma (0.4 Gy at 11 months) and myxosarcoma (0.25 Gy at 11 months). The RBE increased from 5 at 0.4 Gy to 13.8 at 0.25 Gy, when compared with γ-rays (Montour *et al*., 1977). [The Working Group noted that the γ-ray source was not described.]

A total of 551 adult female Sprague-Dawley rats were exposed to 0.43-MeV neutrons at doses of 0 (167 controls), 1, 4, 16 or 64 mGy and the incidences of mammary tumours were examined histologically up to the age of 14 months. At the

Table 12. Mammary tumours in five strains of rat after single whole-body exposure to 0.5 Gy of fission neutrons

Strain	No. of rats with mammary tumours/no. of unirradiated rats	No. of rats with mammary tumours/no. of irradiated rats	All mammary tumours (%)	Fibroadenomas and adeno-fibromas (%)	Adeno-carcinomas (%)	Regressed tumours (%)
Long-Evans/Simonsen	0/15	19/34	56	11	5	0
Sprague-Dawley/Harlan	0/14	20/36	56	8	8	1
Buffalo/Simonsen	1/15	10/34	29	7	3	0
Fischer-344/Simonsen	0/15	9/35	26	6	1	0
Wistar-Lewis/Simonsen	0/14	2/36	5.5	0	1	1

From Vogel & Turner (1982)

end of the study, exposure to 1 mGy was found to have induced a higher incidence (15%) of adenocarcinomas and all other tumours than in the controls (12%). The incidences at the other doses were 18% at 4 mGy, 31% at 16 mGy and 58% at 64 mGy. The first tumours appeared five months after exposure to 1 mGy, three months after 4 mGy, four months after 16 mGy and two months after 64 mGy; in controls, the first tumour appeared at eight months. RBEs of about 100 for the low doses and about 8 for the high doses were reported with reference to an acute dose of 250-kVp X-irradiation (Shellabarger, 1976).

The role of prolactin in the induction of mammary tumours after low-dose whole-body irradiation with fission neutrons was examined in groups of 16–18 female Wistar/Furth rats, seven weeks of age, that were exposed to 0 (control), 0.048, 0.089 or 0.195 Gy of neutrons (mean energy, 2.0 Mev) [γ-ray component not specified]. To promote the development and growth of radiation-induced mammary tumours from dormant initiated cells, prolactin-secreting pituitary tumours (MtT.W95) were grafted subcutaneously 25 days after irradiation. In a further experiment, MtT.W95 were grafted only in tumour-free animals 12 months after irradiation. The rats died naturally or were killed when moribund, and mammary tumours were identified histologically as adenocarcinoma or fibroadenoma. Only 1/48 rats developed mammary tumours after neutron irradiation alone, while 20/48 rats developed mammary tumours when MtT.W95 were grafted 25 days after irradiation. The incidences at each dose were 6/16, 5/15 and 9/17, respectively. When MtT.W95 were grafted in tumour-free animals 12 months after irradiation, the incidences were 4/15, 3/15 and 4/15 at the respective doses (Yokoro *et al*., 1980, 1987).

A total of 767 male and female Sprague-Dawley rats, three months of age, were exposed by whole-body irradiation to fission neutrons at doses of 0.012, 0.02, 0.06, 0.1, 0.3, 0.5 (irradiation period, one day), 1.5, 2.3 (irradiation period, 14 days), 3.9 (irradiation period, 23 days), 5.3 or 8 Gy (irradiation period, 42 days) from a neutron reactor (1.6 MeV; neutron:γ-ray ratio, 3:1) and were observed for the induction of pulmonary neoplasms for life. Tumours were identified histopathologically. The lung tumours included bronchogenic carcinomas, bronchoalveolar carcinomas, lung carcinomas, adenomas and sarcomas. The numbers of animals with lung carcinomas were dose-dependent up to doses of 2.3 Gy, with a reduced mean survival. The numbers of animals with lung carcinoma or adenomas also increased at doses up to 2.3 Gy, but decreased at higher doses. An apparent life-shortening was observed at higher doses (Table 13) (Chmelevsky *et al*., 1984). [The Working Group noted that no data were given on controls.]

A total of 596 male Sprague-Dawley rats, three months of age, were exposed by whole-body irradiation to fission neutrons at 0.016 (mean of the two doses, 0.012 and 0.02), 0.08 (0.06 and 0.10) or 0.40 (0.32 and 0.49) Gy with a mean energy of 1.6 MeV (neutron:γ-ray ratio, 3:1). The duration of exposure was 20 h at 0.016 Gy and 22 h at the other doses. A group of 579 controls was available. The animals were observed for life. Lung carcinomas (bronchogenic and bronchoalveolar) and lung sarcomas were

Table 13. Pulmonary tumours in Sprague-Dawley rats after exposure to fission neutrons

Reference	Dose (Gy)	Irradiation period	No of animals	No. of animals examined	Mean survival (days)	No. of animals with lung carcinomas			No. of animals with lung sarcomas
						Total	Broncho-genic	Broncho-alveolar	
Chmelevsky	0		NR	NR	NR	NR	NR	NR	NR
et al. (1984)	0.012	1 day	150	148	752	4	3		
	0.02	1 day	150	149	741	2	1	1	1
	0.06	1 day	80	77	679	4	1	3	–
	0.1	1 day	78	75	669	6	5	1	–
	0.3	1 day	75	71	584	9	4	5	2
	0.5	1 day	75	72	525	10	7	3	2
	1.5	14 days	40	94	487	14	5	11	3
	2.3	14 days	60	99	450	18	9	10	1
	3.9	23 days	20	20	390	–	–	–	–
	5.3	42 days	19	19	340	4	2	3	1
	8	42 days	20	20	240	2	1		–
Lafuma *et al.*	0		586	579	754	5	4	1	1
(1989)	0.012	20 h	150	149	757	4	3	1	3
	0.02	20 h	150	149	742	2	1	1	2
	0.06	22 h	80	77	679	4	1	3	–
	0.10	22 h	78	75	669	6	5	1	–
	0.32	22 h	75	72	583	9	4	5	2
	0.49	22 h	75	74	522	10	7	3	2

NR, not reported

identified by gross and histological examination. As shown in Table 13, increased incidences of animals with bronchogenic or bronchoalveolar carcinomas were observed. The RBE was 30–40 at the dose of 0.1 Gy and > 50 at the dose of 0.016 Gy in relation to acute ^{60}Co γ-irradiation (Lafuma *et al*., 1989).

A group of 114 female Wistar rats, three to four months of age, were irradiated locally in the region of the liver with 0.2 Gy of neutrons at 14-day intervals for up to two years, for a total of 50 fractions and a total dose of 10 Gy and were observed for life. A group of 114 controls was available. The first liver tumour appeared one year after the beginning of irradiation. At the end of the study, 45 irradiated animals had liver tumours. Of the 83 liver tumours that were classified histologically, 14 were hepatocellular adenomas, 18 were hepatocellular carcinomas, 28 were bile-duct adenomas, nine were bile-duct carcinomas, one was a haemangioma and five were haemangiosarcomas; eight animals had Kupffer-cell sarcomas (Spiethoff *et al*., 1992).

3.1.3 *Rabbit*

A total of 20 male and 18 female adult Dutch rabbits, 7–18 months of age, were irradiated ventro-dorsally with doses of 1.8–5.5 Gy of fission neutrons of about 0.7 MeV mean energy at a dose rate of about 23 Gy h^{-1} with γ-ray contamination of about 2.7 Gy h^{-1}. A control group of 17 rabbits was available. The rabbits were kept for life (six to nine years) and were killed when moribund. Full autopsies were carried out, and the tissues were studied histologically. The mean age at death was significantly lower after the doses of 3.7 Gy and 4.1–5.5 Gy (Student's *t* test). Increased incidences of subcutaneous fibrosarcomas were observed, with 0/17 in controls, 4/15 at 1.8 Gy, 10/16 at 3.7 Gy and 5/7 at 4.1–5.5 Gy. Osteosarcomas were found in 0, 1, 2 and 2 rabbits in the respective groups, and basal-cell tumours of the skin were found in 0, 10, 5 and 1 rabbits, respectively. The RBE for neutrons in relation to acute γ-irradiation was estimated to be 3–3.5 (Hulse, 1980).

3.1.4 *Dog*

A total of 46 male beagle dogs, one year of age, were exposed to fast neutrons with a mean energy of 15 MeV in one of three dose-limiting normal tissues, spinal cord, lung and brain. The radiation was given in four fractions per week for five weeks to the spinal cord, for six weeks to the lung or for seven weeks to the brain. A group of 11 controls was available. The animals were observed for life, and tumours were identified grossly and microscopically. No tumours were reported in the unirradiated controls. Nine neoplasms developed within the irradiated fields in seven dogs receiving fast neutrons, comprising a haemangiosarcoma of the heart (10 Gy to the hemithorax region), an oligodendroglioma and a glioblastoma in the left basal nuclei (13.33 Gy to the brain), an osteosarcoma in the subcutis, an adenocarcinoma of the lung and a haemangiosarcoma of the heart (15 Gy to the hemithorax region), a neuro-

fibroma of the cervical nerve (17.5 Gy to the spinal cord), an osteosarcoma of the vertebrae and a myxofibrosarcoma of the subcutis (26.25 Gy to the spinal cord). The incidence of neoplasia was 15%, and the latent period for radiation-induced cancers varied from 1 to 4.5 years (Bradley *et al.*, 1981).

3.1.5 *Rhesus monkey*

Nine rhesus monkeys (*Macaca mulatta*), three years of age, were exposed by whole-body irradiation to neutrons (^{235}U; energy, 1 MeV) at doses of 2.3, 3.5, 3.8, 4.1 or 4.4 Gy at a rate of 0.08 Gy min^{-1} [γ-ray component unspecified]. A few hours after irradiation, the monkeys were grafted intravenously with $2–4 \times 10^8$ autologous bone-marrow cells (in Hank balanced salt solution) per kg bw. A group of 21 monkeys served as unirradiated controls. Between 4 and 10 years after irradiation, seven animals died with various malignant tumours, including glomus tumours in the pelvis, scrotum and subcutis, sarcomas or osteosarcomas in the humerus, osteosarcomas in the calvaria and papillary cystadenocarcinoma of the kidney and cerebral astrocytoma and glioblastoma. Benign tumours (islet-cell adenoma, subcutis haemangioma and skin fibroma) were also reported. No malignancies were observed in the 21 untreated controls. A RBE of approximately 4 was reported in relation to an acute dose of 300-kVp X-radiation. The latency for death with neoplastic disease after irradiation with fission neutron was 7 years (Broerse *et al.*, 1981, 1991).

3.1.6 *Relative biological effectiveness*

As shown in Table 14, neutrons were generally more carcinogenic than X-rays and γ-rays. Additional studies not described in the text are included in the Table.

3.2 Prenatal exposure

Mouse: Groups of pregnant female BC3F$_1$ [(C57BL/Cne × C3H/HeCne) F$_1$] mice were exposed to 0, 0.09, 0.27, 0.45 or 0.62 Gy of fission neutrons (mean energy, about 0.4 MeV; γ-ray contamination, about 12% of the total dose; minimum and maximum fast neutron dose rates, about 0.049 and 0.248 Gy min^{-1}) on day 17 of gestation and were allowed to deliver their offspring, which were observed for life. Liver tumours were examined histologically. A total of 379 offspring were necropsied. The incidences of liver adenomas and carcinomas were increased to 11, 31, 29 and 52% with the respective neutron doses but decreased to 18% after exposure to the highest dose of 0.62 Gy (Table 15). An RBE of 28 at 0.09 Gy was reported in relation to an acute dose of 250-kVp X-radiation (Di Majo *et al.*, 1990; Covelli *et al.*, 1991a,b).

