SOURCES AND EFFECTS OF IONIZING RADIATION

United Nations Scientific Committee on the Effects of Atomic Radiation

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ANNEX G

Biological effects at low radiation doses

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INTRODUCTION

1. Biological effects of ionizing radiation in humans, due to physical and chemical processes, occur immediately following the passage of radiation through living matter. These processes will involve successive changes at the molecular, cellular, tissue and whole organism levels. For acute whole-body exposures above a few gray from radiation of low linear energy transfer (LET), damage occurs principally as a result of cell killing. This can give rise to organ and tissue damage and, in extreme cases, death. These effects, termed early or deterministic, occur principally above a threshold dose that must be exceeded before they are manifested as clinical damage, although damage to individual cells will occur at lower doses. Protracted delivery of such high doses over several hours or days will usually result in effects of lower severity. Information on the early effects of radiation in humans was reviewed in the UNSCEAR 1993 and 1982 Reports [U3, U6].

2. A second type of damage can occur at late times after exposure. This damage consists primarily of damage to the nuclear material in the cell, causing radiation-induced cancer to develop in a proportion of exposed persons or hereditary disease in their descendants. Although the probability of both cancer and hereditary disease increases with radiation dose, it is generally considered that their severity does not. They are termed stochastic effects and were reviewed in the UNSCEAR 1977, 1988, and 1994 Reports [U2, U4, U7].

3. Direct information on radiation-induced cancer is available from epidemiological studies of a number of human populations. These include the survivors of the atomic bombings in Japan and groups that have been exposed to external radiation or to incorporated radionuclides, either for medical reasons or occupationally. Such studies provide quantitative information on the risk of cancer at intermediate to high doses and are reviewed in Annex I, "*Epidemiological evaluation of radiation-induced cancer*". At lower levels of exposure, however, quantitative estimates of risk are not so readily obtained, and inferences need to be made by downward extrapolation from the information available at higher doses.

4. In the case of radiation-induced hereditary disease, studies on human populations have not provided quantitative information, so risk estimates have to be based on the results of animal studies. There is again the difficulty that quantitative data are available only following exposures to intermediate to high doses. Information on radiation-induced hereditary disease has been reviewed previously by the Committee [U3, U4].

5. For the majority of situations in which human beings are exposed to ionizing radiation in the home, in the natural environment, and in many places of work, the principal concern is the consequence of exposure to low doses and low dose rates. For the purposes of radiation protection, the establishment of the expected incidence of cancer or hereditary disease following radiation exposure is presently based on the hypothesis that the frequency of their induction increases proportionally with radiation dose. A linear, no-threshold dose-response relationship has generally been adopted by national and international bodies for assessing the risks resulting from exposures to low doses of ionizing radiation (see, e.g. [I2, U4]). This hypothesis implies that the risk of cancer increases (linearly) with increasing exposure and that there is no threshold, i.e. no dose below which there is absolutely no risk. As yet no definitive experimental data are available on this issue (see Chapter IV).

6. Experimental and epidemiological data on which quantitative evaluations of the risk of cancer following exposure to low-LET radiation are based come principally from studies involving exposures at moderate to high doses and dose rates. Most organizations have extrapolated linearly and then applied a reduction factor to estimate risks at low doses and low dose rates. This reduction factor has been variously termed a dose and dose rate effectiveness factor (DDREF) [I2], a dose-rate effectiveness factor (DREF) [N1], a linear extrapolation overestimation factor (LEOF), and a low dose extrapolation factor (LDEF) [P1, P14]. The basis for the application of such a reduction factor was described in the UNSCEAR 1993 Report [U3]. For high-LET radiations, such as neutrons and alpha particles, no reduction factor has generally been applied, because the dose response for radiation-induced cancer and hereditary disease is essentially linear between the lowest dose at which effects have been observed and that at which cell killing becomes a factor in the dose response [I2, U3, U4].

7. There has been extensive debate as to the shape of the dose-response relationship below the range at which effects can be directly measured. It has been argued that irradiating cells and tissues with small radiation doses can result in an adaptive response that reduces the amount of damage caused by subsequent radiation exposure [U2, W6] or even results in a beneficial effect, termed hormesis [A9, T11, W13]. There have been suggestions that, at very low doses, radiation may have no effect at all; these suggestions are based on the proposition that there could be a threshold for a response, in the same way as there is for clinically observed deterministic effects. This situation may arise, for example, if damage to a number of cells is needed before any adverse effect occurs or if interaction between cells is a prerequisite for an effect [K19, M34]. An apparent threshold may also arise if the latent period between exposure and the appearance of a cancer exceeds the normal lifespan of the individual [R1, R14].

8. Several mechanistic models have been proposed to describe the effects of radiation at the different levels of biological organization. There has been considerable effort in developing such models to quantitatively describe cellular survival, repair and transformation, based on the stochastic (probabilistic) process of energy deposition in radiosensitive

targets representing elements of cell structure, or employing track structure concepts. Other models have concentrated on representing the processes of repair and misrepair of damaged cell structures. In general, a mechanistic model should, apart from quantitative description of available data, have a predictive capability and offer crucial tests of its validity. Some mechanistic models support the linear no-threshold expressions used to fit epidemiological data, while others point to power law dose-effect relationships, implying a zero initial slope. There have been suggestions that the limits of dose-based quantities have been reached and that fluence and an action cross section are more appropriate concepts for assessing damage to cells. No quantitative attempt has, however, been made to apply these concepts in radiological protection [S5].

9. It has been recognized by the Committee for some time that information is needed on the extent to which both total dose and dose rate influence the induction of cancer and hereditary disease. A number of considerations are important in determining the risks of exposures to radiation at low doses and low dose rates. These include (a) careful analysis of epidemiological studies to determine the lowest doses at which effects are statistically evident, (b) examination of the shape of the dose-response relationships in the low-dose region using available experimental and epidemiological data, and (c) assessment of the possibilities for extrapolation to lower levels of dose based on an understanding of the mechanisms involved in the radiation response of tissues. Extrapolation based on mechanistic considerations can, in principle, be made using information on relevant biological factors such as cellular/molecular targets for tumour initiation, the nature of radiation-induced damage to deoxyribonucleic acid (DNA) and the fidelity of its repair, together with information on adaptive responses and cellular surveillance. Many of these factors were discussed in the UNSCEAR 1993 and 1994 Reports [U2, U3].

10. The objective of this Annex is to examine the sources of data that are available for assessing the risks of radiation-induced cancer and hereditary disease at low doses for both sparsely ionizing (low-LET) and densely ionizing (high-LET) radiation and their associated uncertainties. This Annex brings together information reviewed by the Committee in separate specialized Annexes, material from previous UNSCEAR reports, and additional data from dosimetric and cellular studies, epidemiological investigations, recent advances in molecular biology, and developments in mechanistic models. The aim is to provide an overview of the data available on the relationship between radiation exposure and the induction of cancer and hereditary disease, with emphasis on the extent to which radiation effects can be observed at low doses. This information, coupled with knowledge on the mechanisms of damage to cells and tissues, provides a basis for informed judgements to be made about the likely form of the dose response at exposures below those at which direct information is available.

11. Dose-response relationships for radiation effects in cellular systems are reviewed in Chapter I. Considered first of all is the definition of a low dose and a low dose rate, as they may be described either physically or biologically. This will depend upon the level of biological organization considered. Also addressed are theoretical aspects of the interactions of radiation with cells and tissues; the influence of track structure on radiation response; the concept of dose as it applies to tissues, cells, or subcellular targets; and the possible implications for dose-response relationships. The results of cellular studies are then reviewed. The range of endpoints of these studies include cell killing, cell transformation, chromosome aberrations, and mutation, which occur principally as a consequence of damage to the nuclear material in individual cells.

12. The results of animal studies related to radiation-induced cancer and hereditary disease are considered in Chapter II. For tumour induction, animal studies have demonstrated that dose-response relationships can be complex, depending on the age, gender, and species or strain of the animal, the sensitivity of individual tissues, the tumour type, and the dose rate. The results obtained for dose-response relationships for life-shortening and tumour induction with different animal models following exposure to external radiation or incorporated radionuclides are illustrated, and information is presented on the extent to which animal data can provide information on the risks of exposure at low doses.

13. In the case of damage to germ cells, the mutational events resulting from DNA damage generally arise as a simple function of dose and dose rate and depend principally on the radiation sensitivity of the specific gene locus. Dose-response relationships are reviewed in Chapter II. Radiation-induced hereditary effects were comprehensively examined by the Committee in the UNSCEAR 1986, 1988, and 1994 Reports [U2, U4, U5].

14. Epidemiological studies give information on dose-response relationships for tumour induction and provide the basis for quantitative risk estimates for human populations. The available data have been the subject of substantive reviews by the Committee [U2, U4, U5], and a further review is contained in Annex I, "*Epidemiological evaluation of radiation-induced cancer*". The information available on dose-response relationships is described in Chapter III, with emphasis on the extent to which data are available at low doses. These data relate to the consequences of exposure *in utero* as well as the exposure of infants, children, and adults.

15. The direct information on tumour induction, both from experimental and epidemiological studies, is insufficient, on its own, to elucidate the shape of the dose-response relationship at low doses. In Chapter IV, present knowledge is examined on the mechanisms of radiation tumorigenesis that can be used to gain further insight into effects at low doses. Emphasis is placed on gaps in knowledge and the consequent uncertainties. This topic was last reviewed by the Committee in the UNSCEAR 1993 Report [U3], and other issues relevant to those discussed here are considered in

Annex F, "*DNA repair and mutagenesis*" and Annex H, "*Combined effects of radiation and other agents*".

16. As modern molecular methods are developed and applied, the understanding of the mechanisms of tumorigenesis has, in recent years, increased substantially. At the same time there has been an equivalent increase in knowledge of radiation action on cellular DNA; of control of the reproductive cell cycle; of the mechanisms of DNA repair, genomic maintenance, and mutagenesis; and of non-mutational mechanisms of stable cellular changes. All this information could be relevant to assessing the shape of the dose response for both radiation-induced cancer and hereditary disease at low doses and dose rates and the effects of radiation quality at exposures below those at which direct information is available.

17. An important aim of Chapter IV is, accordingly, to highlight the critical elements of the current understanding of the mechanisms of tumorigenesis in order to relate them to data on dose-effect relationships and permit extrapolation to doses beneath those at which quantitative information is available. In Chapter V, the judgements developed in Chapter IV are used to examine biologically based computational models that may in turn be used to assess the risk of radiation-induced cancer at low doses and low dose rates.

I. CELLULAR EFFECTS

18. Damage to DNA, which carries the genetic information in chromosomes in the cell nucleus, is considered to be the main initiating event by which radiation damage to cells results in the development of cancer and hereditary disease [U3]. Either one or both strands of the DNA helix in cells may be damaged or broken, resulting in cell death, damage to chromosomes, or mutational events. Radiation is thought to have an effect on DNA either through the direct interaction of ionizing particles with DNA molecules or through the action of free radicals or other chemical intermediates produced by the interaction of radiation with neighbouring molecules. Damage can also be caused to other cellular structures, resulting in death or sublethal damage in individual cells; such damage does not in general result in radiation-induced cancer or hereditary disease. An exception is damage to cells that results in fibrosis, as this seems to be a precursor to the development of some tumour types (see Chapter II). It is also possible that other more indirect mechanisms can influence tumour development.

19. This Annex is concerned with the examination of the biological effects of radiation at low radiation doses. It is appropriate, therefore, to consider first how these should be defined. The designation of low doses and low doses rates has been considered in earlier reports by the Committee [U3, U5] and is summarized here briefly. The following Sections then consider radiation damage to DNA, relative biological effectiveness (RBE) of radiations of different quality, and the influence of track structure on cellular response. Cellular studies related to the determination of dose-response relationships for chromosome aberrations, cell transformation, and mutation induction in somatic cells are then summarized.

A. DESIGNATION OF LOW DOSES AND LOW DOSE RATES

20. In interpreting the responses of cells and tissues to ionizing radiation, judgements need to be made as to the bounds for low and high doses of low-LET radiation. In the 1993 UNSCEAR Report [U3], the physical and biological factors that need to be considered in making these evaluations were examined in the context of the doses and dose rates below which it would be appropriate to apply a reduction factor when assessing risks (per unit dose) at low doses and low dose rates from information on risks obtained at high doses and dose rates.

21. The following Sections deal with physical and biological approaches to designating exposures that may be considered to be either low-dose or low-dose-rate and with experimental data that can give information on the dose-response relationship for stochastic effects in cells either *in vitro* or *in vivo*.

1. Physical factors

22. Various models have been developed to account for the features of dose-response relationships obtained in experimental studies. A common aspect of many of these models is that a single radiation track, for any radiation quality, is taken to be capable of producing the initial damage and hence the cellular effect. The fundamental physical quantity used to define the deposition of energy in organs and tissues from ionizing radiation is the absorbed dose. The tissue or organ absorbed dose, D_T, is generally taken to be the mean energy absorbed in the target organ or tissue divided by the mass, T. This definition of the absorbed dose does not, however, characterize the fluctuation of energy absorption resulting from the stochastic nature of the energy deposition events (tracks) in individual cells. The fluctuation in the energy deposition between cells in a tissue is generally disregarded but can be significant when the possible effects of ionizing radiation on cells at low doses are considered. The number of independent tracks within each cell follows a Poisson distribution, and thus the numbers of cells receiving zero or few tracks will depend on the fluence of tracks through the organ or tissue.

23. The physical factors that can influence the effect of radiation on cells and tissues are generally well understood as a result of advances that have taken place in recent years in

microdosimetry at the cellular and subcellular levels [B31, B32, G6, G12, P13, R18]. A microdosimetric argument for defining low doses and low dose rates can be based on statistical considerations of the occurrence of independent radiation tracks within cells or nuclei. For ^{60}Co gamma rays, for example, and a spherical cell or nucleus (taken to be the sensitive target) assumed to be 8 μm in diameter, there will be, on average, one track per nucleus when the averaged absorbed dose is about 1 mGy [B31, B32]. If the induction of damage in the nucleus depends on energy deposition in single nuclei, with no interaction between them, a departure from linearity is unlikely unless there have been at least two independent tracks within the cell nucleus. The number of tracks within cells follows a Poisson distribution, as illustrated in Table 1, with the mean number of tracks proportional to the average absorbed dose. For average tissue absorbed doses of 0.2 mGy from low-LET ^{60}Co gamma rays, for example, spherical nuclei of say 8 μm diameter would each receive, on average, about 0.2 tracks. In this case, just 18% of cells would receive any radiation track at all and less than 2% of cells would receive more than one track. Halving the exposure would simply halve the fraction of the total cells affected, and so, at such low doses, the dose-effect should be linear. This microdosimetric argument for a low dose (taken here to be 0.2 tracks per cell) would apply to biological effects where the energy deposited in a cell produces effects in that cell and no other cell. It might apply, for example, to cell killing, the induction of chromosome aberrations, and mutations. Its applicability to cell transformation and cancer induction is less certain. It would need modification if, for example, the pro-

Table 1
Proportions of a cell population traversed by tracks for various mean doses [a] from gamma rays and alpha particles

Mean tracks per cell	*Percentage of cells in population suffering*						*Percentage of hit cells with only one track*
	0 track	*1 track*	*2 tracks*	*3 tracks*	*4 tracks*	*>5 tracks*	
0.1	90.5	9	0.5	0.015	-	-	95.1
0.2	81.9	16.4	1.6	0.1	-	-	90.3
0.5	60.7	30.3	7.6	1.3	0.2	-	77.1
1	36.8	36.8	18.4	6.1	1.5	0.4	58.2
2	13.5	27.1	27.1	18	9	5.3	31.3
5 [b]	0.7	3.4	8.4	14	17.5	56	3.4
10 [b]	0.005	0.05	0.2	0.8	1.9	97.1	0.05

a Approximately 0.1 mGy for gamma rays, 300 mGy for alpha particles.
b At these values appreciable proportions of the cell population will incur more than five tracks.

bability of an effect was influenced by a subsequent track at a later time, as could be the case for multi-stage carcino-genesis, or if there was interaction between cells in the development of a specific radiation effect, as, for example, has been suggested for so-called bystander effects. This is con-sidered further in Chapter IV.