Table 14. Relative biological effectiveness (RBE) of neutrons for various end-points, in relation to dose and energy

Species	Strain	Effect	Dose (Gy)	Energy (MeV)	RBE	Reference
Mouse	RF/Un	Myeloid leukaemia	0.001	1 and 5	1.8	Upton *et al.* (1970)
		Thymic lymphoma	0.001	1 and 5	3.3	
	RFM	Lung tumour	0.25	NR	25	Ullrich *et al.* (1979)
		Lung tumour	0.10		40	
	BALB/c	Lung adenocarcinoma	0.001	NR	19	Ullrich (1983)
		Mammary tumour	0.001		33	
	CBA/H	Myeloid leukaemia	0.001	NR	13	Mole (1984)
	$B6C3F_1$	Lymphoreticular tumour	0.001	~0.85	2–5	Thomson *et al.* (1985b)
		Lung tumour	0.001		23–24	
		Decreased survival	0.001		15	
	RFM	Myeloid leukaemia	0.001	NR	2.8	Ullrich & Preston (1987)
	$BC3F_1$	Decreased survival	0.01	1.5	12	Covelli *et al.* (1988)
	SAS/4	Lung tumour (male)	< 1	7.5	4.5	Coggle (1988)
		Lung tumour (female)	< 1	7.5	7.4	
	$BC3F_1$	Liver tumour[a]	0.09	0.4	28	Di Majo *et al.* (1990)
		Liver tumour[b]	0.17	0.4	13	
	$B6C3F_1$	Liver tumour (male)	0–2.0	2.13	15	Takahashi *et al.* (1992)
		Liver tumour (female)	0–2.0	2.13	2.5	
	CBA/Cne	Decreased survival (male)	0–0.4	0.4	24	Di Majo *et al.* (1996)
		Decreased survival (female)	0–0.4	0.4	8.6	
		Harderian gland tumour (male)	0–0.4	0.4	20	
		Harderian gland tumour (female)	0–0.4	0.4	9.5	
		Malignant lymphoma (male)	0–0.4	0.4	11	
		Myeloid leukaemia (male)	0–0.4	0.4	2.3	
	C57BL/Cnb	Malignant tumour	0.125–1	3.1	5-8	Maisin *et al.* (1996)

Table 14 (contd)

Species	Strain	Effect	Dose (Gy)	Energy (MeV)	RBE	Reference
Rat	Sprague-Dawley	Mammary tumour	0.001–0.04	0.43	100	Shellabarger (1976)
		Mammary tumour	0.016–0.064	0.43	8	
	Sprague-Dawley	Mammary tumour	0.4	14.5	5	Montour *et al*. (1977)
		Mammary tumour	0.025	14.5	14	
	Sprague-Dawley	Mammary fibroadenoma	0.001	2.43	50	Shellabarger *et al*. (1980)
	ACI	Mammary adenocarcinoma	0.001	2.43	100	Shellabarger *et al*. (1982)
	WAG/Rij	Mammary adenocarcinoma	0.001	0.5	15	Broerse *et al*. (1986)
		Mammary fibroadenoma	0.001	0.5	13	
	Sprague-Dawley	Mammary fibroadenoma	0.001	0.5	7	
	Sprague-Dawley	Lung carcinoma	0.016	1.6–2.1	50	Lafuma *et al*. (1989)
		Lung carcinoma	0.1	1.6–2.1	30–40	
Rabbit	Dutch	All tumours	1.8–5.5	2.5	3–3.5	Hulse (1980)
Rhesus monkey		All tumours	2–4	1	4	Broerse *et al*. (1981)

NR, not reported
[a] Irradiation on day 17 of gestation
[b] Irradiation at three months of age

Table 15. Incidences of liver tumours in male $BC3F_1$ mice exposed *in utero* to a whole-body dose of fission neutrons

Dose (Gy)	No. of mice autopsied	No. of mice with tumours		Incidence (%)
		Adenoma	Carcinoma	
0	230	24	2	11
0.09	49	15	0	31
0.27	42	9	3	29
0.45	25	10	3	52
0.62	33	5	1	18

From Di Majo *et al*. (1990)

3.3 Parental exposure

Mouse: Groups of male C3H mice, seven weeks of age, were exposed by whole-body irradiation to neutrons (^{252}Cf; mean energy, 2.13 MeV) at total doses of 0, 0.5, 1 or 2 Gy and were mated two weeks or three months later with unexposed C57BL females. On day 18 of gestation, some pregnant mice were killed to detect dominant lethal mutations. The incidence of dominant lethal mutations increased in a dose-dependent manner only after postmeiotic exposure, at two weeks. The other pregnant mice were allowed to deliver, and a total of 387 offspring were killed at the age of 14.5 months. Although tumours were found in various organs, only the incidence of liver tumours correlated with exposure to ^{252}Cf radiation, and these tumours were examined histologically. As shown in Table 16, the numbers of liver tumours per male offspring of male mice exposed to 0.50 or 1 Gy ^{252}Cf at either the postmeiotic or the spermatogonial stage were significantly higher than those in unirradiated controls. No increase in the incidence of liver tumours was observed in female offspring. The offspring of male parents irradiated with 2 Gy two weeks before mating did not survive more than two days after birth (Takahashi *et al*., 1992; Watanabe *et al*., 1996).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Transmission and absorption in biological tissues

The interaction of neutrons with biological material cannot be discussed outside the context of ionizing radiation in general, and the reader is referred to the Overall introduction for a fuller discussion. Neutrons with the lowest energy distribution, in

Table 16. Incidences of liver tumours in F_1 offspring of male C3H mice exposed to ^{252}Cf neutrons and mated with unexposed C57BL mice two weeks or three months after irradiation

Paternal dose (Gy)	Sex of offspring	Two weeks (postmeiotic)		Three months (spermatogonial)	
		No. of mice	Liver tumours (%)	No. of mice	Liver tumours (%)
0	Male	31	3.2	33	9.1
0.5		44	43.2*	20	30.0
1		39	15.4	22	22.9
2		0		19	5.3
0	Female	30	3.3		
0.5		58	1.7	18	5.6
1		35	0	24	0
2		0		14	0

From Takahashi *et al*. (1992); Watanabe *et al.* (1996); *$p < 0.01$

thermal equilibrium with their surroundings, are called 'thermal neutrons' and typically have an energy < 0.5 eV. Neutrons with energies between 0.5 and 100 eV are known as 'epithermal', or 'resonance' neutrons. Neutrons with energies up to about 500 keV are usually considered 'intermediate' in energy, and neutrons above 500 keV are called 'fast'. Most neutrons emerging from a fission reaction are fast, but in a reactor their energies are slowed down (moderated) to thermal energies to allow a chain reaction to proceed. The neutron energy spectrum outside a reactor is typically dominated by intermediate energy neutrons. The distribution of energy from neutrons in tissue is different from that from X- or γ-radiation. At low doses, only a small fraction of cells in a tissue is traversed. For example, l cGy of 1-MeV neutrons will traverse about one in 20 cells, whereas low-LET radiation may give rise to five traversals per cell (see Overall introduction, section 3.1).

Techniques for measuring exposure to neutrons are described in the Overall introduction (section 2.1.1).

4.2 Adverse effects other than cancer

Less information is available about the deterministic effects in humans of neutron radiation than of low-LET radiation because fewer patients are treated with neutrons than with low-LET radiation. Although there was a neutron component present in the radiation released by the nuclear explosion at Hiroshima, the effects of neutrons alone

are difficult to separate out accurately. The information about the biological effects of neutrons is derived from studies of patients treated with neutrons of various energies and from experimental studies with animals exposed to neutrons of similar energies and to fission neutrons (for reviews see UNSCEAR, 1982; ICRP, 1990, 1991; Engels & Wambersie, 1998).

4.2.1 *Modifying factors*

A characteristic property of neutrons is that their effects are modified considerably less by dose rate, dose fractionation, oxygenation and cell cycle stage than are the effects of low-LET radiations.

(*a*) *Dose rate and fractionation*

In the case of dose rate and fractionation, the difference between neutrons and low-LET radiation can be attributed to the difference in the capability of the exposed cells to repair the damage induced by the different radiation qualities. With increasing LET, the size of the shoulder on the survival curve decreases and the slope increases. The characteristic reappearance of a shoulder, which is observed with fractionated exposure to low-LET radiation, is either much less pronounced or absent with neutrons. The reduction in repair appears to become maximal as the LET approaches 100 keV μm^{-1}.

(*b*) *Effect of oxygen*

In general, cells and tissues are more radiosensitive when exposed to low-LET radiation in the presence of oxygen than under hypoxic conditions. The 'oxygen enhancement ratio' is the ratio of the doses required to produce a given level of a specific effect in the presence and absence of oxygen. The ratio for photons is in the range of 2.5–3.0. With increasing LET values above 60 keV μm^{-1}, the oxygen enhancement ratio for survival of human kidney cells decreases until it becomes 1 at LETs of about 180 keV μm^{-1} and higher. It was the low oxygen enhancement ratio that encouraged use of neutrons in cancer therapy (Field & Hornsey, 1979).

(*c*) *Cell cycle*

Radiosensitivity varies with the age of a cell, with maximum resistance to cell killing late in S phase. The variation in radiosensitivity is less for neutrons than for low-LET radiation. In synchronized Chinese hamster cells exposed to neutrons, the D_0 for S-phase cells was about 25% higher than that for cells in G_1, whereas with X-radiation the difference was nearly 90% (Sinclair, 1968). In clonogenic cells of the jejunal crypt, the variation in cell survival throughout the cycle was about 30% greater with γ-radiation than with 50-MeV neutrons (Withers *et al.*, 1974).

4.2.2 *Effects in normal tissues*

There is considerable sparing of tissues after exposure to low-LET radiation because they can recover from sublethal damage; markedly less sparing is seen with exposure to neutrons. Since exposure frequently involves a number of relatively small dose fractions, the RBE for damage to tissues may be relatively high. Furthermore, slow repair may occur in slowly dividing or late-responding tissues after low-LET but not after high-LET radiation. Table 17 shows the RBE_m values (see section 1.2), calculated on the basis of the linear–quadratic model, for a number of representative end-points in tissues. The RBE_m values are higher and the α:β ratios are lower for the late-responding tissues than for the early-responding tissues, which have more rapid cell renewal. In reviewing their experience of the radiosensitivity of tissues in patients undergoing neutron radiotherapy, Laramore and Austin-Seymour (1992) stressed the steepness of the dose–response curves for the induction of damage to normal tissue, which renders the therapeutic window rather narrow.

(*a*) *Skin*

The responses of mouse skin to high- and low-LET radiations are qualitatively similar, as are the time courses of the effects. The influence of the neutron energy is reflected in the RBE, which is about 7–8 for 2–3-MeV neutrons and about 3–5 for 15–25-MeV neutrons (Denekamp *et al.*, 1984). The RBE for late effects (3.2–3.4) is greater than that for early effects in pig skin exposed to γ-rays or 50-MeV (Be) neutrons (Withers *et al*., 1977).

(*b*) *Gastrointestinal tract*

(i) *Oesophagus*

Death within 8–40 days due to either obstruction or perforation of the oesophagus can occur in mice exposed to high doses of neutrons to the thorax. Geraci *et al*. (1976) reported an RBE of 1.9 for 8-MeV neutrons generated by bombarding a beryllium source with 22-MeV deuterons. Phillips *et al*. (1974) obtained an RBE of 4 with 15-MeV monoenergetic neutrons.

(ii) *Small intestine*

The murine crypt microcolony assay (Withers & Elkind, 1970) has been used to determine the RBE of single and fractionated doses of neutrons. Gueulette *et al.* (1996) reported RBE values for single doses of fast neutrons used for therapy at seven facilities in five countries, determined from the doses of each neutron source that resulted in 20 crypt microcolonies per circumference of the small intestine relative to the dose of ^{60}Co γ-radiation that caused the same effect. The RBEs were 1.5–2.2. Withers *et al*. (1993) reported RBE values in mice of 3.2–4.6 for neutrons produced by cyclotrons with deuteron energies of 16, 22, 35 and 50 MeV. Composite survival curves for crypt clonogenic cells after exposure to single doses were constructed from

Table 17. Maximum values of relative biological effectiveness (RBE_m) for tissue damage induced by fast neutrons and α:β ratios of the dose–response relationships for reference radiation (X- or γ-rays)

Tissue	End-point	Species	Neutron energy (MeV)	α:β (Gy) photons	RBE_m	Reference
Skin	Moist desquamation	Human	7.5	10.0	4.5	Field & Hornsey (1979)
Haematopoeitic system	LD_{50} at 30 days	Mouse	14.0	5.0	2.0	Broerse & Barendsen (1973)
Respiratory system	LD_{50} at > 30 days	Mouse	7.5	3.0	6.8	Field & Hornsey (1979)
Central nervous system	Late effects	Rat	14.0	3.0	7.2	Van der Kogel (1985)
Kidney	Late effects	Mouse	7.5	2.2	8.6	Joiner & Johns (1988)

data obtained with multiple fractions, and RBEs were calculated from the ratio of the α values for each neutron energy and γ-radiation. These ratios were considered to be RBE_m values. The RBE increased with decreasing neutron energy, which is consistent with the results of other studies (Hall *et al.*, 1979; see ICRP, 1990).