24. To develop the microdosimetric argument for assessing a low dose, knowledge is required of the sensitive volume in the cell. A sphere of 8 μm diameter, as described above, is typical of the size of some cell nuclei, although they may be larger or smaller. If only a part of the nucleus responds autonomously to radiation damage and repair, then a smaller sensitive volume may be more appropriate, and the estimate of a low dose would increase. Figure I illustrates, for various volumes, the specific energy of low-LET radiation that would correspond to this microdosi-metric criterion of a low dose when less than 2% of cells receive more than 1 track. Thus for a nucleus of diameter 4 μm, a low dose (0.2 tracks per cell, on average) would be about 0.8 mGy, and for 32 μm it would be about 0.01 mGy. As described in the UNSCEAR 1993 Report [U3], this definition of a low dose could also take into account information on the time characteristics for DNA repair, which would give a low dose rate of 10^{-3} mGy min^{-1}, or be based on only a single track traversing a cell in a lifetime (say, 60 years), allowing essentially no scope for track interactions, which would give a low dose rate of 10^{-8} mGy min^{-1}.

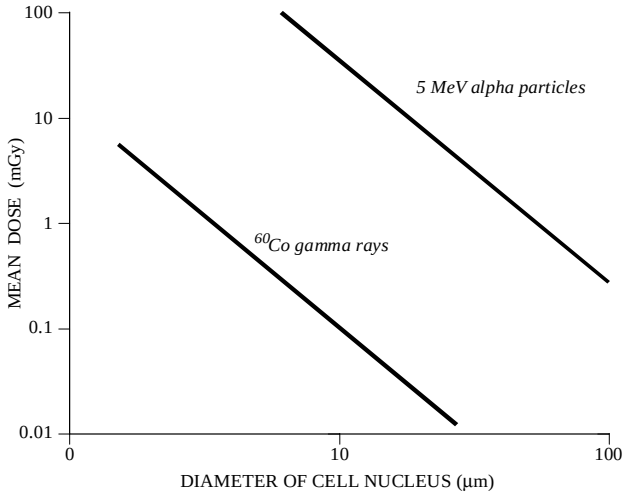

Figure I. Mean dose from an average of 0.2 radiation tracks per cell nucleus as a function of diameter (<2% of nuclei receive more than one track).

25. The situation is quite different for exposures to high-LET radiation. When a tissue receives an average dose of 1 mGy from alpha particles, only about 0.3% of the nuclei are struck by a track at all; the remaining 99.7% are totally unirradiated. When a single track does strike, it delivers to the nucleus a very large dose, of about 370 mGy on average. In individual nuclei the dose may be any value up to about 1,000 mGy [G22, G23].

2. Biological approaches

26. ***Biological approaches.*** The definition of a low dose and a low dose rate can also be based on direct observation of damage in experimental systems or in epidemiological studies. One approach to assessing a low dose is based on parametric fits to observed dose-response data for cellular effects at low to intermediate doses, below those at which cell killing will become important. For the induction of cellular damage, the incidence, I, of an effect can then be related to the dose, D, by an expression of the form

$$I(D) = \alpha D + \beta D^2 \quad (1)$$

in which α and β, the coefficients for the linear and quadratic terms fitted to the radiation response, are constants and are different for different endpoints. This equation has been shown to fit data on the induction of chromosome aberrations in human lymphocytes [U3]. It can also be extended to cover cell killing as described in Section I.B.1. For some types of unstable chromosome aberrations in human lymphocytes, the α/β quotient (which corresponds to the average dose at which the linear and quadratic terms contribute equally to the biological response) is about 200 mGy for ^{60}Co gamma rays [L34], and thus the response is essentially linear up to about 20 mGy, with the dose-squared term contributing only 9% of the total response. Even at 40 mGy, the dose-squared term still contributes only about 17% to the overall response. On this basis a low dose might be judged to be 20–40 mGy.

27. Another approach to assessing the low dose range can be based on animal studies. The results of studies designed to examine the effect of dose and dose rate on tumour induction were comprehensively reviewed in the UNSCEAR 1993 Report [U3]. The results obtained with experimental animals, predominantly mice, comparing the effect of various dose rates of low-LET radiation on the induction of leukaemia and solid tumours have suggested that, on average, a dose rate of about 0.06 mGy min^{-1} over a few days or weeks may be regarded as low. At lower dose rates no further reduction in tumour incidence, per unit exposure, was obtained. The choice by the Committee in the UNSCEAR 1986 Report [U5] of a low dose rate to include values up to 0.05 mGy min^{-1} appears to have been based on dose rate studies in experimental animals.

28. The analysis of information from epidemiological studies, in particular the data from the survivors of the atomic bombings in Japan, can also be used for estimating a low dose. Analysis of the dose response for mortality from solid cancers in the range 0–4 Gy (adjusted for random errors) has suggested an α/β quotient from a minimum of about 1 Gy, with a central estimate of about 5 Gy [P1, P14]. An α/β quotient of 1 Gy suggests that at a dose of 100 mGy the dose-squared term contributes less than 10% to the response and at 200 mGy still less than 20%. It was suggested in the 1993 UNSCEAR report that for solid tumour induction in humans, a low dose could be taken to be less than 200 mGy [U3]. There is, in practice, little evidence of a departure from linearity up to about 3 Gy. In the case of leukaemia in the survivors of the atomic bombings, where there is a significant departure from linearity at doses above about 1.5 Gy, a central estimate of α/β has been calculated to be 1.7 Gy, with a minimum value less than 1 Gy [P1, P14]. On the basis of this central estimate, the dose-squared term would contribute about 10% to the response at a dose of 200 mGy and about 23% at 500 mGy. A low dose might therefore be considered to be any exposure up to about 200 mGy [U3].

29. On the basis of these various analyses of physical and biological data, the Committee concluded in the UNSCEAR 1993 Report [U3] that for the purpose of assessing the risk of tumour induction in humans at low doses and dose rates of low-LET radiation, a reduction factor (dose and dose rate effectiveness factor, DDREF) should be applied, either if the total dose is less than 200 mGy, whatever the dose rate, or if the dose rate is below 0.1 mGy per min^{-1} (when averaged over about an hour), whatever the total dose. The various approaches to assessing a low dose and low dose rate from low-LET radiation are summarized in Table 2.

Table 2
Alternative criteria for upper limits of low dose and low dose rate for assessing risks of cancer induction in humans (low-LET radiation)
[U3]

Basis of estimation	*Low dose (mGy)*	*Low dose rate (mGy min^{-1})*
UNSCEAR 1986 Report [U5]	200	0.05
UNSCEAR 1993 Report [U3]		0.1 [c]
Linear term dominant in parametric fits to single-cell dose responses	20-40	–
Microdosimetric evaluation of minimal multi-track coincidences in cell nucleus	0.2	10^{-8} (lifetime) 10^{-3} (DNA repair)
Observed dose-rate effects in animal carcinogenesis	–	0.06
Epidemiological studies of survivors of the atomic bombings in Japan	200	–

a Approximately 0.1 mGy for gamma rays, 300 mGy for alpha particles.
b At these values appreciable proportions of the cell population will incur more than five tracks.
c Averaged over about an hour.

30. For high-LET radiation, the experimental data suggested that little consistent effect of dose rate or dose fractionation on the tumour response at low to intermediate doses has been obtained. It was therefore concluded [U3] that there was no need to apply a reduction factor to risks calculated at high doses and dose rates.

B. DAMAGE TO DNA

31. Cells are able to repair both single- and double-strand breaks in DNA over a period of a few hours, but this repair can be imperfect, resulting in long-term cellular damage and mutation. It has been assumed in previous reports by the Committee that damage to DNA causing mutational events in germ cells is the result of a single biological event but that carcinogenesis is a multi-stage process in which the radiation can induce one or more of the stages involving damage to DNA and interference with cellular homeostatic mechanisms [U3].

32. The vast majority of endogenous DNA lesions take the form of DNA base damage, base losses, and breaks to one of the sugar-phosphate backbone strands of the double helix [A14, W7]. Such single-strand damage may be reconstituted rapidly in an error-free fashion by cellular repair processes since the enzyme systems involved will, for all these lesions, have the benefit of the DNA base sequence on the undamaged strand acting as a template on which to recopy the damaged or discontinuous code. Single ionizing tracks of radiation will induce a preponderance of such single-strand damage as a result of energy loss events occurring in close proximity to a single DNA strand in the double helix. A cluster of such events within the diameter of the DNA duplex (about 2 nm) has, however, a finite probability of simultaneously inducing coincident damage to both strands. In support of this, an approximately linear dose response for double-strand break induction by low-LET radiation has been observed [J6], confirming that breakage of both strands of the duplex may be achieved by the traversal of a single ionizing track and does not require multiple-track action. There is also evidence that a propor-tion of radiation-induced double-strand breaks are complex and involve locally, multiply damaged sites, LMDS [J6]. On the basis of a body of experimental evidence it may be judged that the ratio of low-LET radiation-induced single-strand DNA breaks plus base damages to double-strand breaks is around 50:1. The probability of a double-strand break per cell has been judged to be about 4 per cell per 100 mGy [G10].

33. A fraction of radiation-induced double-strand damage will be repaired efficiently and correctly, but error-free repair of all such damage, even at the low abundance expected after low-dose exposure, is not anticipated. Unlike damage to a single strand of the DNA duplex, a proportion of double-strand lesions, perhaps that component represented by LMDS, will result in the loss of DNA coding from both strands. Such losses are inherently difficult to repair correctly, and it is believed that misrepair of such DNA double-strand lesions is the critical factor underlying the principal hallmarks of stable mutations induced by ionizing radiation of various qualities [T2, T14]. Double-strand DNA losses may in principle be repaired correctly by DNA repair recombination, but such damage may be subject to error-prone repair, which can result in the formation of gene and chromosomal mutations that are known to characterize malignant development [C23]. This interpretation would, however, be flawed if cellular repair processes were totally effective in repairing damage in the case of small numbers of double-strand breaks in the affected cells. In such a case, a threshold dose before any response could occur would be possible The most basic, although not necessarily sufficient, condition for a true dose threshold would be that any single track of the radiation should be unable to produce the effect. Thus, no biological effect would be observed in the true low-dose region, where cells are traversed only by single tracks. This is considered further in Chapter IV.

1. Dose response for low-LET radiation

34. The approach that has been frequently used to describe both the absolute and the relative biological effectiveness of a given radiation exposure from low-LET radiation is based on the assumption that the induction of an effect can be approximated by an expression of the following form:

$$I(D) = (\alpha D + \beta D^2)\, e^{-(p_1 D + p_2 D^2)} \qquad (2)$$

where α and β are coefficients of the linear and quadratic terms for the induction of stochastic effects and p_1 and p_2 are linear and quadratic terms for cell killing. This equation has been shown to fit much of the published data on the effects of radiation on cells and tissues resulting from damage to DNA in the cells, including the induction of chromosome aberrations, mutation in somatic and germ cells, and cell transformation.

35. The nature of the initial damage to DNA was considered in the UNSCEAR 1993 Report [U3]. The theoretical considerations were described in terms of the general features of target theory, because the insult of ionizing radiation is in the form of finite numbers of discrete tracks. On this basis, it was proposed that the nature of the overall dose response for low-LET radiation could be subdivided into a number of regions:

(a) *Low-dose region*. At the lowest doses, a negligible proportion of cells (or nuclei) would be intersected by more than one track, so the dose response for single-cell effects would be dominated by individual track events acting alone and would therefore be expected to increase linearly with dose and be largely independent of dose rate;

(b) *Intermediate-dose region.* In this dose region, where there may be several tracks per cell, it has been commonly assumed that tracks act independently if a linear term (α) can be obtained by curve-fitting to equations such as (1). For most of the experimental

dose-response data used for curve-fitting, the lowest dose at which a significant effect is obtained is usually towards the higher end of this dose region, when individual cells may, in fact, have been traversed by considerable numbers of tracks. The assumption of one-track action for this region considers that the relevant metabolic processes of the cell are not influenced by the additional tracks in any way that could alter the expression of the ultimate biological damage of each individual track. On this assumption, it is conventional to interpolate linearly from this region to zero dose to deduce the effectiveness of low doses and low dose rates of radiation. Such interpolation is based on the coefficient α in equation (1) and on the assumption that it remains unchanged down to very low doses and very low dose rates. There are a number of radiobiological studies, mostly with cells *in vitro* but also from animals exposed at different dose rates, that suggest that this common assumption is not universally valid.

(c) *High-dose region.* In this region, where there are many tracks per cell, multi-track effects are clearly seen as non-linearity of dose response, with upward or downward curvature of the dose response. The simpler forms of the dose-response relationship that are observed experimentally can commonly be fitted by a general polynomial with terms for dose and dose rate. At high doses, a separate term is needed to account for the effects of cell killing.

36. Equation (1), or some modification of it, has been conventionally used to estimate the biological effectiveness of radiation at minimal doses, assuming a constant value of α from the intermediate-dose region down to zero dose, with independence of dose rate. There are, however, instances in which this may not apply.

37. The assumption of such a dose response for single-cell stochastic effects may not hold if there are significant multi-track events in the intermediate-dose region. Such events could include, for example, the induction of multiple independent steps in radiation carcinogenesis, cellular damage-fixation processes influencing repair of DNA damage; the induction of enhanced repair by small numbers of tracks; multiple tracks or enhancement of misrepair; and variations in cell sensitivity with time. The dose response may also be modified if the biological effect of interest required damage to more than one cell or if it was influenced by damage to additional or surrounding cells.

2. Dose response for high-LET radiation

38. There are extensive radiobiological data indicating that high-LET radiations (neutrons and alpha particles) have a greater biological effect, per unit of average absorbed dose, than low-LET radiation. The influence of radiation quality on a biological system is usually quantified in terms of its relative biological effectiveness (RBE). The RBE of a specific radiation, R, can be defined as the absorbed dose of reference radiation required to produce a specific level of response divided by the absorbed dose of radiation, R, required to produce an equal response, with all physical and biological variables, except radiation quality, being held constant as far as possible. This definition does not depend on the dose response for the two radiations being the same, it simply depends on comparing the dose to give a specific level of effect for a particular endpoint. Low-LET radiation (x rays or gamma rays) is normally used as the reference radiation. A particular form of RBE is RBE_m, which is the maximum RBE that would be obtained at low doses and low dose rates. Various authors and committees (see for example [M18, S35, U6]) have reviewed the relevant biological data. A comprehensive review of the literature relevant to the determination of values for RBE may be found in NCRP Report No. 104 [N6]. Maisin et al. [M53] reported information on tumour induction in 7- or 21-day old C57BL mice exposed to 3.1 MeV neutrons (0.5, 1 or 3 Gy) or 250 kVp x rays (0.125, 0.25, 0.5 or 1 Gy). When the incidence of all malignant tumours and of hepatocellular cancer was fitted to a linear of linear-quadratic function, an RBE in the range 5 to 8 was obtained.