(*c*) *Haematopoietic system*

The effects of neutrons and comparisons of their effectiveness with that of low-LET radiation have been determined from survival curves for progenitor cells, such as colony forming cells in the haematopoietic system, or from dose–response relationships for lethality expressed as LD_{50} at 30 days. Broerse *et al*. (1978) determined an RBE of about 2.0 for the occurrence of bone-marrow syndrome in rhesus monkeys exposed to fission neutrons. In studies of the effects of neutrons and mixed-field radiation in large animals in the 1950s and 1960s (see Alpen, 1991 for review), the RBE for fast neutrons, based on the LD_{50} at 30 days in dogs and goats, was about 1.0. In contrast, the RBEs for lethality in small rodents were about 2.0–2.5. Two factors are important: the characteristic effects of radiation are less affected by body mass in small animals than in large animals, and the RBE of neutrons for lethality is based on damage to the gut in rodents whereas the bone-marrow syndrome predominates in large animals such as dogs.

Accidental exposures and the atomic bombing of Hiroshima exposed humans to a mixture of fission neutrons and γ-radiation. The effect of mixed radiation on the haematopoietic syndrome has been studied in dogs (MacVittie *et al*., 1991) in which the RBE for the LD_{50} at 30 days was about 1.7 on the basis of midline doses of ^{60}Co γ-radiation relative to mixed neutron and γ-radiation, the neutrons having an average energy of 0.85 MeV. The RBE based on the D_0 for granulocyte–macrophage colony-forming cells harvested from rib and pelvic bone-marrow aspirates 24 h after exposure of the dogs was reported to be about 2.

In a study of the survival of canine bone-marrow progenitor cells after exposure *in vitro* to ^{60}Co γ-radiation and fission neutrons (mean energy, 0.85 MeV), the D_0 values were about 77 cGy and 28 cGy, respectively, giving an RBE of about 2.8. The higher RBE of fission neutrons is consistent with neutron energy-dependence and with the RBE values of 1–2 reported for higher neutron energies. The RBE values for effects on the haematopoietic system are generally lower than those for solid tissues, which is consistent with the relatively small amounts of sublethal damage and repair in bone-marrow cells exposed to γ-radiation. The D_0 of the survival curve after γ-irradiation of bone-marrow progenitor cells isolated from dogs exposed *in vivo* to 7.0 cGy of γ-rays per day for 500 or 1000 days was reported to be significantly higher (2–2.5-fold) than that of cells from unirradiated dogs, whereas the increase in radioresistance to neutrons was much smaller. The mechanism of the acquired resistance is not known (Seed & Kaspar, 1991).

The determination of RBEs in deep tissues of large animals, including humans, requires accurate estimation of the doses of neutrons and of the reference radiation at

the target tissue. In the experiment of MacVittie *et al.* (1991), the neutron:γ-radiation ratio was 5.4:1 in air but 1.7:1 at midline. The absorbed dose to the bone marrow and the resultant change in the neutron:γ ratio are not known. Inhomogeneity of the dose to the bone marrow is a confounding factor.

The effects of single and fractionated doses and low dose rates of fission neutrons on the survival of colony-forming units in the bone marrow were studied in $B6CF_1$ mice. The RBE was 2.6 for inactivation by a single dose but somewhat higher for fractionated doses. When mice were exposed to 0.96 Gy of neutrons or 2.47 Gy of γ-radiation in nine fractions, the populations of colony-forming units in femur cells had not returned to control levels by three months, but this sustained depression of progenitor cells contrasted with the number of circulating leukocytes, which was maintained at a normal level by some compensatory mechanism (Ainsworth *et al*., 1989).

(*d*) *Central nervous system*

The brain is considered to be relatively radioresistant, but damage to normal tissue has been a limiting factor in the treatment of brain tumours with neutron radiotherapy. In a small number of patients treated with 15.6 Gy of 16-MeV neutrons, severe injury and progressive dementia occurred. When the contaminating γ-radiation dose was included, the total dose was about 17.6 Gy. Damage to the vasculature was thought to account for lesions in normal brain tissue. A neutron dose of about 13 Gy can cause changes such as cerebral oedema (UNSCEAR, 1982).

Van der Kogel *et al*. (1982) described a so-called early type of damage that takes about five to six months to develop after exposure to low-LET radiation. The target is the glial cells responsible for myelinization. Late injury to the vasculature develops within two to five years after single doses of low-LET radiation. Similar lesions and particularly the earlier type of damage occur after neutron irradiation. RBE_m values of about 5–7 have been estimated for 7.5-MeV and 14-MeV neutrons (Van der Kogel, 1985), and values of 6–10 were determined for degeneration of the white matter (White & Hornsey, 1980; Hornsey *et al*., 1981).

(*e*) *Reproductive system*

The effects of neutrons on the testis and in particular on the survival of type B spermatogonia, a highly radiosensitive cell type, have been reported. D_0 values of about 28 cGy for γ-radiation and 4–9 cGy for neutrons were observed (Hornsey *et al*., 1977). Loss of testicular weight as a function of the dose of high-LET radiation and the survival of various types of spermatogonia have been used to assess the effects of neutrons (for references to individual studies see UNSCEAR, 1982; ICRP, 1990).

The effectiveness of 1-MeV, 2.3-MeV and 5.6-MeV fast neutrons in killing type B spermatogonia in mice was determined by scoring the number of preleptotene spermatocytes 48 h after the start of irradiation, because the surviving type B spermatogonia would have developed to this stage at that time. A decrease in the number of sperma-

tocytes was considered to indicate accurately the loss of spermatogonia to the spermatogenesis process. D_0 values were determined from the loss of spermatogonia as a function of neutron and X-radiation dose. The survival curves were exponential. The RBE_m values were 5.7 for fission neutrons of 1.0 MeV mean energy and 4.6 and 3.0 for the 2.5-MeV and 5.6-MeV neutrons, respectively (Gasinska *et al*., 1987).

(*f*) *Renal system*

Stewart *et al*. (1984) used local irradiation of the kidney in mice to determine RBE values for changes in urine output, isotope clearance and haematocrit induced by single and multiple fractions of 3-MeV neutrons. The repair capacity of the kidney was very limited: the RBE for a single dose of about 6 Gy was approximately 2.4 and increased to 4.5–5.1 with eight fractions of about 1 Gy of neutron radiation.

(*g*) *Respiratory system*

Damage to the lung induced by neutron radiation occurs both early, described as pneumonitis, and late after exposure, in the form of fibrosis. In contrast to most other tissues, the lung does not show significant differences in the RBE values for early and late effects. The RBE values based on the LD_{50} 60–180 days after exposure of mice to 7.5-MeV neutrons were reported to be 1.5 after single doses and about 3.4 after 15 fractions (Hornsey *et al*., 1975). Parkins *et al*. (1985) studied the effects of irradiation of the mouse thorax with up to 20 fractions of 3-MeV neutrons or 240-kVp X-radiation on relative breathing rate and found an RBE_m value of about 7.

(*h*) *Ocular lens*

The effects of neutrons on the lens in humans and experimental animals were reviewed by Medvedovsky and Worgul (1991), who reported that neutron-induced changes in the lens are indistinguishable from those produced by low-LET radiation, but neutrons are quantitatively more effective, the incidence being higher and the latent period shorter per unit dose. Reduction of the dose rate has little or no influence on the effectiveness of neutrons to induce cataracts.

The induction and development of lens opacities depend on how much of the total volume of the lens is irradiated and on the dose, the age at exposure and the radiation quality. The effectiveness of neutrons depends on their energy, the most effective energy being ≤ 1 MeV. The induction of various types of lenticular lesions has been used to assess the effect of radiation for the purposes of radiation protection, in order to prevent the induction of cataracts.

(i) *Cataracts in humans*

Some of the physicists involved in testing the cyclotron developed cataracts (Abelson & Kruger, 1949). Although the doses were not measured precisely, it was estimated that the lens opacities occurred as a result of exposure to < 1 Gy of mixed γ- and neutron radiation. If this estimate is correct, the threshold dose was one-half to

one-fifth that estimated for γ-radiation alone, which would indicate an appreciable RBE for the neutron component.

Data on the induction of cataracts in humans by high-LET radiation come from two sources. Roth *et al*. (1976) reported on the incidence of cataracts in patients treated with 7.5-MeV neutrons and found slight, permanent loss of vision in patients exposed to a total dose of 2.2 Gy in 12 fractions, with an RBE estimated to be about 2.5. The second source of information on the effects of neutrons on the eye is studies of the survivors of the atomic bombings, who have been examined for over three decades. The estimated threshold doses to the eye were reported to be 0.06 Gy (95% CI, 0–0.16) of neutrons and 0.73 Gy (95% CI, 0–1.39) of γ-rays, and the RBE was calculated to be approximately 32 (95% CI, 12–89) (Otake & Schull, 1990). Concern has been raised about errors in the dosimetry for this population in general but about the neutron component of the radiation released during the nuclear explosion over Hiroshima in particular (see section 1.3.3). The risk for cataract per unit dose was studied in persons who reported epilation after the atomic bombing and in those with no epilation, in two studies. The authors of one study attributed the difference between the two groups of survivors to a 48% random error in the dose estimates (Neriishi *et al.*, 1995), while the others concluded that it was not possible to decide whether the differences in the frequency of cataracts was due to differences in individual radiosensitivity or to random errors in the dose estimate (Otake *et al*., 1996). Because of these uncertainties, the data for experimental animals are important.

(ii) *Cataracts in experimental animals*

Bateman *et al*. (1972) reported high RBE values for radiation-induced lens opacities in mice on the basis of the presence of flecks and other minor changes, which also occurred in unirradiated mice but at later ages. Neutrons thus shortened the latency to the appearance of these lesions. Data for 430-keV neutrons suggested that the relationship of the RBE to the neutron dose (D_n) in grays could be described as:

$$RBE = 4\sqrt{1+1.5/D_n}\ .$$

At the lowest dose, the RBE was about 100.

Di Paola *et al*. (1978), using similar techniques, obtained RBE values of 9–21 with decreasing doses of 14-MeV neutrons from 0.38 to 0.01 Gy.

Despite differences in the methods of scoring lenticular opacities, Worgul *et al*. (1996) noted a reasonable degree of agreement in the results for neutron-induced cataracts in most species. They suggested that the RBE for cataractogenesis increases from < 10 at doses ≥ 1 Gy to > 100 at doses ≤ 10 mGy. The commonly used RBE of 20 is not consistent with their results for very low doses, because at a neutron dose of 2 mGy the RBE could be estimated to exceed 250. There is no evidence that the RBE for clinically significant cataracts in humans reaches such high values.

It has become possible to detect very small radiation-induced lesions in the lens, and the estimates of threshold dose have become thresholds of detection. For the purposes of radiation protection, it is the threshold dose for clinically significant opacities (some loss

of vision) that is important. Fortunately, the treatment of cataracts has become so effective that the impact of radiation-induced cataracts has been reduced greatly. The experimental data for the induction of lenticular lesions by radiation are some of the best available for examining the relationship between RBE and dose and for testing the validity and consistency of models of the action of radiation. Lesions in the ocular lens can be assessed quantitatively at much lower doses of radiation than is the case for most, if not all, other tissues.

4.3 Radiation-sensitivity disorders

High-LET ionizing radiation kills mammalian cells more efficiently per unit dose than does X-radiation or γ-radiation (Cox *et al*., 1977a,b; Barendsen, 1985; Goodhead, 1988). Studies of the relationship between the RBE of various forms of radiation and energy deposition in cells can provide additional insight into the mechanisms of the early events in carcinogenesis, such as DNA damage and mutations. It is of interest, therefore, to consider the response to neutron radiation of cells in persons with syndromes such as ataxia telangiectasia, who are known to be sensitive to X-radiation and γ-radiation.

Hypersensitivity to low-LET ionizing radiation is a common characteristic of cells from patients with the chromosomal breakage syndrome ataxia telangiectasia (Taylor *et al*., 1975; Chen *et al*., 1978; Cox *et al*., 1978; see the monograph on 'X-radiation and γ-radiation', section 4.3.1). Cells from such patients have also been reported to be more sensitive than control cells to high-LET radiation, but the difference in sensitivity decreased as the LET of the radiation increased (Cox, 1982). Other characteristics of cells from patients with this syndrome include reduced inhibition of DNA synthesis after exposure to γ-radiation (Edwards & Taylor, 1980; Houldsworth & Lavin, 1980; Ford & Lavin, 1981) or to X-radiation (Painter & Young, 1980; De Wit *et al*., 1981) and greater and more prolonged accumulation of cells in the G_2 phase of the cell cycle after irradiation (Imray & Kidson, 1983; Ford *et al*., 1984; Bates & Lavin, 1989).

Exposure of control lymphoblastoid cell lines and cell lines from patients with ataxia telangiectasia to neutrons of a mean energy of 1.7 MeV affects cell survival and the incorporation of [^{3}H]thymidine into DNA. In addition, neutrons influence the progression of cells through the cell cycle. While high-LET radiation was considerably more effective in killing cells from the patients than from controls, the relative sensitivity of the two cell types was variable in the case of low-LET radiation. While fibroblasts from patients with ataxia telangiectasia were hypersensitive to X-radiation and γ-radiation, their radiosensitivity to α-particles was comparable to that of control cells (Lücke-Huhle *et al*., 1982). In a later study, Lücke-Huhle (1994) failed to observe increased killing by densely ionizing α-particles of cells from these patients when compared with control cells, indicating that the RBE for inactivation of cells from patients with ataxia telangiectasia is much less dependent on ionization density than that of control cells, for which it reaches a maximum of approximately 4 at a LET

value of 100 keV μm^{-1} (Cox *et al*., 1977a,b). In fibroblasts from these patients, the maximum RBE was ≤ 2 at 100 keV μm^{-1} (Cox, 1982). These data suggest that the lesions induced in DNA by high-LET radiation are inefficiently repaired in both cell types and the two can be distinguished only on the basis of DNA damage induced by low-LET radiation, which is readily repairable in controls. In a study with two lymphoblastoid cell lines from patients with ataxia telangiectasia, fast neutrons (mean energy, 1.7 MeV) were considerably more effective than γ-rays in inducing cell death. Fast neutrons inhibited DNA synthesis to the same extent in cells from patients with this syndrome as in those from controls (radioresistant DNA synthesis), but the long-term delay in G_2/M phase was greater in the cells from the patients, as was observed after γ-irradiation (Bates & Lavin, 1989). Thus, a correlation between G_2/M delay and cell killing was seen in these lymphoblastoid cells, regardless of the LET value of the radiation (Houldsworth *et al*., 1991); this was not the case with fibroblasts from these patients (Lücke-Huhle *et al*., 1982).

In keeping with the data on the survival of fibroblasts, marked differences in the rejoining kinetics of γ-radiation-induced double-strand breaks in DNA were found between control cells and those from patients with ataxia telangiectasia, but similar kinetics of rejoining of these breaks was observed after exposure to ^{241}Am α-particles (Coquerelle *et al*., 1987). When the production of micronuclei was determined in lymphocytes from such patients after irradiation, the increase over that in control cells was less pronounced after exposure to neutrons than after exposure to γ-rays (Vral *et al*., 1996).

4.4 Genetic and related effects

4.4.1 *Humans*

Chromosomal aberrations were examined in lymphocytes from eight men aged 24–56 who were exposed during a criticality accident to mixed γ-radiation and fission neutrons at doses estimated to range from 0.23 to 3.65 Gy. The neutrons contributed about 26% of the total dose. Five of the men received doses that were estimated to exceed 2.3 Gy, and the three others received lower doses. The blood samples were drawn about 2.5 years after the irradiation; blood from five unirradiated subjects was used as a control. Only chromatid-type aberrations were found in the controls. In the subjects exposed to the higher doses, the frequency of aneuploid cells was 7–23%, and gross aberrations, such as rings, dicentrics and minutes, were found in 2–20% of the cells. The men who received doses of 0.23–0.69 Gy also had abnormalities but at a much lower frequency (Bender & Gooch, 1962). Analysis of blood samples from the same persons 3.5 years after exposure showed that they still had chromosomal aberrations but in most cases at a somewhat lower frequency (Bender & Gooch, 1963).

Chromosomal aberrations in peripheral blood cells were scored in a study of 17 patients who received tumour therapy with 14-meV neutrons at a rate of about

0.2 Gy min^{-1} with a distance of 80 cm between the source and the skin. Treatment consisted either of daily doses of 0.65–0.80 Gy or of 12 exposures of 1.3 Gy in three fractions per week. The doses of contaminating γ-rays were 5–15% depending on the field size and the depth of the tumour. The intercellular distribution of dicentric chromosomes showed predominantly overdispersion. A positive correlation was found for dicentrics with a total skin dose of 0.8–15.6 Gy, and for total chromosome-type damage (dicentrics, centric rings and excess acentrics). The authors concluded that there was a significant correlation with therapeutic dose, despite the complex influences of biological and physical factors on the aberration yield (Schmid *et al.*, 1980).

[The reports summarized below became available after the meeting of the Working Group, although members of the Group were aware of the existence of some of these publications. In view of their importance for the evaluation, they are included in the monograph for completeness.

[The men studied by Bender and Gooch (1962, 1963) were further examined 7 (Goh, 1968), 8 and 10.5 (Goh, 1975) and 16 and 17 years (Littlefield & Joiner, 1978) after the accident. At 16–17 years, six of the men still had residual chromosomal aberrations; three men who had received the high doses had the highest frequency, and the two who had been exposed to the highest dose had around 10% aberrant cells.

[In a criticality accident in 1965 in Mol, Belgium, a man received doses to the bone marrow estimated to be 500 cGy of γ-radiation and 50 cGy of neutrons. Only 24 mitoses good enough for analysis were obtained. The aberrations included deletions, translocations, dicentrics and rings; some cells had two or even three dicentrics. On the basis of results available at the time on cells exposed *in vitro*, the total dose (mean homogeneous equivalent dose) corresponding in effect to low-LET radiation was estimated to be 470–500 cGy, in good agreement with the physical estimates (Jammet *et al*., 1980).

[An accident in Vinca, Yugoslavia, in 1958 resulted in the exposure of six persons to neutrons and γ-radiation. More than 50% of the dose was estimated to be neutrons, and the doses were estimated to be 165–227 cGy of neutrons and 158–209 cGy of γ-rays. Five years after the accident, the frequency of structural aberrations in the peripheral lymphocytes was 8–28% (Pendic & Djordjevic, 1968). Nineteen years after the accident, the frequency of aberrations in four men had declined somewhat to 10–22% (Pendic *et al*., 1980).

[The persistence of chromosomal aberrations in patients who received fractionated neutron therapy (average bone-marrow dose, < 100 to > 1000 cGy) to tumours located at various sites was evaluated recently (Littlefield *et al.*, 2000). Neutron-induced dicentrics and rings disappeared from the peripheral circulation within the first three years after exposure, while translocations persisted for more than 17 years.]

4.4.2 *Experimental systems*

(*a*) *Mutations* in vivo

(i) *Germ-cell mutations*

Visible dominant mutations: In mice, the spontaneous rate for visible dominant mutations is approximately 8×10^{-6} per gamete per generation. Exposure to fission neutrons (mean energy, 0.7 MeV) gave rise to a spermatogonial mutation rate of 25.5×10^{-5} per gamete per Gy (Batchelor *et al*., 1966).

Dominant lethal mutations: When male mice were exposed to fission neutrons four to five weeks before mating with untreated females (postgonial stage), the rate of dominant lethal mutations was approximately 25×10^{-2} per gamete per Gy (Grahn *et al*., 1979). When males were irradiated in the stem-cell stage, no effect of dose rate was observed after single or weekly exposures to neutrons, both of which gave a dominant lethal mutation rate of 40×10^{-3} per gamete per Gy (Grahn *et al*., 1979).

Experimental evidence of the nature of radiosensitive targets in immature (resting) mouse oocytes led to new experimental designs that permitted measurement of radiation-induced genetic damage in these cells. Such damage has been detected after exposure to monoenergetic 0.43-MeV neutrons, and the genetic sensitivity of the immature oocytes has been compared with that of maturing oocytes. Recoil protons from 0.43-MeV neutrons produce short ionization tracks (mean, 2.6 μm) and can therefore deposit energy in the DNA without simultaneously traversing and damaging the hypersensitive plasma membrane. With these neutrons, dose–response relationships were obtained for both chromosomal aberrations and dominant lethal mutations in oocytes from females irradiated 8–12 weeks earlier, when the oocytes were immature. The intrinsic mutational sensitivity of immature mouse oocytes appeared to be similar to that of maturing oocytes (Straume *et al*., 1991).

Recessive visible mutations: In male mice, irradiation of post-spermatogonial stages with neutrons at doses of up to 1 Gy resulted in recessive visible mutation rates of $100–150 \times 10^{-6}$ per locus per Gy, with no effect of dose rate (Russell, 1965). In female mice, a rate of 145×10^{-6} per locus per Gy was reported for this type of mutation after single doses of fission neutrons (0.3, 0.6 and 1.2 Gy) (Russell, 1972).

Specific locus mutations: One system for studying mutation induction in mice comprises a series of 12 genes, most of which affect coat colour, six or seven of which are usually tested as a group (Cattanach, 1971). Neutrons show an inverse dose-rate effect, low dose rates of high doses being much more effective. In contrast to spermatogonia, oocytes are difficult to analyse for mutations (Batchelor *et al*., 1969). A complicating factor is the time of conception after irradiation: with neutrons at low dose rates, mutations could be recovered in litters conceived within seven weeks of irradiation, but later litters had no mutations (Russell 1967).

Comparison of the effects of high-LET and low-LET radiation: Male $B6CF_1$ mice were exposed to once-weekly doses of either fission neutrons or ^{60}Co γ-radiation for up to one year and mated periodically to screen for the induction of dominant lethal

mutations. The doses of neutrons were 0.0013–0.027 Gy week^{-1} and those of γ-radiation were 0.05–0.32 Gy week^{-1}. Data on both pre- and postimplantation fetal deaths were obtained. Age- and time-dependent factors made no consistent, significant contribution to the mutation rate; such factors could include changes in radiosensitivity and in spontaneous rates and any cumulative damage to the stem-cell population. Direct comparison of these data with data for males exposed to single doses confirmed that weekly neutron irradiation was significantly more effective than single doses in inducing postimplantation fetal losses, whereas single doses of γ-rays were more effective than the same dose divided into weekly fractions. The RBE of neutrons increased from 5 to 12 for single and weekly doses. The rates of preimplantation loss, although significant, were not considered to be a sensitive measure of genetic injury at the low doses used (Grahn *et al*., 1986).

Young adult male B6CF$_1$ mice were exposed to single whole-body doses of fission neutrons or ^{60}Co γ-radiation. Post-spermatogonial dominant lethal mutations, the incidence of reciprocal chromosomal translocations in spermatogonia, the incidence of abnormal epididymal sperm four to six weeks after exposure, and testicular weight loss three to six weeks after exposure were measured. The responses to neutron doses of 0.01–0.4 Gy and γ-radiation doses of 0.23–1.45 Gy were analysed in detail, although more limited data from a fourfold higher dose range were integrated into the analysis. Significant effects were seen at 0.01 and 0.025 Gy of neutrons, consistent with extrapolation from higher doses, with the exception of dominant lethal mutations, which occurred in significant excess of expectation. The dose–response relationships were linear or linear–quadratic, depending on the end-point, radiation quality and dose range. For translocation frequencies, the D^2 term in the linear–quadratic dose–response function (see section 5, Overall introduction) was negative for neutron and positive for γ-ray irradiations. The RBE values for testicular weight loss and abnormal sperm were between 5 and 6 over the full dose range and were between 7 and 9 at lower doses (< 0.1 Gy) for translocations. The RBE values for postimplantation loss and total dominant lethal rates were 5–6 at doses > 0.1 Gy and 10–14 at doses < 0.1 Gy. The values for preimplantation loss were between 15 and 25 at doses > 0.1 Gy and possibly higher < 0.1 Gy. The authors suggested that the unusual results at the lower doses may be explained by variation in cell sensitivity, cell selection, probability of neutron traversal per cell, variance of magnitude of the energy deposition events, dose rate and DNA repair (Grahn *et al*., 1984).