39. It is apparent from the studies summarized above that the RBE for high-LET radiation is dependent on the biological response being studied. For early effects in tissues caused by cell killing (e.g. skin burns, cataracts, and sterility), an ICRP task group [I13] concluded that for a range of tissues and for both neutrons and alpha particles, the RBE was generally less than 10. For damage to the lung from inhaled alpha particles causing fibrosis and loss of fluid into the lung (pneumonitis), the RBE for rats and beagle dogs was estimated to be between 7 and 10. Similarly, for the induction of chromosome aberrations in human blood lymphocytes by alpha particles from ^{242}Cm, RBE values of about 6 have been obtained in comparison with x rays and 18 in comparison with gamma rays [E12]. For the induction of micronuclei (caused by fragmentation of chromosomes) in lymphocytes by alpha particles from plutonium, an RBE of 3.6 has been found at low doses (<1 Gy), and for DNA double-strand breaks in Ehrlich ascites tumour cells, RBEs in the range 1.6–3.8 have been reported [B40].

40. In a few experimental studies a biological effect has been obtained for alpha particle irradiation although a similar effect has not been found with low-LET radiation. Studies in which sister chromatid exchanges (SCE) have been measured in human lymphocytes in the G_0 stage of the cell cycle give a measurable frequency of SCEs following exposure to alpha particles from ^{241}Am, but no effect of x-ray irradiation was obtained. From the definition of RBE given above, this implies an infinite RBE, although it is solely a consequence of there having been no observable effect of x rays at low doses [A16]. Similar results have been reported for SCEs in Chinese hamster ovary cells irradiated in the G_1 phase of the cell cycle by ^{238}Pu alpha particles or x rays. High values of RBE, up to about 245, have also been reported [N10] for sperm head abnormalities in mice when the effect of external exposure to x rays was compared with the effects of tissue-incorporated ^{241}Am. This may partly be accounted for by the heterogeneous distribution of ^{241}Am incorporated in the testis; it is known that actinides such as ^{241}Am tend to concentrate in interstitial

tissue in the mouse testis, in close proximity to the developing sperm cells.

41. For tumour induction, a number of studies have demonstrated that both high- and low-LET radiation may induce cancer in a range of tissues. Data relevant to the choice of RBEs for neutrons and alpha particles are summarized below. Values of RBE obtained for long-term effects can be useful for transferring information on risks calculated following exposure to high-LET to assess risks in populations exposed to low-LET radiation, and vice versa.

(a) Neutrons

42. Values for RBE_m obtained for various biological endpoints in mammals and in mammalian cells for fission neutrons compared with gamma rays are summarized in Table 3. Similar reviews have been published by UNSCEAR [U5] and Sinclair [S35]. Information on the variation of RBE_m with neutron energy comes partly from data from cellular studies, in particular using point mutations, chromosomal aberrations, and cell transformation as endpoints.

Table 3
Estimated RBE_m values for fission neutrons compared with gamma rays
[N6]

Endpoint	*RBE_m*
Cytogenetic studies, human lymphocytes in culture	34–53
Cell transformation	3–80
Genetic endpoints in mammalian systems	5–70
Life shortening (mouse)	10–46
Tumour induction	16–59

43. There is uncertainty in the value of RBE_m for fission neutrons. This uncertainty comes principally from how the data for low-LET radiation, mainly for cancer induction and life shortening in mammals, are extrapolated to low doses and low dose rates. The derivation of values for RBE_m is illustrated in Figure II. The straight line A of slope α_H represents the dose-response relationship for high-LET radiation. The data points shown in the Figure are representative of data for low-LET radiation and can be extrapolated to low doses by the linear relationship B of slope α_{LH} or by curve C, based upon a linear-quadratic dose-response relationship. Curve D represents the extrapolated linear portion with slope α_{LL} of the low dose response of curve C. The ratio of the slopes of curves A (slope α_H) and D (slope α_{LL}) represents RBE_m.

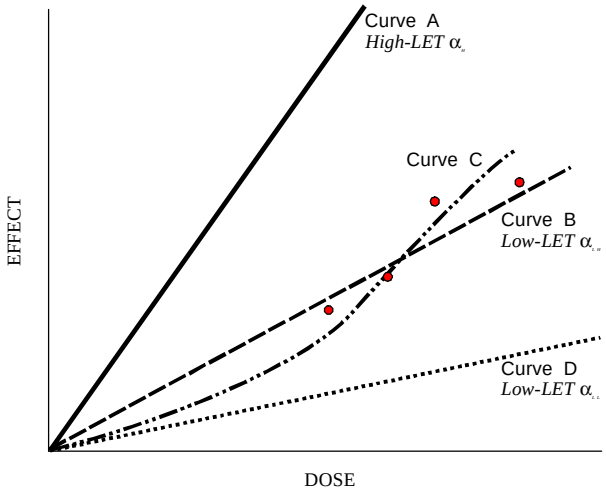

Figure II. Typical dose-effect relationship for low- and high-LET radiations [M18].

44. There are few data in whole animals that measure the variation of RBE_m for specific tumour induction or life shortening with neutron energy. Knowledge of the variation of RBE_m with neutron energy is confined to cellular studies. Chromosomal aberrations in human lymphocytes [E12, E13] indicate a monotonic decrease by a factor of about four from 1 MeV to 14 MeV. Mutations in a human hamster hybrid cell line (A_L) indicated [H29] a monotonic decrease by a factor of about seven from 0.3 to 14 MeV. The oncogenic transformation of C3H10T½ cells showed a more erratic variation with neutron energy [M15], but an overall variation by a factor of three from 230 keV to 14 MeV. The cellular data suggest a decrease in RBE_m by a factor of about four from 100 keV with an increase of neutron energy. There are very few experimental data at lower neutron energies. Some cellular data observing chromosome aberrations in human lymphocytes [E14] suggest an RBE_m close to that for fission neutrons, whereas similar data from Sevan'kaev et al. [S36] suggest lower values.

(b) Alpha particles

45. Alpha particles have a very short range in tissue. For the highest energy natural alpha particles from ^{226}Ra and its decay products with an energy up to about 7.8 MeV, the maximum range is about 80 μm; for 5 MeV alpha particles from ^{239}Pu, the range is about 40 μm. These dimensions may be compared with the dimensions of the cell nucleus, which range from about 5 to 10 μm in diameter. The dose that a single alpha particle delivers crossing the cell nucleus, considered to be the radiosensitive target, is highly variable. It may range from very low doses for particles that graze the nucleus, to more than 1 Gy for particles crossing the diameter. Thus the concept of average tissue dose is a considerable simplification, and individual cells in a tissue will receive very different doses. Furthermore, alpha-emitting radionuclides

may be deposited on the surfaces of organs within the body; this is the case, for example, for radon decay products deposited in the lung and for plutonium isotopes accumulated by the skeleton. There can therefore be a very heterogeneous distribution of alpha particle dose within an organ (or tissue). The specified dose depends on whether an average organ dose or the mean dose to a particular localized tissue volume is calculated. In practice, average organ dose is usually calculated.

46. When it comes to choosing an appropriate RBE to use for estimating the risk of tumour induction in organs and tissues, there are rather few data available. The main difficulty is that comparable patterns of exposure are needed for both the alpha-emitting and the reference radiation (x rays or gamma rays). Although extensive data are available on tumour induction for both of these radiations on their own, their effects have been directly compared much less frequently. Published data relevant to the estimation of RBE_m are available for the induction of bone sarcomas and lung tumours in experimental animals, from studies on cells in culture and, to a limited extent, from epidemiological studies on human populations. Relevant data are summarized in Table 4.

Table 4
Estimated RBE_m values for alpha particle irradiation and for gamma rays

Endpoint	*RBE_m*	*Ref.*
Bone tumours		
Dogs	26	[N6]
Mice	25	[N6]
Dogs	5.4 (4.0–5.8)	[G16]
Lung tumours		
Various species	30 (6–40)	[I5]
Dogs	10–18	[B41]
Rats	25	[H30]
Dogs	36	[H30]
Cell transformation (C3H10T½)	10–25	[B42]
Cell mutation		
Human lung cells HF19	up to 7.1 [a]	[C27]
Chinese hamster cells V79	up to 18	[T16]
Chromosome aberrations	5–35	[E12, P20]
Germ cell mutations (chromosome fragments, chromosome translocations, dominant lethals)	22–24	[S37]

a Compared with x rays (from [M18]).

3. Influence of track structure

47. As described above, it is commonly observed that high-LET radiations (neutrons and alpha particles) are much more effective per unit dose than low-LET (electrons and photons), in producing cellular effects such as chromosome aberrations and mutations or for effects in animals such as cancer and life shortening. Despite this, the number of DNA breaks produced per gray is not very different for high- and low-LET radiations. Yet it is these breaks that lead to chromosomal and mutation events in cells and eventually to cancer. The explanation lies either in the difference in the efficiency/fidelity of double-strand break repair after high- and low-LET radiation, or in the difference between the spatial distribution of the initial physical events (ionizations and excitations) which lead, via double-strand breaks, to aberrations and mutations in the cell. If the second explanation is true, there is some biological relevance to the distribution of initial events of energy deposition around tracks of charged particles, i.e. to track structure.

48. Computer programmes based on Monte Carlo techniques are now available to calculate on a scale of nanometres or smaller the exact position of ionizations and excitations in the track of charged particles [N9]. Examples for a 500 eV electron and a 4 MeV alpha particle are given in Figure III [G10]. Electrons meander by scattering and may travel in any direction. In contrast, heavy charged particles (from protons to much heavier ions) essentially travel in straight lines on a well-defined path. They pass their energy on to secondary electrons, which wander from the path of the ion. Generally, ions of higher velocity produce higher energy electrons that can travel further from the path of the ion. As an example, Figure IV shows calculations of the fraction of energy deposited within a distance, r, of a track for protons of energy from 0.3 to 20 MeV. For 0.3 MeV protons, at least 99% of the energy is deposited within 30 nm of the centre of the track. For a 20 MeV proton, some 2% of the energy is deposited more than 1 μm away from the path of the particle track.

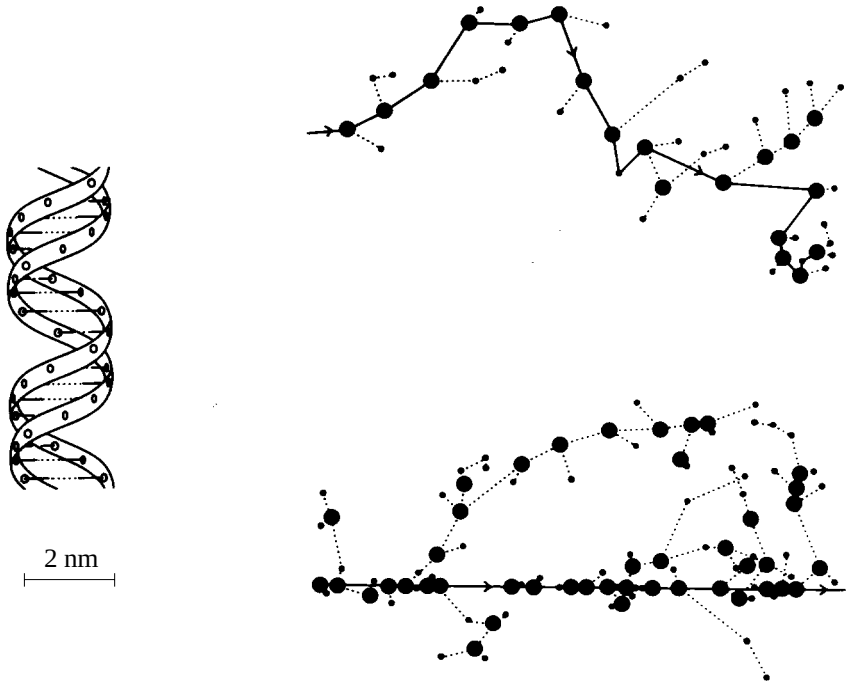

Figure III. Simulated low-energy track (upper panel: initial energy 500 eV) and simulated short portion of an alpha-particle track (lower panel: initial energy 4 MeV). A section of DNA is shown to give a perspective on dimensions [G10].

49. By choosing particle type and energy, it is possible to select particles of the same LET but markedly different track structures, and when such experiments are done then different biological effects are found [C24]. Clearly, track structure is important for understanding how radiations of different quality cause differences in RBE, although opinions vary as to which particular features of track structure and which objects and characteristic distances in the cells relate to given biological endpoints. As an example, Savage [S32] considers that for producing chromosome aberrations, DNA breaks may exchange over distances up to one or two hundred nanometers. Thus, energy around a particle track deposited within volumes of the order of 100 nm may be important. Other dimensions may apply to other biological effects. Some analytic models employing volume sizes as fitted parameters have been quite successful in representing quantitative relationships between RBE and LET for biological endpoints such as cell survival or transformation in irradiated mammalian cell cultures [C25, C29, K24, K28].

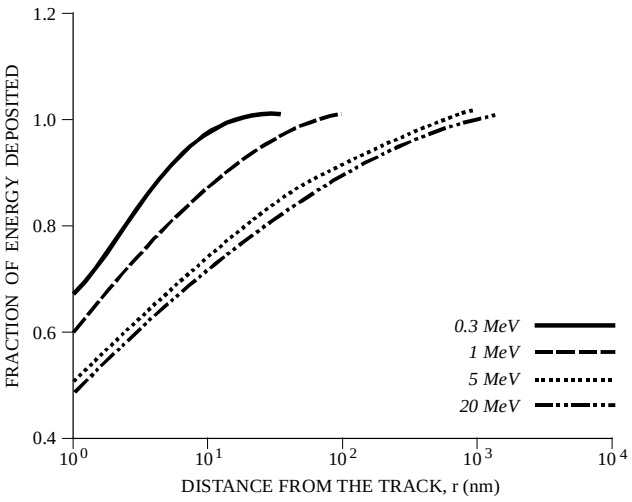

Figure IV. Fraction of energy deposited at distances from a proton track less than a specified value, r [E16].