Male mice heterozygous for the Rb(11.13)4Bnr translocation were irradiated for 14.5 min with either 0.15 Gy of fission neutrons or 0.6 Gy of X-rays. These mice are known to show high levels of spontaneous autosomal non-disjunction (20–30%) after anaphase I. The effects of the irradiation on this process were determined in air-dried preparations of primary and secondary spermatocytes. The induced effects were studied at intervals of 2 and 3 h after the start of the irradiation and assessed by scoring: univalents in primary spermatocytes; deletions, aneuploid chromosome counts and precocious centromere separation in secondary spermatocytes; and chromatid gaps and

breaks in both cell types. The two types of radiation induced comparable levels of chromosomal damage. The RBE value for neutrons relative to X-rays was calculated to be 5.4 for the meiosis I stage and 3.3 for the meiosis II stage. According to the authors, the significantly higher incidence of cells showing damage at meiosis II than at diakinesis/meiosis I does not indicate a difference in radiation sensitivity, but is the consequence of the different chromosomal processes taking place during the time between irradiation and fixation (Nijhoff & de Boer, 1980).

(ii) *Somatic mutations*

Hprt: Mutation induction was measured at the *Hprt* locus in splenic lymphocytes of $B6CF_1$ mice 56 days after whole-body irradiation with fission-spectrum neutrons. Lymphocytes were cultured for 12–16 days in the presence of 5×10^4 feeder cells (syngeneic lymphocytes irradiated with 50 Gy γ-radiation). Animals were exposed to either single doses of neutrons (1.5 Gy) or fractionated doses delivered over two weeks (0.25 Gy × 6; total, 1.5 Gy). The frequency of *Hprt* mutant induction by the single 1.5-Gy dose was $5.98 \pm 1.51 \times 10^{-5}$ (SE). Multiple doses of neutrons (total, 1.5 Gy) gave rise to a mutation frequency of $8.71 \pm 5.39 \times 10^{-5}$ (SE) (Kataoka *et al*., 1993).

Oncogenes: Point mutations at codon 12 of the K-*Ras* oncogene were analysed by an 'enriched' polymerase chain reaction method in 25-year-old paraffin-embedded samples of normal lung tissue and lung adenocarcinoma tissue from mice that had been exposed to radiation. Significantly more K-*Ras* codon-12 mutations (100%) were observed in normal lung tissue from mice exposed 24 times to once-weekly neutron radiation than in normal lung tissue from sham-irradiated mice (50%; $p < 0.05$). Lung adenocarcinomas from these irradiated mice also had a significantly higher frequency of point mutations in codon 12 of K-*Ras* than lung adenocarcinomas from mice exposed to γ-radiation once a week for 24 or 60 weeks (50%), but the higher frequency was not significantly different from that in spontaneous lung adenocarcinomas from mice (75%; $p > 0.05$). Sequencing of two of the mutants revealed a K-*Ras* 13(Asp) point mutation (Zhang & Woloschak, 1998). [The Working Group noted that it cannot be concluded that the codon-12 mutations were induced by the radiation or arose in clones initially transformed by the radiation.]

N-*Ras* mutations were examined in DNA samples extracted from the spleens of CBA/Ca mice that had developed myeloid leukaemia after exposure to radiations of various qualities. Seventeen cases of myeloid leukaemia comprising five cases of neutron-induced and 12 cases of photon (three γ-radiation and nine X-radiation)-induced myeloid leukaemia were included, with 12 DNA samples from the bone-marrow cells of control mice. Mobility shifts revealed by polymerase chain reaction and single-strand conformational polymorphism indicated mutations only in exon II of the N-*Ras* gene. Such mutations were more prevalent in samples from mice exposed to fast neutrons. Silent point mutations, i.e. base transitions at the third base of codons 57, 62 or 70, were present only in mice that had developed myeloid leukaemia after

exposure to fast neutrons. The higher frequency of N-*Ras* mutations in neutron-induced myeloid leukaemia suggested that fast neutrons are more effective in inducing genomic instability at the N-*Ras* region of the genome. More importantly, N-*Ras* mutations appear not to be the initiating event in radiation leukaemogenesis. This conclusion was supported by the finding of N-*ras* mutations only in mice with an overt leukaemic phenotype and not in animals with minimal tissue infiltration of leukaemic cells, suggesting that the disease may be present before the N-*Ras* mutations (Rithidech *et al.*, 1996).

A protocol was developed to induce thymic lymphomas in RF/J mice efficiently by a single acute dose of neutron radiation. Activated *Ras* genes were detected in 4 of 24 of the tumours analysed. One of the tumours contained a K-*Ras* gene activated by a point mutation in codon 146. Activating *Ras* mutations at position 146 have not previously been detected in any known human or animal tumour. The spectrum of *Ras* mutations detected in neutron radiation-induced thymic lymphomas was different from that seen in thymic lymphomas induced by γ-radiation in the same strain of mice (Sloan *et al*., 1990). A novel K-*ras* mutation in codon 146 was also found in thymic lymphomas induced by neutrons (Corominas *et al*., 1991).

(iii) *Cytogenetic effects*

Sister chromatid exchanges were scored in bone-marrow cells from three-month-old rats as a function of time after exposure to 2 Gy of whole-body radiation with 1-MeV fission neutrons. This dose reduced the mean survival time to 445 days after irradiation and induced more than one tumour per animal; by 200 days after irradiation, all of the animals bore tumours at autopsy, but the bone-marrow was not a significant target for tumour induction. In controls, the mean number of sister chromatid exchanges per cell remained constant from 3 to 24 months of age (2.38 per cell; SD, 0.21), but irradiation induced two distinct increases in the frequency: the first occurred during the days following exposure and the second between days 150 and 240. Thereafter, the values levelled off at 3.37 per cell (SD, 0.39) until day 650. Between the two increases (i.e. days 15–150), the number of sister chromatid exchanges dropped to control values. Analysis of the distribution per cell showed that the changes were not confined to a particular cell population. These results suggest that, in irradiated rats, the second increase in sister chromatid exchange coincides with tumour growth, whereas the first increase may be due to DNA damage that is rapidly repaired (Poncy *et al*., 1988).

A modified mouse splenocyte culture system was standardized and used to evaluate the induction of micronuclei and chromosomal aberrations for the purposes of biological dosimetry after exposure to X-radiation and fission neutrons *in vivo* and/or *in vitro*. After irradiation with 1-MeV fission neutrons *in vivo* and culturing of mouse splenocytes, linear dose–response curves were obtained for the induction of micronuclei and chromosomal aberrations. The lethal effects of neutrons were shown to be significantly greater than those of a similar dose of X-radiation. The RBE was 6–8 in

a dose range of 0.25–3 Gy for radiation-induced asymmetrical exchanges (dicentrics and rings) and about 8 for micronuclei in a dose range of 0.25–2 Gy (Darroudi *et al*., 1992).

The induction of reciprocal translocations in rhesus monkey stem-cell spermatogonia was studied by analysing primary spermatocytes at metaphase. The animals were exposed to 1 Gy of γ-radiation at dose rates of 140 or 0.2 mGy min^{-1} or to 0.25 Gy of 2-MeV neutrons at 36 mGy min^{-1}. Reduction of the dose rate from 140 to 0.2 mGy min^{-1} did not lower the frequency of recovered translocations from 0.43% induced by the γ-radiation. The RBE for neutrons in relation to X-radiation was 2.1, which is clearly lower than the value of 4 obtained for mice (Van Buul, 1989).

(*b*) *Cellular systems*

(i) *DNA damage*

Radiolysis of water results in numerous products; the most reactive and the most damaging to DNA is the •OH radical. This radical either abstracts •H from deoxyribose and bases or reacts with the bases of all nucleotides. Consequential to these reactions, conformational changes occur in DNA, which lead to the generation of lesions. These lesions include single- and double-strand breaks and modifications of deoxyribose and bases (some of these are alkali-labile sites that are revealed as single-strand breaks after alkaline treatment), intrastrand and interstrand cross-links and DNA–protein cross-links (Burns & Sims, 1981). The RBE of neutrons (in relation to γ-radiation) for generation of these lesions is often higher than 2.5, but there is no qualitative difference in the results of exposure to these types of radiation.

Irradiation of pBR322 plasmid DNA in solution with neutrons or γ-radiation resulted in half the yield of single-strand breaks and a 1.5-times higher yield of double-strand breaks with neutrons as compared with γ-rays (Spotheim-Maurizot *et al.*, 1990, 1996). Scavenging of •OH radicals with ethanol inhibited all neutron-induced single-strand breaks but only 85% of the double-strand breaks, whereas with γ-irradiation the formation of both single- and double-strand breaks was completely inhibited. The results suggest at least three different origins for neutron-induced double-strand breaks. The occurrence of around 30% of these breaks can be explained by a radical transfer mechanism, as proposed by Siddiqi and Bothe (1987), for γ-radiation. In this model, a radical site is transferred from a sugar moiety of the cleaved strand to the complementary intact strand, which occurs with a probability of about 6%. Around 55% of neutron-induced double-strand breaks may be due to the non-random distribution of radicals in high-density tracks of the secondary particles of neutrons, which results in a simultaneous attack of the two strands by •OH radicals. The first two processes are both •OH-mediated and are therefore sensitive to ethanol. The direct effect of fast neutrons and their secondaries (recoil protons, α-particles and recoil nuclei) can account for the remaining 15% of double-strand breaks, which are not inhibited by scavengers (Spotheim-Maurizot *et al.*, 1990). Consistent with this

view, Pogozelski *et al*. (1999) found that the decrease in yields of strand breaks in plasmid pBR322 with increasing •OH scavenging capacities was not as pronounced for fission neutrons as for γ-rays. In contrast, damage to restriction fragments or oligodeoxyribonucleotides induced by fission neutrons can be almost completely suppressed by thiols (Savoye *et al.*, 1997; Swenberg *et al.*, 1997).

In an 80-base-pair DNA fragment exposed to fast neutrons, the probability of strand breakage at a given nucleotide site was not determined by the nature of the nucleotide but by its flanking sequence. The sequence-dependence is due to variations in the accessibility of the H4′ and H5′ atoms. Fitting the experimental results with the calculated reaction probabilities suggested that a C4′-centred radical develops into a strand break three times more efficiently than a C5′-centred radical, and that half of the breaks occur via the 4′ path and half via the 5′ path (Sy *et al.*, 1997).

DNA lesions induced by fast neutrons in L5178Y mouse lymphoma cells were classified into three types on the basis of their repair profiles: rapidly repaired breaks (half-time, 3–5 min), slowly repaired breaks (70 min) and unrepairable breaks. The rates of repair of the first two types of break were almost the same as those of corresponding damage induced by low-LET radiation. Neutrons induced less rapidly repaired damage, a nearly equal amount of slowly repaired damage and more unrepairable damage when compared with equal doses of γ-radiation or X-radiation (Sakai *et al*., 1987).

The induction and repair of breaks was studied by alkaline elution (Kohn & Grimek-Ewig, 1973) of DNA from Chinese hamster V79 and human P3 epithelial teratocarcinoma cells after exposure to fission-spectrum neutrons (mean energy, 0.85 MeV) and ^{60}Co γ-radiation in the biological dose range. The fission-spectrum neutrons induced fewer direct single-strand breaks per gray of absorbed dose than γ-radiation (Peak *et al.*, 1989). Measurements of cell survival had already indicated incomplete recovery of the cells after exposure to neutrons (Hill *et al.*, 1988). Whereas most single-strand breaks caused by exposure to fission-spectrum neutrons can be rapidly repaired by both hamster and human cell lines, a small but statistically significant fraction (about 10%) of the single-strand breaks induced by exposure to 6 Gy of neutrons was refractory to repair. In contrast, all measurable single-strand DNA breaks induced by 3 Gy of γ-radiation were rapidly repaired (Peak *et al.*, 1989).

Neutron irradiation has been reported to cause single-strand breaks, with RBEs varying from 0.3 to nearly 2 in assays with various cellular and extracellular systems and neutron energies (see, e.g. Van der Schans *et al.*, 1983; Prise *et al.*, 1987; Vaughan *et al.*, 1991). The RBEs for double-strand break induction by neutrons are usually about 1, although higher values have been reported. The breaks differ from those induced by γ-rays mainly in the fact that they are less readily repaired, as described below.

Monolayers of L-929 mouse fibroblasts were irradiated with fast neutrons or 250-kVp X-rays and treated simultaneously with dinitrophenol to prevent the DNA strands from rejoining; single-strand breaks induced in DNA were measured by the alkaline

sucrose sedimentation method. The RBE for single-strand breaks was about 1.6, which is essentially the same as that measured from cell survival (Moss *et al.*, 1976).

The effects on cellular viability and the kinetics of induction and repair of DNA strand breaks in HeLa cells were examined after exposure to a thermal neutron beam and compared with those after γ-irradiation. The survival curve had no initial shoulder. The RBEs of the neutron radiation were 2.2 for cell killing (ratio of D_0 values), 1.8 and 0.9 for single-strand breaks measured by alkaline sedimentation and alkaline elution, respectively, and 2.6 for double-strand breaks, determined by neutral elution (Bradley & Kohn, 1979). No difference was observed between thermal neutrons and γ-rays in respect of the repair kinetics of single- and double-strand breaks. It was suggested that the effect of the intracellular nuclear reaction, $^{14}N(n,p)^{14}C$, is mainly responsible for the high RBE values observed (Maki *et al*., 1986).

The effects of 2.3-MeV (mean energy) neutrons and 250-kVp X-rays on cell survival and DNA double-strand break induction and repair (measured by neutral elution) were investigated in Chinese hamster V79 cells. The lethal effects of neutrons were shown to be significantly greater than those of a similar dose of X-rays (RBE, 3.55 at 10% survival), but the RBE for double-strand break induction, in a dose range of 10–50 Gy, was 1. Radiation-dependent differences were found in the pattern of repair. A fast and a slow repair component were seen in both cases, but the former was reduced after neutron irradiation. Since the amount of slow repair was similar in the two cases, proportionally more unrejoined breaks were seen after exposure to neutrons. The results were similar when the elutions were conducted at pH 9.6 and pH 7.2 (Fox & McNally, 1988).

DNA double-strand break induction and rejoining, measured by field-inversion gel electrophoresis, were compared by cell survival in mutant (XR-V15B) and wild-type parental (V79B) hamster cell lines after low-dose neutron and X-irradiation. Neutrons did not induce more double-strand breaks than X-rays. Even with low doses of neutrons, a visible increase was found in the formation of a smaller subset of DNA fragments, which arise only after very high doses of X-rays. In both cell lines, double-strand breaks induced by neutrons were rejoined more slowly than those induced by X-radiation. At long repair times (4 and 17 h), there were no significant differences between neutrons and X-rays in the fractions of unrejoined double-strand breaks. The authors proposed that neutron-induced double-strand breaks have a higher probability of becoming lethal because they are more likely to be misrepaired during the slow stage of rejoining (Kysela *et al*., 1993).

Irradiation of viable CHO AA8 cells on ice with 4–25 Gy of either ^{60}Co γ-radiation or d(20 MeV)Be neutrons (mean energy, 7.5 MeV) produced similar resistance to rewinding of nuclear DNA supercoils after treatment with ethidium bromide. The recovery from the effects of 12 Gy of either radiation was also similar, leaving no detectable residual damage. The discrepancy between these data and the reduced ability of neutrons to produce DNA breaks, as defined by the alkaline elution assay, is explained by the discontinuous deposition of energy associated with neutron irra-

diation. A microdosimetric analysis suggested that neutron radiation interacts with DNA at sites that are on average 5–10 times further apart than those that interact with γ-radiation. The long DNA sequences that result from neutron irradiation are consequently eluted inefficiently during alkaline elution, giving a reported RBE of approximately 0.3. Restrictions in the rewinding of individual supercoils are not dependent on the inter-ionization distance and thus give rise to an RBE of approximately 1. Furthermore, the complete removal of DNA damage, as measured by this technique, supports the hypothesis that the toxicity of neutrons is associated with incorrect, not incomplete, rejoining of the DNA molecule (Vaughan *et al.*, 1991).

The relative sensitivity of Chinese hamster ovary cells to fast neutrons and γ-rays was studied with a panel of mutants characterized by defects in the nucleotide excision repair pathway. These could be further subdivided into mutants that were defective in nucleotide excision repair alone, in base excision repair alone, in DNA-dependent protein kinase-mediated DNA double-strand break repair or in the distinct but overlapping pathway for the repair of DNA cross-links. None of the mutants defective in nucleotide excision repair showed different sensitivities to fast neutrons and γ-radiation. In contrast, deficiency in the base excision repair pathway resulted in significant primary sensitization to both types of radiation (2.0-fold to γ-radiation and 1.8-fold to neutrons). Deficiency in the double-strand break repair pathway mediated by DNA-protein kinase resulted in marked but again similar primary sensitization to γ-radiation (4.2-fold) and neutrons (5.1-fold). Thus, none of the repair pathways examined showed a preferential role in the repair of damage induced by low-LET and intermediate-LET radiations; this resulted in an essentially consistent RBE of approximately 2 in the cell lines studied (Britten & Murray, 1997).

(ii) *Chromosomal aberrations*

Many studies have been performed of radiation-induced chromosomal aberrations in mammalian cells—often human lymphocytes. Comparisons of the effects of radiation have often been based on the number of dicentric chromosomes induced, although premature chromosome condensation is also an end-point for comparison. The RBEs of neutron irradiation have been determined for dicentrics or for dicentrics plus centric rings in human lymphocytes isolated from peripheral blood exposed to neutrons with different energies (Table 18). Analysis of dicentrics revealed RBE values of 5, 6 and 14 for neutrons of mean energy 21, 14 and 6.5 MeV, respectively, produced on a beryllium target [^{9}Be(d,n)^{10}B] (Fabry *et al.*, 1985).

The yield of chromatid-type aberrations induced by either fission neutrons or X-radiation can be potentiated by post-irradiation treatment with hydroxyurea and caffeine when the cells are irradiated in G_2; however, the frequencies of neutron-induced chromatid-type aberrations are not potentiated by treatment with cytosine arabinoside, except at the highest dose used. In contrast, chromatid aberrations induced by X-radiation were strongly potentiated by cytosine arabinoside. These results indicate that

Table 18. Relative biological effectiveness (RBE) of neutrons for chromosome-type dicentrics (or dicentrics plus centric rings) induced in human peripheral lymphocytes irradiated *in vitro* (reference radiation, ^{60}Co γ-rays; constant dose rate, 0.5 Gy min^{-1}; Lloyd *et al*., 1975)

Source	Neutron energy (MeV)	Absorbed dose rate (Gy min^{-1})	Sampling time	RBE for 2.0–0.02 aberrations per cell	RBE_m	Reference
d, T						
Japan	~ 14.1	–	–	1.2–5.9[a]	14.5	Sasaki (1971)
Germany	~ 15.0 (γ < 4%)	0.12	48 h	1.1–3.6	9.0	Bauchinger *et al*. (1975)
Glasgow, Scotland	~ 14.7 (γ ~ 7.5%)	0.30	48 h	1.7–6.6	16.7	Lloyd *et al*. (1976)
Harwell, England	~ 14.9 (γ ~ 3%)	0.25	48 h (O_2)	2.2–6.6	16.2	Prosser & Stimpson (1981)
			(N_2)	1.2–2.1	4.3	
^{3}H(α,n)^{4}He						
Russian Federation (NG–150M)	14.7 (γ < 10%)	0.36–1.85	50–52 h	1.7–3.8	9.0	Sevan'kaev *et al*. (1979a,b)
d, Be						
Harwell, England (VEC)	~ 20	~ 0.50	52–72 h (with BrdU)	1.4–11.3	29.2	Barjaktarovic & Savage (1980)
Hammersmith, England (cyclotron)	~ 7.6 (γ < 10%)	0.30	48 h	2.1–11.9	30.4	Lloyd *et al*. (1976)
Louvain, Belgium (cyclotron)	~ 6.2 (γ low)	0.05	48–53 h	1.0–8.3	21.5	Biola *et al*. (1974)
Japan	~ 2.03	–	–	2.2–17.4[a]	43.3	Sasaki (1971)
Li/Be						
Russian Federation	~ 0.04 (γ < 7%)	0.01	50–52 h	2.4–6.8	16.5	Sevan'kaev *et al*. (1979a,b)
(KG-2.5 accelerator)	~ 0.09 (γ < 4%)	0.03		1.1–10.8	28.0	

Table 18 (contd)

Source	Neutron energy (MeV)	Absorbed dose rate (Gy min^{-1})	Sampling time	RBE for 2.0–0.02 aberrations per cell	RBE_m	Reference
Fission						
France (CEA/Crac)	Max, ~ 10 (γ very high + thermal)	–	46–53 h (data corrected for γ)	2.8–22.3	57.4	Biola *et al*. (1974)
France (CEN/Triton)	Max ~ 10 (γ ~ 30–50%)	0.03–0.07	46–53 (data corrected for γ)	2.7–21.6	55.7	Biola *et al*. (1974)
France (CEN/Harmonie)	Max ~ 1.5 (γ ~ 5%)	0.12	46–53 h	2.0–16.1	41.3	Biola *et al*. (1974)
Sofia, Bulgaria (IRT-2000)	Max ~ 3	–	52 h	0.8–6.5	16.9	Todorov *et al*. (1973)
Aldermaston, England	~ 0.9 (γ < 10%)	0.03	48 h	2.2–18.0	46.4	Lloyd *et al*. (1976)
Argonne, USA (JANUS)	~ 0.85 (γ ~ 3%)	0.06	48–50 h	2.3–18.3[a]	45.6	Carrano (1975)
Russian Federation (BR-10)	~ 0.85 (γ < 5%)	0.06–2.6	50–52 h	2.8–19.9	51.1	Sevan'kaev *et al*. (1979a,b)
Harwell, England (BEPO)	~ 0.7 (γ ~ 10%)	0.50	48 h	2.6–20.6	53.2	Lloyd *et al*. (1976)
Harwell, England (BEPO)	~ 0.7 (γ ~ 10%)	0.50	48–56 h	2.6–21	54.1	Scott *et al*. (1969)
Harwell, England (GIEEP)	~ 0.7 (γ ~ 15%)	0.0005 0.0011	48–46 h	2.5–20.4 3.1–25.2	52.2 65.0	Scott *et al*. (1969)
Italy (TAPIRO)	~ 0.4 (γ ~ 10%)	0.002–0.07	48 h	2.6–22.2	57.1	Vulpis *et al*. (1978)
Russian Federation (BR-10)	~ 0.35 (γ < 5%)	0.04–0.4	50–52 h	4.1–32.6	83.9	Sevan'kaev *et al*. (1979a,b)
Russian Federation (BR-10)	Thermal (γ < 5%)	0.005	50–52 h	1.3–20.6	53.3	Sevan'kaev *et al*. (1979a,b)

Table 18 (contd)

Source	Neutron energy (MeV)	Absorbed dose rate (Gy min^{-1})	Sampling time	RBE for 2.0–0.02 aberrations per cell	RBE_m	Reference
National Radiological Protection Board (^{252}Cf)	~ 2.13 MeV	0.12–0.17	48 h	1.8–14.8	38.2	Lloyd *et al.* (1978)

Adapted from Savage (1982)
[a] Dicentrics plus centric rings

neutrons produce a smaller proportion of lesions, the repair of which can be inhibited by this compound, than X-radiation (Antoccia *et al*., 1992).

Several radiosensitive Chinese hamster cell lines have been studied to explore the relationship between radiation-induced DNA lesions and chromosomal aberrations. The frequency of radiation-induced aberrations in *Xrs* mutants, which are deficient in double-strand break repair, was higher than in control cells. In a radiosensitive hamster cell line (V-C4), which has no detectable defect in double-strand break repair, the frequencies of X-radiation-induced aberrations are higher than those found in wild-type V79 cells. After treatment with fission neutrons, however, the frequency of aberrations is similar to that in V79 cells, indicating that V-C4 cells are defective in repair of X-radiation-induced lesions other than double-strand breaks. Apparently, these other lesions may also lead to aberrations (Natarajan *et al.*, 1993).