C. CELLULAR DAMAGE

50. A range of assays has been developed for evaluating radiation damage to cells either *in vitro* or *in vivo*. These include survival curves, cell transformation, induction of mutations, and chromosome aberrations. Various models have been developed to describe these radiation-induced cellular effects and their dependence on dose, dose rate, and radiation quality. Some of these models have been summarized by Goodhead [G6, G7]. The near-consensus view of the critical damage to single cells that has resulted from these studies is that single radiation tracks from ionizing radiations can lead to cellular damage. The various models that have been developed assume *inter alia* that cellular damage arises from DNA double-strand breaks, either singly or in pairs; pairs of DNA single-strand breaks; localized clusters of radiation damage in unspecified molecular targets or in DNA; unspecified single or double lesions, probably in DNA, but qualitatively similar and independent of radiation quality; or damage to DNA and associated nuclear membranes [U3]. Such models indicate that a single track, even from low-LET radiation, has a finite probability of producing one, or more than one, double-strand break in DNA in a cell nucleus. Hence the cellular consequences of a double-strand break, or of interactions between them, should be possible even at the lowest of doses or dose rates. This would not, however, be the

outcome if cellular repair processes for single or small numbers of double-strand breaks were totally efficient, in which case a threshold for the response might be anticipated. There is no evidence for repair being 100% efficient, however, although experimental assays to test the hypothesis have limited resolution.

51. The following Sections illustrate dose-response relationships that have been obtained for chromosome aberrations, for cell transformation, and for mutagenesis in somatic cells. Emphasis is placed on assessing effects at low doses. Other cellular effects, including changes in gene expression, are examined in Annex F, "*DNA repair and mutagenesis*" and noted in Chapter IV of this Annex. In a number of cellular systems, an adaptive response has been described in which a small initial radiation dose can modify the effect of a subsequent larger dose. Some examples of studies demonstrating this response are given below.

1. Chromosome aberrations

52. The scoring of chromosome aberrations in human peripheral blood lymphocytes provides a sensitive method for biological dosimetry. It also provides a valuable approach to assessing dose-response relationships for chromosome mutations. By scoring dicentric aberrations in the full genome of about 1,000 cells, average whole-body doses of about 100 mGy from x rays or gamma rays may be detected and higher doses estimated. Calibration curves have been prepared by a number of laboratories for a wide range of radiations. All dose-effect curves for low-LET radiation conform to equation (2) up to 5–10 Gy. At higher doses, saturation of the curve can occur when yields of dicentrics approach five per cell [E8].

53. The difficulty of experimentally demonstrating the presence or absence of a threshold for single cellular events can be illustrated by work on the induction of chromosome aberrations in lymphocytes by x rays. In assessing radiation exposure by the analysis of aberrations in blood lymphocytes, measurements are normally made of the incidence of both dicentrics and total aberrations. These are unstable aberrations, and they will slowly disappear from peripheral blood. They are, however, more sensitive for the detection of effects at low doses than are stable aberrations.

54. The background incidence of dicentric aberrations in blood lymphocytes observed at metaphase is about 1 in 1,000 cells. As radiation-induced aberrations arise at the rate of about 4 per 100 cells per gray, the ability to detect a dose of 100 mGy would require about 1,000 lymphocytes to be scored, which would take about three man days. Radiation damage at lower doses can be detected, but this requires the assay of proportionately more cells. Investigations at doses much less than 100 mGy require very large numbers of cells to be assayed, which would be likely to exceed the scoring capacity of any single laboratory and cannot, as yet, be satisfactorily undertaken on a routine basis, even with automated scoring techniques.

55. A number of *in vitro* studies of chromosome aberrations published in the 1970s and 1980s gave data that could be fitted with a linear dose-response relationship, although other functions were also reported. Thus, Luchnik and Sevan'kaev [L13] reported a plateau in the dicentric response to gamma rays at doses between 100 and 300 mGy (low-LET), and Kučerová et al. [K9] produced dicentric data for x rays, which might have indicated a threshold at about 150 mGy, although the authors interpreted the data with a linear function. Lloyd et al. [L9] found a linear response in the lower dose region for x rays down to 50 mGy for both dicentrics and total aberrations. Wagner et al. [W1] found a linear-quadratic response using doses in the range 50–500 mGy from 220 kVp x rays. One study by Pohl-Rüling et al. [P15] reported that 4 mGy of 200 kVp x rays produced a significant reduction in aberration frequencies below the control value until doses of 20 mGy and above were received. This was interpreted as evidence for the stimulation of repair mechanisms at doses below a few tens of milligrays. It was only when repair processes were overwhelmed that aberration yields rose following a linear-quadratic response. It was subsequently noted that if the control aberration yield from a similar experiment designed to examine the effect of D-T neutrons (and in which one of the two controls was common to the x-ray study) [P23], then the yield for 4 mGy of x rays was no longer significant [E15].

56. To provide better data on the response at low doses, a coordinated project was carried out by scientists in six laboratories They collaborated in a study to examine the yield of unstable aberrations induced in peripheral blood lymphocytes *in vitro* by x rays [L8]. The study covered doses of 0, 3, 5, 6, 10, 20, 30, 50, and 300 mGy. Cells from 24 donors were examined, and a total of about 300,000 metaphases were scored. Aberration yields significantly in excess of control values were seen at doses down to 20 mGy, and the dose-response data at low doses were consistent with a linear extrapolation from higher doses. The overall dose response up to 290 mGy was fitted with a linear-quadratic dose-response relationship of the form $I = C + \alpha D + \beta D^2$, where I is the incidence of dicentrics, C is a constant equal to the spontaneous dicentric yield, and α and β are the coefficients for the linear and quadratic terms as a function of the dose D (in gray). Values of $C = 0.0012 \pm 0.002$, $\alpha = 0.027 \pm 0.012$, and $\beta = 0.044 \pm 0.042$ ($\chi^2 = 5.2$ for 5 degrees of freedom, df) were obtained as best fits to the data. At doses below 20 mGy, the observed dicentric yields were generally lower than background, but not significantly so. Excess acentric aberrations and centric rings, in contrast, were higher than controls, although the increase was not statistically significant. A number of uncertainties associated with this type of analysis were described in the paper, including differences in scoring by the participating laboratories, and it was concluded that the statistical uncertainties were such that it is unlikely that this technique would ever allow the response for aberrations to be directly measured at doses much below 20 mGy.

57. The complete set of dicentric data published in the paper have been subject to further analysis to determine the extent to which other models could fit the dose-response

information obtained in the study [E2]. A threshold-linear dose response of the form $I = C + \alpha(D - D_0)$ has been used for the analysis in which I is the incidence of dicentrics at dose D, in gray, and D_0 is the threshold dose. With values of C = 0.0013 and α = 0.040, the best estimate of the threshold, D_0, was 0.0097 ± 0.0045 Gy (±1 SE) (χ^2 = 4.0 for 5 df). It may be concluded, therefore, that while the data can be reasonably fitted with a simple linear-quadratic function, the possibility of a threshold for doses up to about 10 mGy cannot be excluded.

58. It has been found that the yield of aberrations following a given radiation dose can be influenced by an earlier radiation exposure. Some of the earliest studies on this so-called adaptive response were carried out in human lymphocytes that had incorporated tritiated thymidine [O6]. The cells were exposed to chronic, low doses from tritium in culture and were subsequently exposed at the relatively high dose of 1.5 Gy from x rays. Approximately half as many chromosome aberrations were induced in cells that had incorporated thymidine as in those that had not. This observation was repeatable, and subsequent experiments showed that exposure to tritium need not be chronic [W14] and that pre-exposure to 10 mGy of x rays could also cause the lymphocytes to become adapted [S25]. Subsequent work showed that the response to low doses took several hours to fully manifest itself [S26] and that it depended on synthesis of proteins (possibly an enzyme), which was inhibited by the addition of cycloheximilidine 4–6 hours after the 10 mGy priming dose of x rays [W15]. It has been postulated that stimulation of the synthesis of enzymes responsible for DNA repair is the key factor in this response [U2]. This type of an adaptive response has been observed in other cellular systems, as for somatic mutations, but it has been most comprehensively studied in human lymphocytes [U2, W13]. This issue is considered further in Chapter IV.

59. The measurement of unstable aberrations in blood lymphocytes has its limitations as a biological dosimeter, because the incidence of dicentrics, rings, and other aberrations decreases with time. In recent years stable chromosome aberrations have been extensively studied, as they provide a method for assessing exposures that occurred some years previously. Some data have been published on dose-response relationships for stable aberrations using fluorescent *in situ* hybridization techniques. However, these aberrations have a higher background yield, which increases and becomes more variable with age and lifestyle of the individual [R21]. Cumulative background radiation exposure accounts for only a small part of the increased frequency with age; clastogenic physiological processes of normal ageing are more important [H34, L50]. This higher and inherently more variable background of stable translocations means that it has not been possible to measure a significant increase in response at doses below 200–300 mGy [G13, L38, N7]. Stable aberrations are, therefore, of little value at present in obtaining information on the shape of the dose response at low doses.

60. Recently chromosome aberrations have been used to examine the effects of radiation in a high-background-radiation area (HBRA) in China and in a control area [J9]. The level of radiation in the high-background-radiation area was 3 to 5 times higher than that in the control area. Overall the cumulative doses in 39 individuals ranged from 31 to 360 mGy for high-background-radiation area and 6.0 to 59 Gy for the controls. The frequency of dicentrics and ring chromosomes (unstable aberrations) increased in proportion to the cumulative dose in the high-background-radiation area group. Such a dose-response relationship was not clear for those in the control area. The increase in the frequency of these aberrations at such an extremely low dose rate suggested that there is no threshold dose for the induction of chromosome aberrations. In contrast, in the case of translocations any effect of radiation, at up to 3 times control levels, could not be detected against the background incidence [H33].

61. For high-LET radiation the dose response obtained following exposure *in vitro* both to alpha particles and to neutrons is generally well fitted with a linear response up to doses of around 1.5 Gy [E8] (Figure V). The lowest doses used in these studies were about 50 mGy (high-LET).

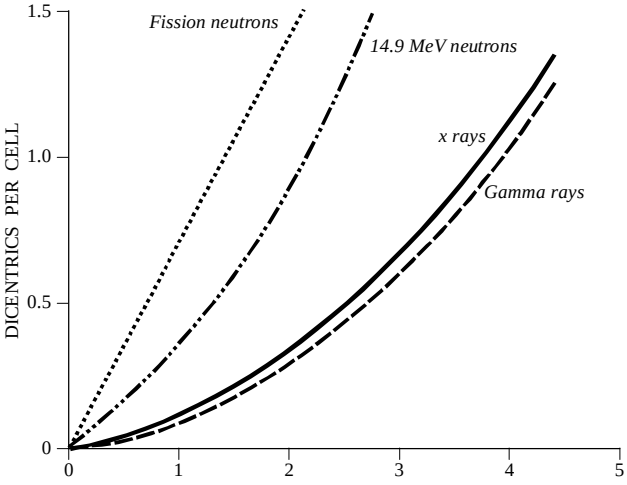

Figure V. Yield of dicentrics in human lymphocytes as a function of dose for some photon and neutron radiations [E8].

2. Cell transformation

62. Cell transformation systems *in vitro* have been widely used to study the initial stages of oncogenesis. Cell transformation describes the cellular changes associated with loss of normal homeostatic control, particularly of cell division, which ultimately results in the development of a neoplastic phenotype. They are considered to be the closest *in vitro* model for carcinogenesis. The only biological endpoint generally accepted as being definitive of oncogenic transformation is the growth of malignant clones in "nude" or immunologically suppressed host animals. As it is not practicable to screen every transformed cell in this way, other endpoints are normally used, such as enhanced growth rate;

lack of contact inhibition and indefinite growth potential; anchorage-independent growth; and the ability to grow in less nutritious media. The specific criteria used to define oncogenic transformation depend on the particular system that is being used.

63. The most common cell transformation systems such as the BALB/c3T3 and the C3H10T½ mouse-embryo-derived lines are based on cell lines derived from rodent fibroblasts. There are disadvantages to using such models for carcinogenesis in humans, and these were reviewed by the Committee in the UNSCEAR 1993 Report [U3]. When transformed C3H10T½ cells are innoculated into suitable hosts, they form fibrosarcomas. These are not typical of the tumour types that arise in humans after exposure to radiation, which are mainly epithelial in origin. Ideally, more relevant cell lines based on human epithelial tissues are needed for studying the mechanisms of tumorigenesis and dose-response relationships, but they have proven to be much more difficult to develop.

64. Both carcinogenesis and transformation are multi-stage processes, although transformation *in vitro* is normally studied in cells that have already undergone one or more of the possible steps involved, in particular, immortalization. Too much reliance on studies of rodent cell lines can, however, lead to errors in interpretation, if directly applied to humans. Thus, a correlation between anchorage-independent growth and the tumorigenic phenotype has been established in rodent cells [F10, O1, S8], which has permitted the selection of neoplastically transformed cells by growth in soft agar. This does not, however, apply to cultured human cells, as normal human fibroblasts are capable of anchorage-independent growth when cultured in the presence of high concentrations of bovine serum.

65. Despite such limitations, a number of characteristics of *in vitro* cell transformation have allowed their use as model systems for studying the early stages of radiation carcinogenesis *in vivo*. These have been summarized by Little [L4] and include a high correlation between animals and cell transformation systems for carcinogenicity of many chemicals; the response of transformed cells to initiation and promotion similar to two-stage carcinogenesis in the tissues of experimental animals; and the provision of quantitative information on the conversion of normal to tumour cells. Cell-based transformation systems should be free of the influence of hormonal and immunological factors, although cell-cell interactions are still possible.

66. Both BALB/c3T3 and C3H10T½ cell lines have been used to measure the oncogenic effects of ionizing radiation (see, for example, [H1, H18, M3]) and chemical agents (see, for example, [B25, R13]), as well as to screen for possible carcinogenic agents (see, for example, [S9]). Dose-response relationships for cell transformation following exposure to low-LET radiation were reviewed in the UNSCEAR 1986 and 1993 Reports [U3, U5] and by Barendsen [B3]. The pattern of response is very dependent on cell-cycle kinetics; nevertheless, in carefully controlled experiments, the results from transformation studies on dose and dose-rate effects agree closely with the results obtained with other cellular effects. There are, however, limitations to the sensitivity at low doses and dose-response data for low-LET radiation are generally available down to doses of around 100 mGy (see, for example, [M35, M36]). Above 3 Gy, cell reproductive death starts to predominate over the transformation frequency per plated cell [B2, B3, H1].

67. For transformation by low-LET radiation, various dose-response relationships have been reported. A linear dose response has been described by a number of authors (see, for example, [B33, H19, H27]), while linear-quadratic or curvilinear relationships have been described by others (see, for example, [B30, H19, H24, M15]). Balcer-Kubiczek and Harrison [B29] reported a linear dose response for the induction frequency, IF, of cell transformation in C3H10T½ cells exposed to single doses of x rays (4 Gy min^{-1}) between 250 mGy and 2 Gy (Figure VI), described by IF = 2.50 ± 0.11 10^{-4} Gy^{-1}. The overall fits to the data were evaluated by comparing χ^2 values. The addition of a quadratic function was found not to be justified by least-squares fitting. For continuous exposures over 1 hour or 3 hours, linear responses were also obtained but with a reduced transformation frequency described by 1.5 ± 0.03 10^{-4} Gy^{-1} and 0.87 ± 0.5 10^{-4} Gy^{-1}, respectively. In this study, no transformation was observed in unirradiated cultures. The laboratory estimate for transformation was less than 0.81 ± 0.04 10^{-5} transformants per viable cell; thus it is reasonable to assume that even the lowest transformation frequency obtained at 250 mGy was due to radiation exposure.