Chromosomal aberrations were scored in BHK21 C13 Syrian hamster fibroblasts exposed in stationary phase to ^{60}Co γ-rays, 250-kV X-rays, 15-MeV neutrons or neutrons of a mean energy of 2.1 MeV produced from the ^{9}Be(d,n)^{10}B reaction. No detectable difference was seen in the responses to ^{60}Co γ-rays and 250-kV X-rays. The RBE for the production of dicentrics, based on the 'one hit' component of the response, was 5 ± 2 for the 15-MeV neutrons and 12 ± 5 for the 2.1-MeV neutrons (Roberts & Holt, 1985).

Micronucleus formation induced by neutrons has been studied in a number of cell types, including human blood lymphocytes and two-cell mouse embryos exposed in late G_2 phase (Molls *et al*., 1981; Mill *et al.*, 1996; Vral *et al.*, 1996).

There is now substantial evidence that ionizing radiation can induce genomic instability in the form of chromosomal aberrations which appear several cell generations after irradiation. When the progeny of neutron-irradiated human epithelial MCF-10A cells were examined for chromosomal aberrations 5–40 population doublings after irradiation, an increase in the frequency of chromatid-type gaps and breaks was observed, but no such effect was observed for chromosome-type aberrations. Neutron-irradiated cells showed consistently increased frequencies of aberrations when compared with unirradiated control cells at all times examined, indicating that neutrons can cause chromosomal instability (Ponnaiya *et al*., 1997).

(iii) *Interchromosomal versus intrachromosomal aberrations*

Many attempts have been made to identify specific biomarkers of radiation as the causal agent of biological effects in cells and tissues. The search has included the examination of chromosomal aberrations for what has been termed a chromosomal 'fingerprint' that would indicate the type of radiation responsible for the aberration. Brenner and Sachs (1994) observed that high-LET radiation, in particular α-particles or fission neutrons, produces a remarkably low ratio of interchromosomal to intrachromosomal aberrations, which is two to three times lower than the ratio recorded after X- or γ-irradiation. The authors proposed use of this ratio as a fingerprint for exposure to high-LET radiation.

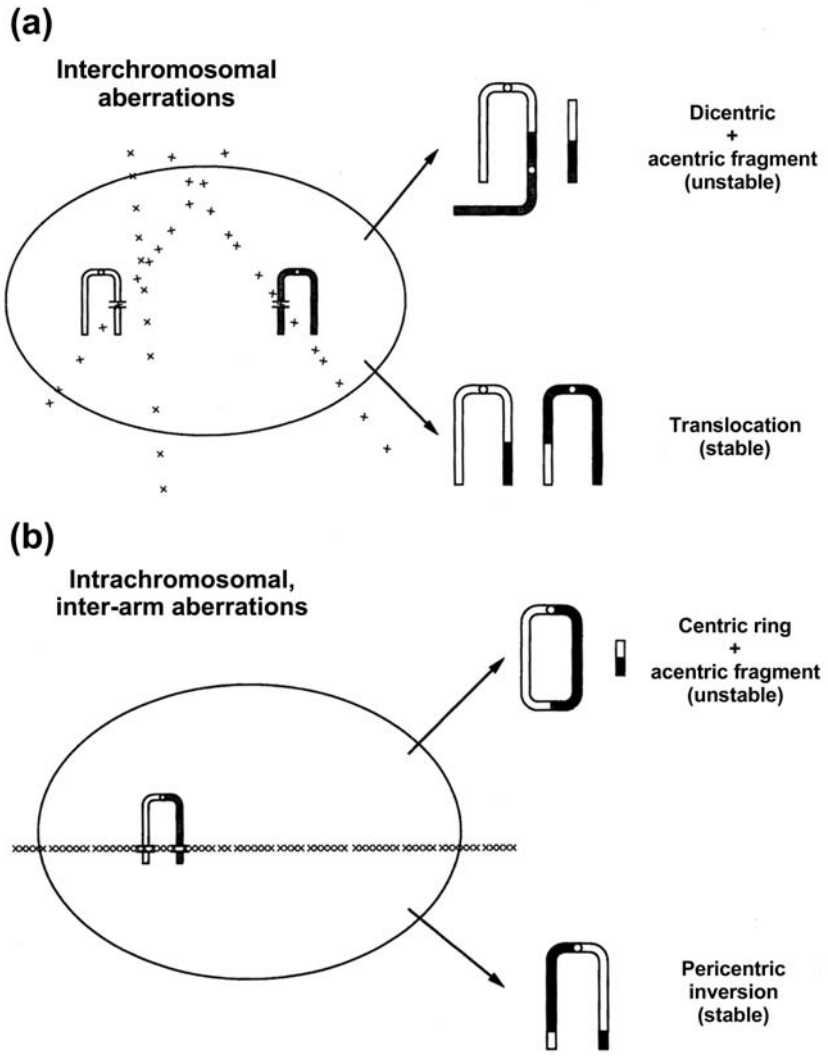
The two types of aberration are illustrated in Figure 5. Exchange-type chromosomal aberrations are interchromosomal if the DNA double-strand breaks that are the initial cause of the lesion occur on different chromosomes. If the double-strand breaks are on different arms of the same chromosome, the lesion is intrachromosomal. If the double-strand breaks were random and all the double-strand breaks were equally likely to interact with one another, the ratio *F* of the interchromosomal to intrachromosomal aberrations would be 90, assuming that all chromosome arms were of equal length. Since chromosome arms are not of equal length and there is an increased probability of interaction between double-strand breaks that are close together, the *F* value is lower and is indicative of lesions induced by low-LET radiations, such as X- and γ-radiation. High-LET radiations, which are densely ionizing because of the inhomogeneity of the energy deposition, induce double-strand breaks that are even closer than those produced by X- or γ-radiation, increasing the yield of intrachromosomal aberrations and resulting in a smaller *F* value. On the basis of many reports of the induction of chromosomal aberrations in humans and other experimental data, it was suggested that the *F* value for densely ionizing radiation was about 6 and that this was significantly lower than the values for X- and γ-radiation and for chemical clastogenic agents. If valid, this approach for determining *F* ratios would have potential use in epidemiological studies, such as those on atomic bomb survivors and persons exposed to radon, in establishing the type of radiation involved (Brenner & Sachs, 1994).

Other authors have both supported (Sasaki *et al*., 1998) and disputed (Bauchinger & Schmid, 1997, 1998) this hypothesis. The report of a workshop set up to examine the use of *F* values concluded that: (1) there was some evidence to suggest that ratios of different chromosomal aberrations might be used as a biomarker of exposure to high-LET radiations; (2) there are large interlaboratory differences in *F* values for the same type of radiation; (3) despite these variations, *F* values do not depend on dose or LET at doses above 1 Gy; (4) further studies are required to establish if *F* values can be used to identify a causal relationship between the observed chromosomal aberrations and specific exposure to radiation. It was suggested that the ratio of intrachromosomal intra-arm to interchromosomal aberrations (designated the *H* ratio) should be examined as a possible fingerprint of exposure to high-LET neutrons (Nakamura *et al*., 1998).

(iv) *Gene mutations*

Since mutation of a given gene is a relatively rare event, the majority of systems for studying radiation-induced mutations involve placing an irradiated cell population under selective pressure so that only the mutant cells are able to survive and can be enumerated. Mutation of genes in a hemizygous (single copy) or heterozygous (two copies but only one active) state is usually studied, to enable measurement. Commonly used mutation systems are based on the loss of enzyme activity, e.g. the enzyme HPRT, which renders cells resistant to the drug 6-thioguanine, the enzyme TK, which confers resistance to trifluorothymidine and the enzyme APRT which confers resistance to

Figure 5. Interchromosomal and intrachromosomal, inter-arm aberrations resulting from ionizing radiations of different quality



Adapted from Brenner & Sachs (1994)
Each cross represents an ionization cluster of sufficient localization and multiplicity to produce a double-strand DNA break. Panel **(a)** shows interchromosomal aberrations resulting, in the case shown here, from two independent, sparsely ionizing radiation tracks. This aberration could also result from two double-strand breaks caused by a single radiation track. Panel **(b)** shows intrachromosomal, inter-arm aberrations resulting, in the case shown here, from a single, densely ionizing radiation track.

8-azaadenine and 2-aminopurine (see section 4.4.2 in the monograph on X- and γ-radiation). The *Hprt* gene is located on the X chromosome, while the *Tk* and *Aprt* genes are on autosomes and must therefore be used in a hemi- or heterozygous state.

The effects of the dose rate of high-LET radiation on mouse L5178Y cells were reported (Nakamura & Sawada, 1988) after exposure to ^{252}Cf (2.13-MeV neutrons). At the high dose rate of 1.2 cGy min^{-1}, ^{252}Cf irradiation produced a linear induction of *Hprt* mutants at relatively low doses but showed reduced effectiveness at higher doses. At the lower dose rate of 0.16 cGy min^{-1}, the initial slope for mutant induction (9×10^{-7} per cGy) was approximately the same as that at the higher dose rate, but the induction curve did not appear to 'turn over' at higher doses. Dose-dependent values for the RBE of high-LET radiation in excess of 10 were found for the low-dose rate in a comparison of neutrons from ^{252}Cf with ^{60}Co γ-radiation.

Human B-lymphoblastoid TK6 cells were used to examine the effectiveness of 4.2-MeV (^{230}Pu, Be) neutrons at dose rates of 0.00014–0.04 cGy min^{-1} for up to 20 days. Neutrons at dose rates < 0.0014 cGy min^{-1} were more effective at inducing mutants than were higher dose rates. The RBE of these low dose rates, relative to 100-kV X-rays, can be calculated to be about 10. When TK6 cells were exposed to beams ranging in atomic number from ^{20}Ne to ^{40}Ar over an energy range of 330–670 MeV per atomic mass unit (amu), mutation induction was evaluated for both the *TK* and the *HPRT* loci for a subset of these beams. The results obtained with the ^{20}Ne ions of 425 MeV per amu (LET, 32 keV μm^{-1}) and ^{28}Si ions of 670 MeV per amu (LET, 50 keV μm^{-1}) closely resembled those obtained after brief exposure to (^{230}Pu, Be) neutrons. Alterations in DNA structure within the *TK* locus of mutants induced by neutrons and by ^{40}Ar ions were similar and were dominated by allele loss. Multi-locus deletions inclusive of the c-*erb*A1 locus were common among the *TK*-deficient mutants induced by these densely ionizing radiations (Kronenberg & Little, 1989; Kronenberg, 1991).

A system involving human–hamster hybrid cells was developed by Waldren *et al*. (1979) from a stable hybrid containing the Chinese hamster genome and one copy of the human chromosome 11. The loss of several markers on this chromosome can be determined, and even complete loss of the chromosome is not lethal. This system has been used to measure mutant frequencies after irradiation with neutrons of various energies (0.33–14 MeV), at doses up to 200 cGy. Significant increases in mutant frequency were found at doses as low as 10 cGy, and dose-dependent RBEs of up to 30—for the 0.33-MeV neutrons—were calculated in comparison with ^{137}Cs γ-radiation (Hei *et al*., 1988).

Fast neutrons produced by proton bombardment of a beryllium target in a cyclotron were used to examine the energy dependence of the induction of mutants at the *Hprt* and *Tk* loci in V79 Chinese hamster cells. The beams of neutrons were produced from protons with 46, 30, 20 and 14 MeV of energy. Gradually increasing cytotoxic and mutagenic effects of the neutrons were noted as the energy decreased. The frequency of induced mutants at the *Tk* gene was higher than at the *Hprt* gene. In a human epithelium teratocarcinoma cell line (P3), the mutation frequency at the *HPRT*

locus, as in V79 cells, increased 2.5–4 fold with decreasing neutron energy (Zhu & Hill, 1994; Sharma & Hill, 1996).

A 1-Gy fission neutron dose from a ^{252}Cf source induced a maximal *Hprt* mutation frequency in synchronized L5178Y mouse lymphoma cells when delivered immediately after release from G_2/M block, whereas the maximal response to ^{60}Co γ-radiation was found in G_1 (Tauchi *et al*., 1993).

The biological effectiveness for mutation induction at the *Hprt* locus in confluent cultures of mouse m5S cells exposed to fission neutrons from ^{252}Cf, relative to γ-radiation, was increased from 4.9 to 7.4 when the dose rate was reduced from 1.8 to 0.12 cGy min^{-1}. The changes in RBE were due mainly to a reduction in the effect of γ-radiation. The authors noted that their observations contrast with reports of proliferating cell cultures and suggested that they could be ascribed to the cell growth conditions used in their experiments (Komatsu *et al*., 1993).