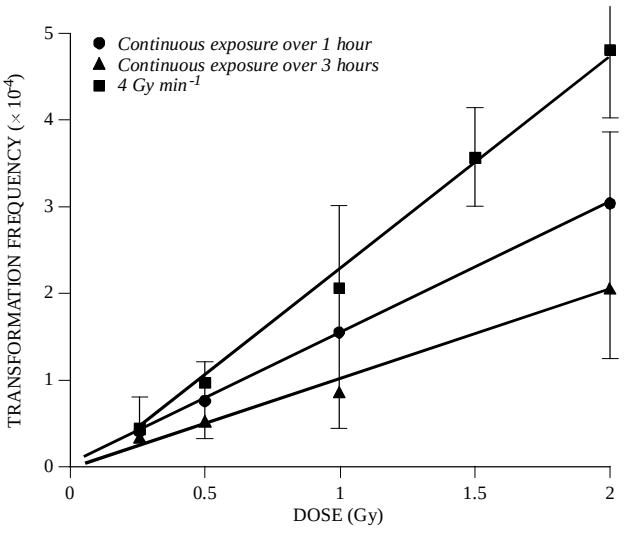

Figure VI. Transformation frequency in C3H10T½ cells following exposure to a single or protracted doses of x rays [B29]. *Lines are fits to the data with a linear dose-response function.*

68. Little [L4] compared results from BALB/c3T3 and the C3H10T½ cell lines. Following exposures between 100 mGy and 3 Gy, the dose response for the BALB/c3T3 cells was nearly linear but that for the C3H10T½ cells could be represented by a linear-quadratic or quadratic relationship (Figure VII).

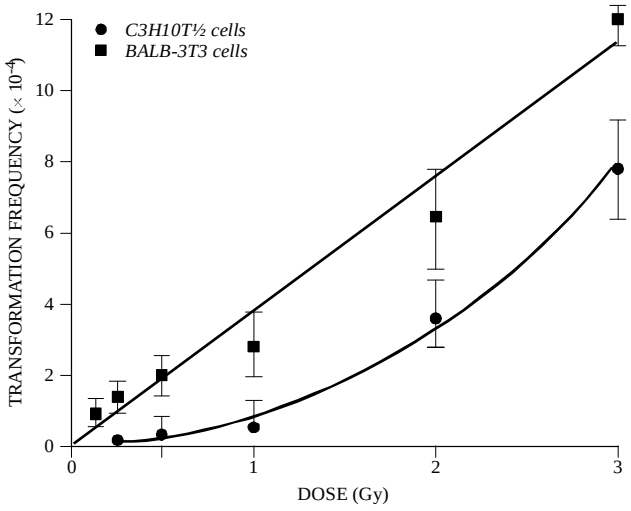

Figure VII. Transformation frequency in C3H10T½ cells and BALB-3T3 cells following exposure to single doses of x rays [L4].

69. Miller et al. [M35, M36] measured the effect on C3H10T½ cells of x-ray doses down to 100 mGy delivered just 24 hours after seeding. They found a plateau in the incidence of transformants per surviving cell between about 300 mGy and 1 Gy, which may have reflected the fact that the cells had not achieved asynchronous growth at the time of exposure.

70. In a major study, six European laboratories collaborated in a study that was specifically designed to address the issue of the dose response at low doses of low-LET radiation with the C3H10T½ transformation system [M14]. Considerable effort went into standardizing techniques in the different laboratories and carrying out extensive intercomparison exercises. One laboratory carried out all the irradiations, and care was taken to ensure that transport conditions did not interfere with the assays. Dose-response data were obtained for exposure to 250 kVp x rays at dose intervals from 0.25 to 5 Gy, and a total of 51,000 petri dishes (of 55 cm^2) were scored. In total, 759 transformed loci were obtained, far in excess of the numbers reported in any other study involving low-LET radiation and the C3H10T½ cell transformation system.

71. The combined data are shown graphically in Figure VIIIa. A regression fit to the data on transformation induction frequency, IF, between 250 mGy and 5 Gy gave a linear fit of the form IF = $(0.83 \pm 0.08)\ 10^{-4}\ Gy^{-1}$. A fit using a linear-quadratic relationship resulted in a non-significant value for the dose-squared term. The authors concluded that the data supported a linear dose-response relationship for cell transformation *in vitro* and that there was no evidence for a threshold dose. A presentation of these data in terms of the numbers of cells at risk might be more relevant. The data from Figure VIIIa are therefore replotted in Figure VIIIb, showing the transformation frequency per cell at risk. This follows the standard bell-shaped curve with a fall in frequency at doses above about 2 Gy, reflecting the effect of cell killing. At the lower end of the curve the response is similar to that shown in Figure VIIIa.

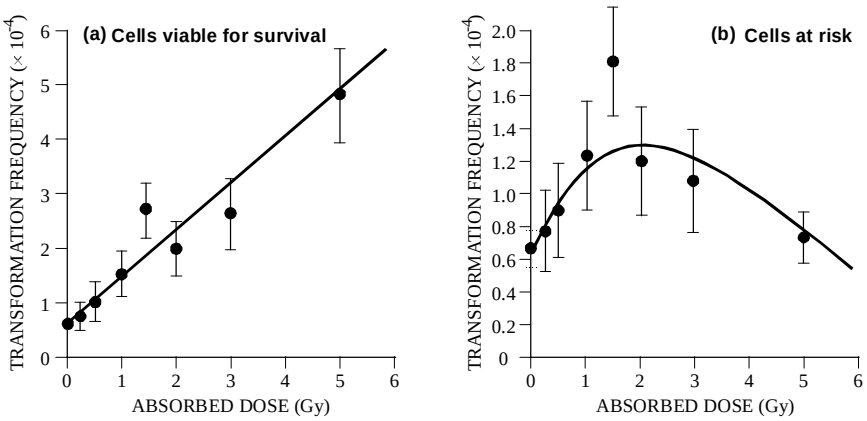

Figure VIII. Transformation frequencies in C3H10T½ cells following exposure to 250 kVp x rays at 2 Gy min^{-1} [M14].

72. These experiments were carried out under as near-identical conditions as possible in the participating laboratories and probably represent the optimum conditions that could be achieved for this type of experiment. The results support a linear dose-response relationship for cell transformation *in vitro* at low doses and do little to support the concept of either a threshold dose or an enhanced supralinear response. Nevertheless, the lowest dose at which effects could be detected was 250 mGy, clearly demonstrating the limitations of the technique for assessing dose-response relationships at low doses. Because of the large amount of scoring needed, it would not have been practicable to obtain information at appreciably lower doses.

73. An adaptive response to low doses of gamma radiation that reduces the effectiveness of a subsequent challenge dose in inducing spontaneous neoplastic transformation has been reported for C3H10T1/2 cells [A20]. In a subsequent study the same group reported that doses of 1–100 mGy from gamma radiation resulted in a suppression of the transformation frequency of C3H10T1/2 cells to levels below that seen for spontaneous transformation of unirradiated cells [A21]. Similar results have been obtained with HeLa x skin

fibroblast human hybrid cells [R22]. In the latter study the frequency of transformation of unirradiated cultures was compared with that of cultures irradiated with 10 mGy from gamma radiation and either plated immediately or held for a further 24 hours at 37°C prior to plating. Pooled data from a number of studies indicated an adaptive response in the case of post-irradiation holding, although the results of four individual studies were quite variable.

74. Exposure to high-LET radiation results in a higher transformation frequency than exposure to low-LET radiation, with a general tendency towards a linear dose-response relationship, but with a tendency to plateau and then fall at high doses (see, for example, [H19, H25, M15, M16]). There is no tendency for the response per unit dose to decrease at low doses or low dose rates, although a number of studies have shown an enhanced effect. As described in the UNSCEAR 1993 Report [U3], the main evidence for this enhanced effect is restricted to 5.9 MeV or fission neutrons, and in more recent studies with monoenergetic neutrons of various energies (see, for example, [M37, M38]) the magnitude of this so-called inverse dose-rate effect has been reduced from a factor of around 9 to a factor of 2 or 3 and has been shown to be radiation-quality dependent. A model has been developed that can satisfactorily explain many experimental results showing this enhanced dose-rate effect [B26, H20].

75. Recently, Miller et al. [M40] carried out a detailed analysis of the effect of transformation of C3H10T½ cells by alpha particle irradiation using charged particle microbeams. Cells in a monolayer in a cell culture dish were irradiated in turn under a highly collimated shuttered beam of alpha particles. The technique permitted measurement of the oncogenic potential of a single or a fixed number of alpha particles passing through a nucleus. The nucleus of each cell was exposed to a predetermined exact number of alpha particles with energy similar to that of radon decay progeny. In parallel with these microbeam studies, "broad beam" alpha particle exposures were also carried out such that cell nuclei received different fluences of alpha particles with mean numbers of 0, 1, 2, 6, and 8. In this case the actual number of "hits" of each cell was determined by Poisson statistics. Thus, if the mean number of traversals of a cell is 1, then 37% are not traversed at all, 37% once, and the remainder are traversed by two or more alpha particles.

76. The authors reported that the measured oncogenicity from exactly one alpha particle was significantly less than from a Poisson distributed mean of one alpha particle, implying that cells traversed by multiple alpha particles were more likely to be subject to transformation. Transformation frequencies for an exact or mean number of one alpha particle per cell were $1.2\ 10^{-4}$ and $3.1\ 10^{-4}$, respectively. The incidence of transformations in the exact single-traversal cells was not significantly different from that in the zero-dose (sham) irradiated cells ($0.86\ 10^{-4}$). The result was taken to imply that the majority of the yield of transformed cells following irradiation with a mean of one alpha particle per cell must come from the minority of cells subject to multiple traversals. While these results suggest a non-linear response at low doses and that the risk is less than might be expected for single-track traversals on the basis of a linear dose response, these conclusion were based on only a single result for each exposure condition and need further replication before any confidence can be placed in them. Nevertheless, they have demonstrated a unique approach to examining the carcinogenic potential of alpha particles at low doses.

3. Mutagenesis in somatic cells

77. The principal mechanism resulting in a neoplastic initiating event is induced damage to DNA, which predisposes target cells to subsequent malignant development (see Chapter IV). There is also strong evidence linking a number of tumours to specific gene mutations. An understanding of the dose-response relationships for this initial mutational change is relevant to an assessment of the effect of low doses on tumour induction. The experimental data have been reviewed by Thacker [T2] and are considered in detail in Annex F, "*DNA repair and mutagenesis*". Considered here is information on dose-response relationships for somatic mutation induction resulting from exposure to both low- and high-LET radiations.

78. A range of mutation systems have been described in the literature, but only a few are sufficiently well defined for quantitative studies. There are also a number of difficulties in interpreting results from somatic cell systems. In particular, the mutation frequency of a given gene is to some extent modifiable, depending on the exact conditions of the experiment. It may also be that a period of time is needed for the mutation to manifest itself. Thus, the true mutation frequency may be difficult to determine, and this can present particular difficulties in studies of dose-response relationships. Several established cell lines, derived from mouse, hamster, or human tissue, have also been used to measure mutation frequencies at different doses and dose rates. Because the cells lines used experimentally can have sensitivities that depend on the stage of the cell cycle, to ensure as consistent a response as possible, it is preferable to use a stationary culture in plateau phase in which only a limited number of the cells will be cycling in the confluent monolayer.

79. The mutation of a single gene is a relatively rare event; the majority of experimental systems are therefore designed to select out cells carrying mutations. Commonly used systems employ the loss of function of a gene product (enzyme) that is not essential for the survival of cells in culture. Thus, cells may be challenged with a drug that they would normally metabolize with fatal consequences. If mutation renders the gene product producing the specific enzyme ineffective, the cell will survive, and thus the mutation frequency can be obtained by measuring the survivors. Frequently used examples of such a system are those employing the loss of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HPRT), which renders cells resistant to the drug 6-thioguanine (6-TG), and of the enzyme thymidine kinase (TK), which gives resistance to trifluorothymidine (TFT). HPRT activity is specified by an X-linked gene,

hprt, while TK is specified by an autosomal gene *tk* and therefore has to be used in the heterozygous state.

80. Mutation induction in a human lymphoblastoid cell line (TK_6) after acute x ray and continuous low-dose-rate gamma irradiation was investigated using the *hprt* and *tk* mutation assays [K20]. The TK_6 cells are radiosensitive, and increases in mutation rate for both 6-TK and HPRT were obtained at acute x-ray doses from 250 mGy to 1 Gy, with a response that could be fitted with a linear function down to zero dose. At high doses (1.5 and 2 Gy), mutation data were difficult to obtain because of a very low surviving fraction (1%–4%). Mutation frequency after continuous gamma irradiation could also be fitted with a linear response and with mutation rates at both 27 mGy h^{-1} and 2.7 mGy h^{-1} that did not differ significantly from those for acute exposure.

81. Evans et al. [E7] examined the effect of dose and dose rate on the mutation frequency at both the *tk* and *hprt* loci in two variants (LY-S1 and LY-R16) of mouse lymphoma L5178Y cells. Mutation at the *tk* locus, resulting from x-ray exposure, was dose-rate-dependent in the LY-R16 variant but not in the LY-S1 variant. This was thought to reflect the deficiency of DNA double-strand break repair in LY-S1. In contrast, with the *hprt* locus, mutation was dose-rate-independent in both strains. The results suggested that mutation at the *hprt* locus is caused by single lesions, with dose-rate-independent repair, whereas for the *tk* locus, interaction of DNA damaged sites is important. In both cases, however, an increased incidence of mutations was obtained at doses down to about 500 mGy, the lowest dose tested. The data could be fitted with a linear dose response, with no threshold up to 3 Gy for LY-R16 cells and 2 Gy for LY-S1 cells.

82. Induction of mutation to 6-TG resistance was examined in a radiation-sensitive mutant strain of mouse leukaemia cells following gamma irradiation at dose rates of 30 Gy h^{-1}, 200 mGy h^{-1}, and 6.2 mGy h^{-1} [F14]. The mutation frequency increased linearly with increasing dose for all dose rates, with no significant difference between the responses at any of the dose rates. The lowest dose tested was 250 mGy.

83. A particularly sensitive mutation assay has been described using the pink-eyed unstable (p^{un}) mutation in the mouse [S23]. This causes a reduction in the pigment in coat colour and eye colour as a result of a gene duplication and reverts to wild type by deletion of one copy. Reversion events are assayed as black spots on the grey coat. The reversion frequency of p^{un} is at least five orders of magnitude greater than that of other recessive mutations at other coat colour loci. Female mice, homozygous for the reversion, were irradiated with various doses of x rays between 10 mGy and 1 Gy and the frequency of reversions measured. Even at a dose of 10 mGy, the incidence of black melanosome streaks was increased threefold. There was a linear dose response over the dose range examined, with no indication of a threshold (Figure IX).

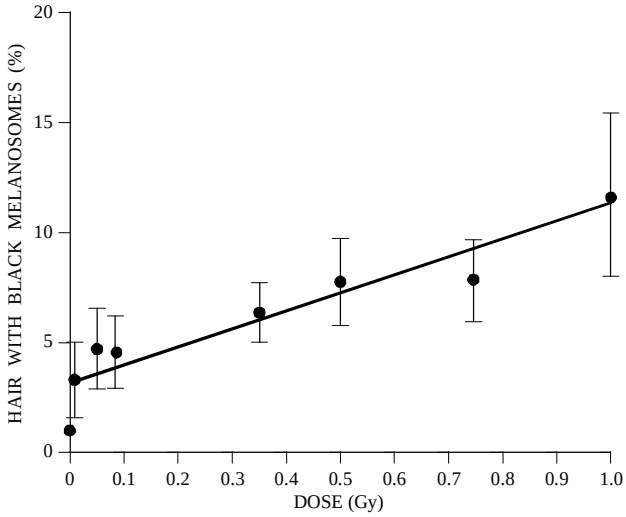

Figure IX. Dose response for x-ray induced reversion of pink-eyed unstable mutations in mice [S23].

84. Albertini et al. [A10] compared the effects of ^{137}Cs gamma rays and alpha irradiation from ^{222}Rn on *hprt* mutations induction in human T cells *in vitro*. For gamma-ray doses between 500 mGy and 4 Gy, the dose response could be represented by either a linear or linear-quadratic relationship, with a significant increase in mutations at the lowest dose. The doubling dose for mutation induction was calculated to be about 0.8 Gy. For alpha particle irradiation, a linear regression gave a best fit to the data for doses between about 0.25 and 0.9 Gy. In this case the doubling dose was calculated to be about 0.2 Gy. This would suggest a relative biological effectiveness (RBE) of about 4 for alpha particle irradiation compared with low-LET radiation.

85. Further comparative data on mutagenesis in human lymphoblastoid cells have been published by Amundson et al. [A11, A12]. Despite being derived from the same donor, two cell lines, WTK1 and TK6, have very different responses to radiation. Alpha particles from a ^{238}Pu source produced about four to five times more *hprt* mutants per gray in WTK1 cells than did x rays. On the other hand, there was little difference between the dose-response curves for the TK6 cells, although alpha particles were somewhat more effective. In contrast, the *tk* locus was only slightly more sensitive to alpha particles than to x rays, although the WTK1 cells were considerably more sensitive than the TK6 cells. The authors considered that the results suggested that WTK1 cells have an error-prone repair pathway that is either missing or deficient in TK6, and they further suggested that this pathway might be involved in the processing of alpha-particle-induced damage. In all these studies the data could be fitted with a linear dose-response function, with no threshold, although the lowest exposure dose used was about 200 mGy for alpha particle irradiation.

86. Data on the response of TK6 cells exposed to the alpha emitter ^{212}Bi have been reported by Metting et al. [M41]. The radionuclide was added directly to the cell suspension as a chelate complex. The incidence of mutations was a simple linear function of the dose from 200 mGy up to 800 mGy. Induced mutant frequencies were 2.5 10^{-5} Gy^{-1} at the *hprt* locus and 3.75 10^{-5} Gy^{-1} at the *tk* locus.

87. An adaptive response of the kind observed in blood lymphocytes [W6, W15] has also been demonstrated to have an influence on mutation induction. Thus, experiments with the *hprt* locus in human lymphocytes have shown that exposure to tritiated thymidine [S27] or 10 mGy from x rays [K21] can markedly decrease the number of mutations induced by subsequent high doses of radiation. The response to low doses from x rays eliminated about 70% of the effect of a challenging dose of 3 Gy.

88. Similarly, when SR-1 mammary carcinoma cells were irradiated with 10 mGy from x rays and subsequently challenged 18 or 24 hours later with 3 Gy from x rays, approximately one half as many mutations were induced as when the cells were irradiated only with 3 Gy [Z3]. Furthermore, the rate of repair of DNA double-strand breaks, which are the lesions responsible for chromosomal breaks, increased in cells that had been pre-exposed.

89. Wolff [W13] pointed out that the data on mutation induction illustrate two aspects of adaptation that are similar to that observed in blood lymphocytes: after the initial dose it takes time for the induction to occur, and once induced it disappears with time. The SR-1 data show that in this system the effect takes more than 6 hours to become effective and it then disappears if 48 hours elapse between the two doses.

90. In addition to being studied in mammalian systems, radiation-induced mutations have also been studied in plant cells. Mutations can occur in stamen hairs in tradescantia that result in the normal dominant blue colour being replaced by recessive pink. This is a sensitive system for detecting effects at low doses. Dose-response curves for pink mutations have shown for 250 kV x rays a linear response between 2.5 mGy and 50 mGy and for neutrons (0.43 MeV) a linear response between 0.1 mGy and 80 mGy [S28]. In neither case was evidence for a threshold obtained.

D. SUMMARY

91. Damage to DNA in the nucleus is considered to be the main initiating event by which radiation causes damage to cells that results in the development of cancer and hereditary disease. Information on the effects of radiation on individual cells can, therefore, provide insight into the fundamental damage that may ultimately give rise to cancer or hereditary disease. It can also provide information on the consequences of damage to other cellular structures, such as the cellular membrane and the cytoplasm, although damage here is less significant in terms of long-term health effects.

92. Double-strand breaks in DNA are generally regarded as the most likely candidate for causing the critical damage to the nucleus that can subsequently manifest itself as a mutation in somatic or germ cells. Single radiation tracks have the potential to cause double-strand breaks and in the absence of 100% efficient repair could result in long-term damage, even at the lowest doses.

93. In examining the effects of radiation at low doses it has been appropriate to consider how they should be defined. A number of physical and biological approaches have been examined for designating low doses and low dose rates. Microdosimetric arguments suggest low doses will be less than 1 mGy. However, radiobiological experiments on cells in culture suggest that acute doses of about 20 mGy are low, while epidemiological studies suggest that a low dose is of the order of 200 mGy, whatever the dose rate. In addition, studies of tumour induction in experimental animals suggest that dose rates of about 0.1 mGy min^{-1} are low, whatever the total dose.

94. A range of assays is available for evaluating radiation damage to cells occurring either *in vivo* or *in vitro* and the form of the dose-response relationships. In the low-dose region, damage from low-LET radiation can be considered to be due to single tracks acting independently, whereas at higher doses multi-track effects can occur, causing non-linearity in the dose response. In the case of high-LET radiation, the dose response is generally found to be linear.

95. The results of studies of the induction by low-LET radiation of chromosome aberrations in blood lymphocytes, of the transformation of cells in culture, and of somatic mutations in mammalian cell systems at low doses all give results that are somewhat variable. They depend on the experimental design and the effort that went into assessing risks at doses of less than about 1 Gy. In the most comprehensive studies the results are consistent with an increasing incidence with increasing dose at low to intermediate doses. Nevertheless, even very extensive studies, which have taken considerable resources, have demonstrated that it is not practical to obtain information on radiation effects at doses much below about 20 mGy for chromosome aberrations, 100 mGy for cell transformations, and 200 mGy for somatic mutations. The exact form of the response for cellular effects at low doses must therefore remain unclear. One exception is a particularly sensitive system based on the assay of reversion events in a gene mutation in pink-eyed unstable p^{un} mutations in the mouse, which cause a reduction in coat colour. A linear dose response, with no indication of a threshold, was obtained over the dose range for x rays from 10 mGy to 1 Gy. Similarly, pink mutations have been induced in tradescantia stamen hairs at doses down to 2.5 mGy from x rays and with a linear response up to about 50 mGy.

96. For high-LET radiation the experimental data again indicate a linear dose response down to the lowest doses that have been tested. In the case of chromosome aberrations, the lowest neutron dose used was about 50 mGy. For mutation induction, little information is available from mammalian cellular systems at doses much below 200 mGy.

97. The relative biological effectiveness (RBE) of high-LET radiation compared with low-LET radiation varies considerably depending on the biological damage and the dose range. In the case of deterministic effects, caused by cell killing, RBE values are generally less than 10. For stochastic effects, values of RBE depend on the dose, reflecting an essentially linear dose response for high-LET

radiation and a linear-quadratic response for low-LET radiation. The maximum value, RBE_m, occurs at low doses. For exposures to both neutrons and alpha particle irradiation, values of RBE_m depend on the biological endpoint but for cytogenetic damage, cell transformation, and tumour induction are generally greater than 10.

98. A so-called adaptive response has been observed for a number of indicators of cellular damage: a small radiation dose reduces the amount of cellular damage caused by a later higher dose. For the induction of unstable chromosome aberrations and of mutation, it has been demonstrated that a small priming dose of low-LET radiation of about 10 mGy can reduce the effect caused by a subsequent higher dose. This adaptive response seems to take a few hours to manifest itself and then lasts for up to about 40 hours. There are no indications that this would modify the shape of the dose response, although it could alter the magnitude of any effect.

99. The information on the lowest doses at which effects from low-LET radiation have been detected in cellular systems are summarized in Table 5. These are principally for endpoints arising from mutations.

Table 5
Lowest doses at which chromosome aberrations and mutations have been detected in experimental systems exposed to low-LET radiation

System	*Endpoint*	*Radiation*	*Lowest dose* [a] *(mGy)*	*Paragraph* [b]	*Ref.*
Human lymphocytes	Unstable chromosomal aberrations	x rays	20	56	[L8]
Human lymphocytes	Stable chromosomal aberrations	gamma rays	250	59	[L38]
C3H10T½ cells	Cells transformation	x and gamma rays	100	66/69	[M35]
Mouse	Pink-eye mutation	x rays	10	83	[S23]
TK_6 cells	*hprt* and *tk* mutation	x rays	250	80	[K20]
Tradescantia	Pink mutation	x rays	2.5	90	[S28]

a Acute exposures (minutes).
b Text paragraph in which endpoints are further discussed.

II. ANIMAL EXPERIMENTS

100. Studies with experimental animals are important for predicting the long-term effects of radiation in humans. Provided the limitations of such studies are acknowledged, considerable information can be obtained on both radiation-induced cancer and hereditary disease. The most directly relevant data come from studies with mammalian species, with the majority of work relevant to assessing effects at low doses and low dose rates having been carried out with rodents and beagle dogs.

101. Studies with experimental animals are valuable for examining the biological and physical factors that may influence tumour induction by radiation. They can be used to examine the form of dose-response relationships over a wide range of doses; the effect of spatial and temporal distribution of dose; and the influence of factors such as sensitivity of individual organs and tissues, age at exposure, radiation quality, and dose protraction or fractionation on the tumour response. Animal models are also of increasing value in understanding the molecular and cellular mechanisms underlying tumour response (Chapter IV). Quantitative risk coefficients for radiation-induced cancer in humans cannot, however, be based on the results of animal studies because there are differences in radiation sensitivity between different mammalian species.

102. Experimental studies of genetic damage in the offspring of irradiated animals, mainly mice, have been used to assess the hereditary effects of radiation. In the absence of any clear evidence from observations in humans on the risks of radiation-induced hereditary disease, animal studies provide information on dose-response relationships as well as quantitative risk estimates. Studies of germ-cell mutations are also relevant to understanding the dose-response relationship for the initial damage to DNA that could ultimately result in the development of cancer.

A. CANCER

103. Data on radiation-induced tumours in experimental animals were extensively reviewed in the UNSCEAR 1977 and 1986 Reports [U5, U7], by NCRP [N1], by Upton [U22], and in a comprehensive monograph on radiation carcinogenesis [U23]. The effect of dose rate on tumour response was examined by NCRP [N1] and in the UNSCEAR 1993 Report [U3]. As noted in the UNSCEAR 1993 Report, the experimental animals used in many studies are inbred strains with patterns of disease that can be very different from those found in humans. Very many studies have used rodents as the experimental animal. Different strains of mice and rats have varying susceptibilities

to both spontaneous and radiation-induced tumours; furthermore, within a given strain, there are frequently differences between the sexes and ages in the incidence and time of onset of specific tumour types. A number of tumour types for which information is available are either not found in humans (e.g. Harderian gland) or appear to require substantial cell killing for their development and thus may exhibit a threshold in the dose response (e.g. ovarian tumour, thymic lymphoma). For a number of other tumours there may be a human counterpart (e.g. myeloid leukaemia and tumours of the lung, breast, pituitary, and thyroid), but even here there can be differences in the cell types involved and in the development of the tumour. Although data for larger animals are not as extensive, broadly similar findings are found for tumour induction in dogs or other species.

104. There are also substantial variations in the rates of turnover of cells and in the lifespan of most experimental animals compared with humans. Furthermore, the development of tumours in both humans and animals is subject to the modifying influence of various internal and external environmental factors, all of which can potentially influence dose-response relationships. Their development will also depend on the genetic background, the physiological state, and the environmental conditions of the animals. All these factors make it difficult to interpret the results of animal studies and to apply them to humans. Nevertheless, most tumours in laboratory animals appear to arise as clonal growths and to develop, as do most human tumours, through stages of initiation, promotion, and progression. They are therefore of considerable value for helping to understand the form of the dose response for tumour induction in humans and the potential for effects at very low doses.

105. While extensive data exist on tumour induction in laboratory animals exposed either to external radiation or to incorporated radionuclides, many of these studies were carried out in the 1960s and 1970s. The ability to detect radiation-induced cancer at low doses depends, as with epidemiological studies, on the number of animals in the study, the spontaneous incidence of the disease, and the radiation sensitivity of the particular tumour type(s). This is illustrated in Table 6. Thus, in the CBA strain of mouse with a very low spontaneous incidence of acute myeloid leukaemia ($1\ 10^{-4}$) and a high sensitivity to induction by radiation (about $1\ 10^{-1}$ Gy^{-1}), only 300 exposed animals and a similar number of controls would be needed to detect a significant increase (p=0.05) in tumour incidence at a whole-body dose of 100 mGy (low-LET). It would also be possible to detect an effect of 10 mGy with groups of about 4,000 animals. In contrast, for the RFM mouse strain, which has a much higher spontaneous rate of the disease ($7\ 10^{-3}$) and a lower sensitivity ($7\ 10^{-3}$ Gy^{-1}), the number of animals needed to detect a significant increase in acute myeloid leukaemia at 100 mGy would be $1.2\ 10^{5}$, an impractically high number of animals.

Table 6
Statistically determined sample sizes of irradiated and control mice needed to detect a significant increase in tumour risk [a]

Mouse strain	*Tumour*	*Sample size*			
		1 000 mGy	*100 mGy*	*10 mGy*	*1 mGy*
RFM	Thymic lymphoma [b]	1 300	$1.2\ 10^{5}$	$1.2\ 10^{7}$	$1.2\ 10^{9}$
RFM	Myeloid leukaemia [c]	1 700	$1.2\ 10^{5}$	$1.2\ 10^{7}$	$1.2\ 10^{9}$
CBA	Myeloid leukaemia [d]	30	300	4 000	$1.3\ 10^{5}$

a p = 0.05.
b Spontaneous incidence $1.3\ 10^{-1}$; risk of $3\ 10^{-2}$ Gy^{-1} assumed.
c Spontaneous incidence $7\ 10^{-3}$; risk of $7\ 10^{-3}$ Gy^{-1} assumed.
d Spontaneous incidence $1\ 10^{-4}$; risk of $1\ 10^{-1}$ Gy^{-1} assumed.