The toxic and mutagenic effects of X-rays and neutrons were compared in the Chinese hamster ovary cell line K1-BH4 and its transformant, AS52, which lacks the normal *Hprt* gene but instead contains a single autosomally integrated copy of the bacterial equivalent, the *gpt* gene. X-radiation and neutrons appeared to be equitoxic in the two cell lines, but both were 10 times more mutagenic to the *gpt* gene in AS52 cells than to the *Hprt* gene of K1-BH4 cells. The apparent hypermutability of AS52 cells probably results from better recovery of multi-locus deletion mutants in AS52 cells than in K1-BH4 cells, rather than a higher yield of induced mutants (Hsie *et al*., 1990).

Chinese hamster ovary cells were exposed to thermal neutrons, and the mutation frequency at the *Hprt* locus was determined. The Kyoto University Research Reactor, which produces thermal neutrons with a very low level of contaminating γ-rays and fast neutrons, was used as the source of radiation. The cells were irradiated in the presence or absence of boric acid. Thermal neutron irradiation was 2.5 times as mutagenic as γ-radiation without boron. In the presence of boron, however, thermal neutron radiation was 4.2–4.5 times as mutagenic as γ-radiation. When the mutation frequency was plotted against the surviving fraction, greater mutagenicity was observed in the presence than in the absence of boron, suggesting that the enhancement of thermal neutron-induced mutation with boron is strongly associated with α-particles released by the ^{10}B(n,α)^{7}Li reaction (Kinashi *et al*., 1997).

(v) *Cell transformation*

Ionizing radiation of low LET is an effective inducer of cell transformation in various systems (see section 4.4.2 in the monograph on X- and γ-radiation). A large number of studies have also been conducted with neutrons, which are even more effective than X- or γ-rays. The RBE values relative to X- or γ-radiation depend on the energy of the neutrons. Miller *et al*. (1989) examined the effect of low absorbed doses of monoenergetic neutrons with energies of 0.23–13.7 MeV on transformation in asynchronous mouse C3H10T1/2 cells. The dose–response curves were linear or

nearly linear for the various neutron energies and curvilinear for the reference X-rays. The RBE values were found to decrease with increasing dose for both cell transformation and survival. The maximal values varied from 13 for 5.9-MeV neutrons to 35 for 0.35-MeV neutrons. Rather lower RBE values were reported in a study with less pure neutron sources (Balcer-Kubiczek & Harrison, 1983): the maximum observed RBE for reactor fission neutrons (with 8–20% γ-ray component) was 3.8, and that for cyclotron neutrons (8% γ-ray component) was 1.2. A subsequent study on fission neutrons at various dose rates gave an RBE for cell transformation of 3 at a high dose rate (0.1 Gy min^{-1}) and 10 at the lowest dose rate studied (0.005 Gy min^{-1}) (Balcer-Kubiczek *et al.*, 1988). In mouse mS5 cells, ^{252}Cf neutrons showed RBE values for cell transformation of 3.3–5.1, depending on the dose rate (1.8–0.12 cGy min^{-1}) (Komatsu *et al.*, 1993).

The claim of Hill *et al.* (1984a,b) that neutron-induced transformation in the C3H10T1/2 system was enhanced by a factor of about 9 at low dose rates triggered much work on dose rates and dose fractionation with respect to the so-called 'inverse dose rate' problem. The effect was confirmed in the same system by several authors (see e.g. Miller *et al.*, 1990) and flatly denied by others (Balcer-Kubiczek *et al.*, 1988, 1991; Saran *et al.*, 1991; Balcer-Kubiczek *et al.*, 1994; Saran *et al.*, 1994). Syrian hamster embryo cells were also reported to show the effect (Jones *et al.*, 1989), and an inverse dose-rate effect of 2.9 was reported for the human hybrid system (HeLa × skin fibroblasts), with fission neutrons of an average energy of 0.85 MeV (Redpath *et al.*, 1990); however, no effect was found in confluent cultures of mouse m5S cells (Komatsu *et al.*, 1993). Several authors reported that the effect is specific to particular sources or energies of neutrons (Elkind, 1991; Miller & Hall, 1991), and there is still some confusion in the area (Masuda, 1994; Brenner *et al.*, 1996). Explanations of the inverse dose-rate effect have involved cell proliferation during irradiation and the postulated existence of a hypersensitive 'window' in the cell cycle (Elkind, 1991).

In experiments with synchronized mouse C3H10T1/2 cells, Miller *et al.* (1995) found that the G_1 phase of the cell cycle (4–6 h after mitotic 'shake-off') was the most sensitive to neutron-induced oncogenic transformation, in contrast to what has been observed with X-radiation where the peak was 14–16 h after 'shake-off', reflecting mostly G_2 cells. Less variation in the response during the cell cycle was seen for neutrons than for X-rays (Redpath e*t al.*, 1995; Pazzaglia *et al.*, 1996).

It is not clear what molecular changes induced by neutrons are responsible for cell transformation. In 5.9-MeV neutron-transformed foci of C3H10T1/2 cells, chromosomal aberrations have been found, but there were no N-*ras* or K-*ras* mutations (Freyer *et al.*, 1996), and it was reported that human keratinocytes transformed by neutrons do not contain mutations in either *RAS* or *p53* (Thraves *et al.*, 1994).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to neutrons normally occurs from a mixed irradiation field in which neutrons are a minor component. The exceptions are exposure of patients to neutron radiotherapy beams and exposures of aircraft passengers and crew. In high-altitude cities, neutrons can constitute as much as 25% of cosmic background radiation. A measure of the societal burden is the annual neutron collective dose per year^{-1}. Those values would be 4.6×10^5 person–Sv year^{-1} for the world population exposed at ground level, 350 person–Sv year^{-1} for nuclear workers and 7500 person–Sv year^{-1} for the passengers and crews of aircraft. The individual average lifetime effective dose of neutrons has been estimated to be 6 mSv for the world population exposed at ground level and 30 mSv for aircrews. The maximal lifetime doses of neutrons are estimated to be 68 mSv for the population of the high-altitude city of La Paz, Bolivia, 46 mSv for long-haul pilots and up to 130 mSv for the small proportion of nuclear workers exposed to neutrons.

5.2 Human carcinogenicity data

There are no epidemiological data adequate to evaluate whether neutrons are carcinogenic to humans.

5.3 Animal carcinogenicity data

Neutrons have been tested at various doses and dose rates with wide ranges of mean energy from various sources (reactors, ^{252}Cf, ^{235}U) for carcinogenicity in mice, rats, rabbits, dogs and rhesus monkeys. Fission-spectrum neutrons were used in most of these studies. Neutrons were also tested for carcinogenicity in mice exposed prenatally and in mice after male parental exposure.

In adult animals, the incidences of leukaemia and of ovarian, mammary, lung and liver tumours were increased in a dose-related manner, although the incidence often decreased at high doses. While a γ-ray component was present in the exposure in most studies, it was generally small, and the carcinogenic effects observed could clearly be attributed to the neutrons. Prenatal and parental exposure of mice resulted in increased incidences of liver tumours in the offspring.

In general, there was no apparent reduction in tumour incidence after exposure to low doses at a low dose rate, but enhancement of tumour incidence was often observed with high doses at a 1ow dose rate. In virtually all studies, neutrons were more effective in inducing tumours than were X-radiation or γ-radiation when compared on the basis of absorbed dose.

5.4 Other relevant data

Neutrons are uncharged particles that are penetrating and interact with atomic nuclei, generating densely ionizing charged particles, such as protons, α-particles and nuclear fragments, and sparsely ionizing γ-radiation. The densely ionizing particles produce a spectrum of molecular damage that overlaps with that induced by sparsely ionizing radiation, but they are more effective in causing biological damage because they release more of their energy in clusters of ionizing events, giving rise to more severe local damage.

Comparison of the effects of neutrons with those of X- and γ-radiation is based on the assumption that the effects are the same qualitatively and differ only quantitatively. The assumption is reasonable with regard to deterministic effects because they are, in general, caused by cell killing. Neutrons are more effective than X- and γ-radiation in causing both early and late deterministic effects. The effectiveness of neutrons is dependent on their kinetic energy and decreases with increasing energy up to about 15 MeV. The effects of neutrons are much less dependent on dose rate, fractionation, cell cycle stage and oxygenation than those of X-radiation and γ-radiation. The relative biological effectiveness of neutrons for the induction of deterministic effects is greater than 1 but not as high as those estimated for induction of cancer in experimental animals. For single doses of 1–5-MeV fast neutrons, the relative biological effectiveness values range from 4 to 12, except in the haematopoietic system for which the values are 2–3. The relative biological effectiveness is higher for later-responding tissues than for early-responding tissues.

For individual cells also, neutron energy is an important factor in the stochastic effectiveness of neutrons. The ability of surviving cells to proliferate and increase cell populations does not appear to depend on the quality of radiation; however, because of the greater effectiveness of neutrons per unit dose, the surviving population is smaller and a longer time is required for the proliferation rate to recover. This may be critical in maintenance of the integrity of a tissue.

Cells from patients with ataxia telangiectasia are hypersensitive to cell killing and to induction of micronuclei by fast neutrons, although the degree of hypersensitivity is less pronounced than for sparsely ionizing radiation.

The spectrum of DNA damage from neutrons includes clustered damage of substantial complexity and consequently reduced repairability. Neutrons are comparable to X- and γ-radiation in producing double-strand breaks, but neutron-induced DNA lesions in mammalian cells are less readily repaired than those produced by sparsely ionizing radiation.

Neutrons are very efficient at inducing transformation in rodent and human cellular systems. The relative biological effectiveness of neutrons has been reported to vary from 3 to 35; whether (or under what conditions) the efficiency of neoplastic transformation is greater at low dose rates remains unclear.

Chromosomal aberrations (including rings, dicentrics and acentric fragments) were induced in the circulating lymphocytes of people exposed in an accident involving release of neutrons in a nuclear plant and in the lymphocytes of patients exposed during neutron therapy. In the former study, there was also an increase in the frequency of numerical aberrations. Within the limits of the studies, the effect was found to be dose-dependent.

Gene mutations and chromosomal aberrations are induced in mammalian cells many times more efficiently by neutrons than by the same absorbed dose of X- or γ-radiation. Fission neutrons have been shown to induce germ-line mutations in mice, including visible dominant mutations, dominant lethal mutations, visible recessive mutations and specific locus mutations. When compared with sparsely ionizing radiation on the basis of absorbed dose, fission neutrons are many-fold more effective. Neutrons have been shown to induce *Hprt* mutations in splenic lymphocytes of mice. Point mutations in K-*Ras* and N-*Ras* oncogenes were found in malignant tissue from mice exposed to neutrons, but the mutations cannot be directly ascribed to the exposure. Neutrons have been shown to induce sister chromatid exchange, dicentrics and rings in mice and reciprocal translocations in rhesus monkey stem-cell spermatogonia.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of neutrons.

There is *sufficient evidence* in experimental animals for the carcinogenicity of neutrons.

Overall evaluation

Neutrons are *carcinogenic to humans (Group 1)*.

In making the overall evaluation, the Working Group took into consideration the following:

- When interacting with biological material, fission neutrons generate protons, and the higher-energy neutrons used in therapy generate protons and α-particles. α-Particle-emitting radionuclides (e.g. radon) are known to be human carcinogens. The linear energy transfer of protons overlaps with that of the lower-energy electrons produced by γ-radiation. Neutron interactions also generate γ-radiation, which is a human carcinogen.
- Gross chromosomal aberrations (including rings, dicentrics and acentric fragments) and numerical chromosomal aberrations are induced in the lymphocytes of people exposed to neutrons.
- The spectrum of DNA damage induced by neutrons is similar to that induced by X-radiation but contains relatively more of the serious (i.e. less readily repairable) types.

- Every relevant biological effect of γ- or X-radiation that has been examined has been found to be induced by neutrons.
- Neutrons are several times more effective than X- and γ-radiation in inducing neoplastic cell transformation, mutation *in vitro*, germ-cell mutation *in vivo*, chromosomal aberrations *in vivo* and *in vitro* and cancer in experimental animals.

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