106. At low radiation doses the number of animals used and their sensitivity is thus important in determining the ability to detect any effect. Animal studies do not generally involve as many individuals as there are in the more extensive epidemiological studies. They do, however, have the advantage that they are planned; the groups of animals exposed are, in general, genetically homogeneous; and the numbers of animals allocated to various dose groups can be chosen to maximize the information obtained. Laboratory animals are exposed to sources of radiation under controlled conditions, and there is much greater certainty associated with the dosimetry. Information may also be available from studies of animals exposed at different dose rates. Data on irradiation of laboratory animals can thus give information for a range of tumour types on the shape of dose-response relationships and provide an estimate of the lowest dose at which a significant effect on the induction of tumours from exposure to ionizing radiation can be observed.

107. Despite a substantial number of research studies on tumour induction in experimental animals potentially available for analysis, there are in practice only a limited number that can help to define the dose-response relationship for cancer induction down to low doses. The range of dose-response relationships that have been obtained in experimental animals and the effect of dose rate on tumour response were reviewed in the UNSCEAR 1993 Report [U3]. Accordingly, only illustrative examples of dose-response relationships are given here for tumour induction following exposure to external radiation and intakes of radionuclides.

1. Dose-response relationships

108. Tumour induction has been demonstrated in laboratory animals exposed to both low- and high-LET radiation. Information on dose-response relationships was previously examined in the UNSCEAR 1986 and 1993 Reports [U3, U5]. In the UNSCEAR 1986 Report, the Committee limited its analysis to those models that appeared to be supported by general knowledge of cellular and subcellular radiobiology. Because most readily induced human tumours, such as those of the breast, thyroid, and lung, as well as leukaemia, did not indicate the existence of a threshold [U5, Annex B, paragraph 108], analyses were confined to the linear no-threshold, the linear-quadratic and the quadratic dose-response relationships. It was considered that these three dose-response relationships provided a general envelope for observation of tumour induction in experimental animals as well as in human populations. In the UNSCEAR 1993 Report [U3], emphasis was placed on examining the effect of dose rate on the tumour response, although information was also given on the dose-response relationships.

109. Dose-response functions other than those adopted in 1986 have also been proposed [E4, G1, U2]. Models that incorporate a threshold assume that there is no response up to some level of exposure, and that thereafter the response increases with dose. Some animals models of tumour induction show this type of response. Dose-response models that incorporate an adaptive response have also aroused some interest. These consider the possibility that stimulated repair of radiation damage as a result of the effect of a toxic agent, including radiation, at low doses would reduce the influence of subsequent, higher doses. The evidence for such an adaptive response was reviewed by the Committee in the UNSCEAR 1994 Report [U2]. Much of the evidence for such a response that is presently available comes from observations of short-term effects in both plants and animals and from studies on cells in culture (Chapter I). Extensive data from animal experiments on dose-response relationships for cancer induction and limited human epidemiological data on low-level exposures have, however, provided no firm evidence that the adaptive response decreases the incidence of late effects such as cancer induction after exposure to low radiation doses. Molecular and cellular studies have shown that DNA damage in the form of double-strand breaks is repairable but that some degree of misrepair is to be expected (Chapter IV). On this basis, it may be concluded that the extent of damage caused by ionization events resulting from exposure to low radiation doses may be influenced by the stimulation of DNA repair mechanisms, but even so, such repair can only be partially effective and for many tumour types cannot entirely eliminate the risk of tumour development following radiation exposure.

110. Published reports of dose-response relationships obtained with various animal species have described responses for different tumour types or life shortening (as a surrogate for tumour induction) using a wide range of functions. Although in many studies dose-effect relationships can be defined by a linear, linear-quadratic, or quadratic response, the data are generally not well defined, particularly at low doses, and alternative fits to the data are also possible. Some animal models also indicate the presence of a threshold for a response. Extensive data are available on a wide range of tumour types including leukaemias and lymphomas arising in haematologic tissue as well as tumours of solid tissues (e.g. lung, liver, and bone). Examples of dose-response relationships for exposures to both external radiation and internally incorporated radionuclides are given below. The studies have been chosen to illustrate the various patterns of dose response that have been obtained with some emphasis on those that give information at low doses.

(a) External radiation

111. ***Life shortening.*** Extensive studies in laboratory animals have reported life shortening as a result of whole-body external irradiation. This is a precise biological endpoint and reflects the early onset of lethal diseases, an increased incidence of early occurring diseases, or a combination of the two. At radiation doses up to a few gray (low-LET), life shortening in experimental animals appears to be mainly the result of an increase in tumour incidence. There is little suggestion that there is a general increase in other non-specific causes of death [G14, M39, S38]. At higher doses, into the lethal range, a non-specific component of life shortening becomes apparent owing to cellular damage to the blood vasculature and other tissues. It was concluded in the UNSCEAR 1993 Report [U3] that life shortening at low to intermediate doses can be used as a basis for examining the factors that influence dose-response relationships for tumour induction.

112. The majority of comprehensive studies that give quantitative information on dose-response relationships for life shortening from exposure to low-LET radiation as well as on factors such as the effects of age, dose fractionation and dose rate have used the mouse as the experimental animal. Substantial differences in sensitivity have, however, been noted between strains and between the sexes. A review of 10 studies involving about 20 strains of mice given single exposures to x or gamma radiation showed that estimates of life shortening ranged from 15 to 81 days Gy^{-1}, although the majority of values (9 of 14 quoted in the review) were between 25 and 45 days Gy^{-1}, with an overall unweighted average of 35 days Gy^{-1} [G14]. In general, in the range from about 0.5 Gy to acutely lethal doses, the dose response was either linear or curvilinear upwards. In male BALB/c mice exposed to acute doses of ^{137}Cs gamma rays (4 Gy min^{-1}), life shortening was a linear function of dose between 0.25 and 6 Gy, with a loss of life expectancy of 46.2 ± 4.3 days Gy^{-1} [M39]. Similar data have been reported on B6CF3 mice irradiated at 17 days before birth or at various times up to 365 days after birth [S38], although the lowest dose used was 1.9 Gy. The effects of acute single doses on life shortening in other species were summarized in the UNSCEAR 1982 Report [U6], although they are not as comprehensive as the data for mice.

113. The effect of dose fractionation appears to be very dependent on the strain of mouse and the spectrum of dis-

eases contributing to the overall death rate. Overall no clear trend in the effect of dose fractionation on life shortening could be found [U3], and the results from a number of studies suggested that when compared with acute exposures, the effects of dose fractionation are small and in some studies have given either small increases or small decreases in life-span. When the effects in mice of acute exposures to low-LET radiation are compared with those of protracted irradiation given more or less continuously, the effectiveness of the radiation decreases with decreasing dose rate and increasing time of exposure. With lifetime exposures there is some difficulty assessing the total dose contributing to the loss of lifespan. The results available suggest, however, that with protracted exposures over a few months to a year, the effect on life shortening is reduced by factors of between about 2 and 5, compared with exposures at high dose rates.

114. The results of a number of studies on life shortening as a result of exposure to high-LET radiation were examined in the UNSCEAR 1993 Report [U3]. The data were all reasonably consistent and suggest that the dose response for life shortening is a linear function of dose, at least for total doses up to about 0.5 Gy, and that neither dose fractionation or dose protraction has much effect.

115. ***Tumour induction.*** In the late 1970s, Ullrich and Storer published a series of studies on tumour induction in mice (see, for example, [U16, U17, U18]). The data have provided comprehensive information on the effects of dose and dose rate on the induction of a range of neoplastic diseases, including myeloid leukaemia and solid tumours of the ovary, pituitary, lung, and thymus.

116. In a large study in female RFM mice, animals were exposed to acute doses from ^{137}Cs gamma rays (0.45 Gy min^{-1}) at 10 ± 0.5 weeks of age [U16]. Groups of animals received a range of doses (0, 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 Gy), were followed for their lifespan, autopsied at death, and diagnosed for various types of neoplastic disease. Dose-response data were obtained for a range of tumour types. A significant increase in the incidence, I (%), of acute myeloid leukaemia was obtained at doses of 1.0 Gy and above. A linear dose response of the form $I = 0.63 + 1.4D$, where D is the dose in gray, adequately described the data, and the doses were not high enough for a cell-killing term to have become apparent. A linear-quadratic model, $I = 0.69 + 0.86D + 0.00227D^2$, also provided a fit to the data, although the dose-squared term was not significant. Ullrich and Storer [U18] published further data on myeloid leukaemia in female RFM mice exposed under similar conditions. The results were similar to those published earlier, but with fewer exposure points (0, 0.5, and 2.0 Gy).

117. The information on myeloid leukaemia induction in mice for these two data sets has been combined in Table 7. An analysis of the combined data carried out for this Annex indicates that the incidence of myeloid leukaemia is increased over controls at doses of about 0.5 Gy and above. The data have been fitted with linear, linear-quadratic, and threshold-linear dose responses. All three models give a good fit to the data, and in the case of the threshold-linear model, a threshold at about 0.22 Gy can be obtained (Figure X, Table 8). These studies by Ullrich and Storer [U16, U18] involved a total of nearly 18,000 mice, and yet the information at low doses is equivocal because of the small numbers of acute myeloid leukaemias occurring. Few other animal studies have been carried out on such a scale, and this clearly illustrates the limited ability of such animal studies to provide detailed information on the effects of whole-body radiation at low doses.

Table 7
Myeloid leukaemia incidence in female RFM mice exposed to acute doses of gamma rays [U16, U18]

Dose (Gy)	*Number of animals*	*Incidence*
0	4 763	0.72 ± 0.10
0.1	2 827	0.72 ± 0.15
0.25	965	0.84 ± 0.30
0.5	1 918	1.17 ± 0.26
1	1 100	1.60 ± 0.41
1.5	1 054	3.6 ± 0.76
2	1 099	3.22 ± 0.43
3	4 133	5.2 ± 0.51
Total	17 859	

Table 8
Model fits to data on myeloid leukaemia in mice exposed to ^{60}Co gamma rays

Function	*C*	*α*	*β*	*D_0*	*χ^2*	*DF*
$I = C + \alpha D$ [a]	0.64 ± 0.09	1.39 ± 0.13	-	-	4.7	6
$I = C + \alpha D\ \beta D^2$ [b]	0.69 ± 0.09	0.87 ± 0.39	0.22 ± 0.15	-	2.7	5
$I = C + \alpha(D - D_0)$ [c]	0.72 ± 0.08	1.55 ± 0.18	-	0.22 ± 0.14	2.6	5

a Linear. *b* Linear-quadratic. *c* Linear-threshold.

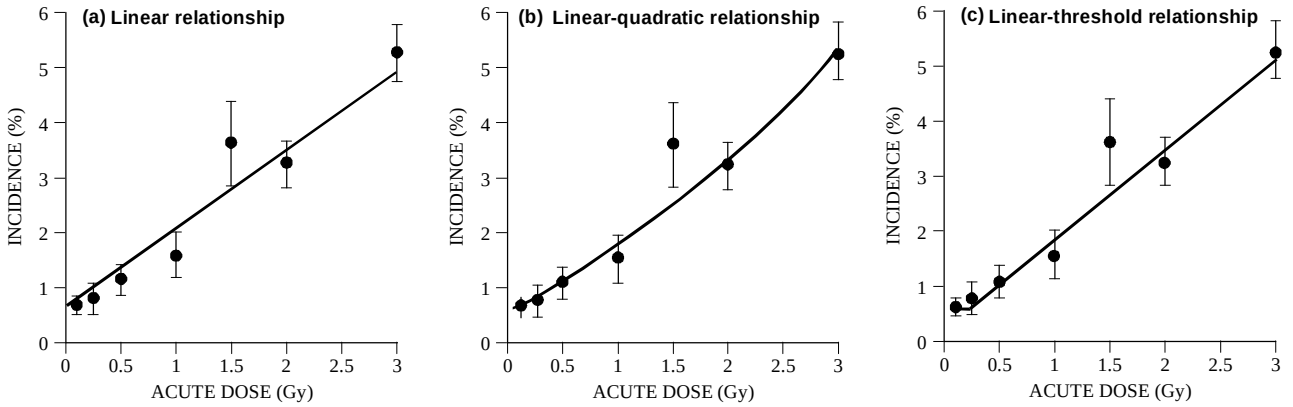

Figure X. Dose-response relationships fitted to data on myeloid leukaemia in female RFM mice [U16, U18].

118. The induction of lung tumours has been compared in female BALB/c mice given doses from ^{60}Co gamma rays in the range 0.5–2 Gy at two dose rates (0.4 Gy min^{-1} and 0.06 mGy min^{-1}) [U18, U24]. Tumour induction was less at low dose rates than at high dose rates. After high-dose-rate exposure, the age-correlated incidence, I (%), could be represented by a linear function, [I(D) = 13.4 + 12D; p>0.5], where D is the absorbed dose in gray. At low dose rates, a linear function also gave a good fit to the data [I(D) = 12.5 + 4.3D; p>0.8]. The data were adjusted for differences in the distribution of ages at death among the treatment groups, and the authors indicated there were no changes with age over the period of irradiation. The differences in slope were taken to indicate variations in effectiveness for tumour induction at the two dose rates. The data were subsequently extended to provide additional information at the high dose rate (0.4 Gy min^{-1}) in the dose range from 0.1 to 2 Gy [U14]. Although the tumour incidence data could again be fitted with a linear dose response [I(D) = 10.9 + 11D; p>0.70], a linear-quadratic dose response [I(D) = 11.9 + 4D + 4D^2; p>0.70] would also give a fit. In this extended analysis, the linear term was very similar to that obtained in the low-dose-rate study, and it was concluded by the authors that the result was consistent with a linear-quadratic response in which the linear term is independent of dose rate, at least for the dose rates used in the study.

119. One of the most extensively studied tumours in the mouse is that arising in the thymus. The dose response for the induction of thymic lymphomas by acute whole-body irradiation found in a number of studies has been of the threshold type (Figure XI). Thus, Maisin et al. [M39] exposed 12-week-old male mice to single or fractionated doses of ^{137}Cs gamma rays (4 Gy min^{-1}) in the dose range from 0.25 to 6 Gy. The dose-response curve was of a threshold type; the incidence of thymic lymphomas rose above that in controls only following exposures at 4 Gy and above. Similarly, Ullrich and Storer [U18, U24] studied the dose-response relationship for thymic lymphoma in female RFM/Un mice. Exposures were at 0.45 Gy min^{-1} and 0.06 mGy min^{-1}. For the highest dose rate, the incidence of lymphoma up to 0.25 Gy increased with the square of the dose, although a threshold for a response up to about 0.1 Gy could not be excluded. Linearity was rejected over this limited dose range. From 0.5 to 3 Gy the increase in incidence with dose was nearly linear. At the lower dose rate the response was best described by a linear-quadratic dose response with a shallow (perhaps zero) initial linear slope, again allowing the possibility of a threshold at low total doses.

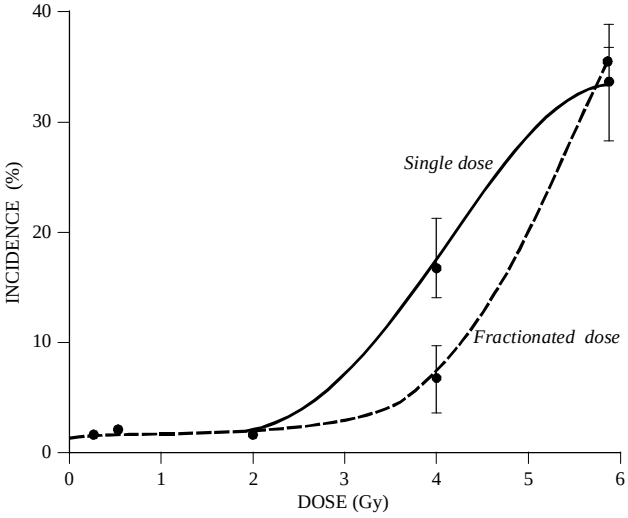

Figure XI. Incidence of thymic lymphoma as a function of dose for single or fractionated x rays [M39].

120. The induction of ovarian tumours in mice exposed to x rays or gamma radiation has also indicated the presence of a threshold in the response for some strains, and this is reflected in a pronounced effect of dose and dose rate [U17, U18, U21, U24]. Thus, in SPF/RFM mice exposed at 0.45 Gy min^{-1}, a significant increase in tumour incidence was obtained at doses from 0.25 to 3 Gy [U18]. The data could be fitted with a linear-quadratic dose response with a negative linear component [I(D) = 2.3 + (−23)D + 1.8 D^2; p>0.25] or by a threshold plus quadratic model [I(D) = 2.2 + 2.3 (D−D*)^2; p>0.75], where the threshold dose, D*, was estimated to be 0.12 Gy. Linear and quadratic dose responses were rejected. This pattern of response is considered to reflect the fact that ovarian tumour development in the mouse seems to follow changes in hormonal status that occur after substantial killing of oocytes. For low-dose-rate exposures, cell killing is less effective, and as a consequence there is a substantial reduction, by a factor of about 6, of

effectiveness in inducing tumours at low dose rates. The results suggested the possibility of a threshold up to about 0.115 Gy.

121. A further substantial study in the mouse has demonstrated that dose-response relationships for tumour induction can vary in different organs and tissues [S39]. Groups of $B6C3F_1$ mice were exposed to various doses between 0.48 and 5.7 Gy low-LET radiation from ^{137}Cs gamma rays. The dose-response curves for tumour induction in the liver, pituitary, ovary, and lungs were convex upwards in the dose region examined, with a significant increase in numbers of tumour at 0.48 Gy. The data suggested a progressive increase with dose up to about 1 Gy. A subsequent gradual increase to the highest incidence obtained was seen and then a declining incidence at doses above about 1.5 to 3 Gy, depending on the tumour type. The results could be interpreted as showing an increasing risk with dose up to the maximum incidence, although the lack of data below 480 mGy limited the ability to elucidate the dose response at low doses. In contrast, the shape of the dose response for bone tumour induction was quite different from that for other solid tumours: the initial slope was concave upwards, with the highest incidence observed in the group given 3.8 Gy. Bone tumour incidence up to about 3 Gy was a function of the square of the dose, and the existence of a threshold could not be excluded because the incidence of bone tumours in groups irradiated with doses below 1.43 Gy was not significantly increased.

122. Variations in sensitivity to radiation-induced mammary cancers in different strains of mice and rats are well known, although the reasons underlying these differences are not well understood. Thus studies of mammary carcinogenesis in Sprague-Dawley, WAG/Rij, and BN/BiRij rats have shown that only in WAG/Rij rats was an appreciable number of carcinomas induced by radiation [V3]. Analysis of data on radiation-induced mammary tumours gave a linear dose-response function for fibroadenomas in Sprague-Dawley rats and for both fibroadenomas and carcinomas in WAG/Rij rats after irradiation with either 0.5 MeV neutrons or x rays. In the case of exposure to x rays, the lowest data point was at 200 mGy (Figure XII).

123. Studies of mammary tumours in mice by Adams et al. [A13] have demonstrated that irradiation resulted in many more transformed mammary cells than are ultimately expressed as tumours. A later study by Ullrich et al. [U26] examined possible reasons for differences in sensitivity in sensitive BALB/c and resistant C57BL and B6CF1 hybrid mice. They demonstrated that variations in sensitivity could be correlated with differences between strains in the sensitivity of the mammary epithelial cells to radiation-induced transformation. Differences in sensitivity could not, however, be accounted for by differences in the number of sensitive cells or by systemic or cellular influences on progression. This observation of inherent differences in sensitivity to radiation-induced tumour initiation may be one approach to understanding the mechanism by which radiation induces cancer in these different mouse strains and may have more general application.

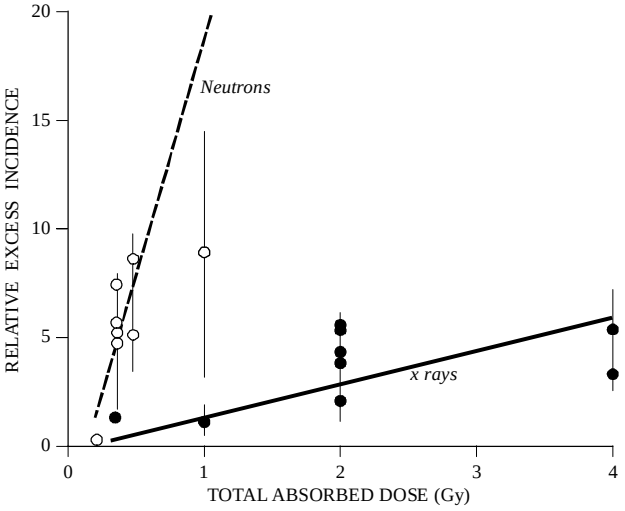

Figure XII. Relative excess incidence of carcinomas in WAG/Rij rats after irradiation with x rays and 0.5 MeV neutrons [B34].

124. A unique experimental system has been described by Tanooka and Ootsuyama [O8, T13] in mice. The backs of female ICR mice were irradiated with beta particles from ^{90}Sr-^{90}Y three times a week throughout life. At radiation doses per exposure between 1 Gy and 11.8 Gy (low-LET), the tumour incidence was 100%. At 0.75 Gy per exposure, however, no tumours occurred in 31 mice over a period of 790 days from the start of irradiation. One osteosarcoma did arise at 791 days and one squamous cell carcinoma at 819 days. This was despite the fact that the cumulative dose was extremely high (305 Gy in 950 days). The appearance of tumours in irradiated mice depended on a fractionated regime: no tumours occurred following single exposures with doses up to 30 Gy. At such doses depilation and severe skin damage occurred. The authors proposed that at the lower dose fractionation regime efficient repair occurs, resulting in an apparent threshold in the tumour response. No histological information was reported, but it seems likely that at these high doses deterministic damage would occur, resulting in the development of a fibrotic response preceding tumour development.

125. Tumour induction in rats and mice exposed to high-LET neutron irradiation was described in the UNSCEAR 1993 Report [U3] and has also been summarized by Broerse [B34] and Fry [F15]. In general the experimental results reviewed indicated that there are differences between tissues in their tumorigenic response following either dose fractionation or reductions in dose rate as compared with acute radiation exposures. Taken together, however, the effects of dose rate and fractionation are small, and for the majority of studies a linear dose response would give a good fit to the data up to about 1 Gy. Exceptions are tumour types for which cell killing seems to play a significant role in tumour induction, as for thymic lymphomas, when a threshold dose may be found. In many cases, however, information is not available down to low levels of exposure. For life shortening at low doses, which has been shown to be the result of tumour induction, again little effect of fractionation or dose rate has been found [F15, U3].

(b) Internally incorporated radionuclides

126. In the case of intakes of radionuclides, many factors in addition to those for external radiation exposure may influence the dose response. For radionuclides such as ^{90}Sr or ^{239}Pu with a long physical half-life and a long biological half-time in the body, radiation exposure after an intake will generally be for the remaining lifespan of the animal, making it difficult to relate tumour incidence to radiation dose. Additional difficulties in interpreting dose-response data arise from the heterogeneous distribution of dose between and within body organs and tissues as well as temporal changes in the distribution of radionuclides, and hence dose, within the body. A key factor in the calculation of the radiation dose is the identification of the sensitive "target" cells at risk. When a radionuclide is uniformly distributed throughout an organ or tissue, as is the case for tritiated water or ^{137}Cs, then the calculation of average tissue dose is sufficient to assess the dose to these critical cells. In other cases, however, as with the bone-seeking radionuclides ^{239}Pu and ^{241}Am, the distribution of dose may be very heterogeneous, and then the calculation of dose to sensitive cells is essential in assessing dose-response relationships.

127. ***Stem cells.*** The International Commission on Radiological Protection (ICRP) has developed a comprehensive set of biokinetic and dosimetric models to enable the calculation of organ doses from inhaled or ingested radionuclides [B36, I9, I10]. These models take account of the distribution and retention of radionuclides in individual organs and the proportion of the energy of decay deposited in different organs. For penetrating photon radiation, it is necessary to take account of crossfire between organs, but in these cases the calculation of average energy absorbed in a tissue is sufficient. For non-penetrating alpha and beta radiations, energy is taken to be deposited in the organ in which the radionuclide is retained. For these radiation types it is necessary in some cases to take account of the distribution of the radionuclide within the organ relative to sensitive target cells. This consideration has been addressed in models developed by ICRP for the respiratory and gastrointestinal tracts, the skin, and the skeleton. In the case of other tissues (for example the liver, kidneys, and spleen), the average tissue dose is calculated on the assumption that sensitive cells are uniformly distributed throughout them. In relation to intakes of radionuclides, only the respiratory tract, the gastrointestinal tract and the skeleton are directly relevant. Radiation doses to the sensitive cells in the skin are, however, important in the case of radionuclides deposited on the surface of the body.

128. ***Respiratory tract.*** The ICRP model for the human respiratory tract [I11] takes account of the distribution of sensitive cells for cancer induction in the extrathoracic region and the bronchial and bronchiolar regions of the lung. For the region of the lung in which gaseous exchange occurs, the alveoli and terminal bronchioles, the dimensions of the structures are considered to be sufficiently small for doses to be calculated on the assumption that sensitive cells are uniformly distributed.

129. The extrathoracic region of the respiratory tract (the nose, oropharynx, and larynx) are lined mainly with stratified squamous epithelium. Excess nasal and laryngeal cancers have been observed in luminizer workers and patients receiving head and neck exposures [B37] but not in atomic bomb survivors or patients treated for spondylitis [D12]. Sinonasal cancers were described in humans as a result of systemic contamination with radium [E11, F13]. Radiation-induced tumours were mainly carcinomas, including basal cell, squamous cell, and epidermoid carcinomas, for which the cells at risk are assumed by ICRP [I11] to be the basal cells of the epithelial layer with their nuclei at average depths of 40–50 μm.

130. The trachea, bronchi, and bronchioles are lined by a pseudostratified, ciliated, columnar epithelium separated from the subepithelial connective tissue by a prominent basement membrane. Radiation-induced lung cancers have been documented in uranium miners, atomic bomb survivors, and therapeutically irradiated patients [B38, I12, P16]. Lung cancers occur predominantly in the bronchial region; there is no evidence that radiation induces tracheal cancer. There are four main classes of tumour observed: squamous cell carcinoma (most frequent), small-cell carcinoma, adenocarcinoma, and large-cell carcinoma. It appears that these tumour types share the same endodermic progenitors [M44, Y5]; the most likely candidate cells for tumour induction were considered by ICRP to be secretory cells [T15]. Basal cells may also be involved, although their role may be limited [J7]. ICRP therefore assumes for dosimetric purposes that the sensitive cells in the bronchial region are secretory and basal cells, with nuclei at average depths of 10–40 μm and 35–50 μm, respectively [I11]. The sensitive cells in the bronchiolar region are taken to be secretory cells, with nuclei at an average depth of 4–15 μm.

131. Estimates of dose to the lung from short-range emitters, particularly alpha emitters, depend on the assumptions made regarding the depth and thickness of the sensitive layer in the bronchi and bronchioles. For example, in a recent sensitivity analysis of doses from radon progeny, a dose range that varied by a factor of 2.6 resulted from consideration of sensitive cell parameters [M45].

132. ***Gastrointestinal tract.*** The current dosimetric model of the gastrointestinal tract makes only a simple generalized allowance for the position of sensitive cells relative to ingested radionuclides [I9]. Doses are calculated separately for the mucosal layer of each region modelled: the stomach, small intestine, upper large intestine, and lower large intestine. For penetrating radiations, the average dose to the wall of each region is used as a measure of the dose to the mucosal layer. For non-penetrating radiations, the fraction absorbed by the mucosal layer is taken to be equal to 0.5v/M, where M is the mass of the contents of that section of the gastrointestinal tract and v is a factor (between 0 and 1) representing the proportion of energy that reaches sensitive cells. The factor of 0.5 is introduced because the dose at the surface of the contents will be approximately half that within the contents for non-penetrating radiations. For beta particles, v is taken to be 1.

For alpha particles, v is taken to be 0.01. This value is based on weak experimental evidence from an acute toxicity study in rats in which the LD_{50} for ingested ^{91}Y was estimated at about 12 Gy while a more than 100 times greater dose to the mucosal surface from ^{239}Pu had no effect [S33].

133. This model is currently being revised. The new model is expected to consider the location of sensitive cells in all regions of the alimentary tract, from the mouth to the large intestine. Radiation-induced cancer in human populations has been documented for the oesophagus, stomach, and colon; the small intestine is not a significant site for cancer induction [B39]. The sensitive cells in the oesophagus are assumed to be the basal layer of the stratified squamous epithelial lining. This epithelium is quite thick (300–500 μm) and is protected by a surface layer of mucus. At the gastro-oesophageal junction, it is abruptly succeeded by a simple columnar epithelium with gastric pits and glands. In the stomach, the sensitive cells for cancer induction are assumed to be the epithelial stem cells, located within but towards the top of the gastric pits, at a depth of about 75–100 μm from the surface. In the small intestine, stem cells are located above the paneth cells, towards the base of the crypts. In the large intestine, stem cells are situated at the very base of the crypts. These locations have been deduced from a variety of cell kinetic, mutational, and regeneration studies in mouse models [P17] and their positions are likely to be qualitatively similar in man. The number of stem cells per colonic crypt in mice has been estimated to be in the range 1–8, and as colonic crypts in man are around six times as large as in mice, it is possible that the number of stem cells per crypt may be greater in man. The depth of the stem cells, measured in human tissue samples, is about 100–150 μm in the small intestine and 200–400 μm in the large intestine [P18].

134. ***Skin.*** The skin is broadly divisible into two component layers: the outer epidermis and the underlying dermis. The epidermis arises from a single basal layer of cells, overlaid by layers of cells with dead layers on the outer surface. The basal layer is separated from the dermis by a basement membrane. This boundary is not flat but undulates, with discrete points known as rete pegs where the epidermis projects down into the dermis. In addition, the basal layer extends around the skin appendages, notably the shaft and base of the hair follicles, which project even deeper into the dermis. At some sites on the body, over 50% of the basal layer stem cells are associated with the hair follicles. Thus, the depth of the basal layer is highly variable. In most body areas it ranges from 20 to 100 μm in the interfollicular sites, but exceptionally (e.g. the finger tips), it can be over 150 μm deep because of increased outer cornification [L46]. The deeper projections associated with hair follicles result in basal cells being situated more than 200 μm deep.

135. There is substantial evidence linking the incidence of non-melanoma skin cancer (NMSC) with exposure to ionizing radiation, including studies on irradiated children and atomic bomb survivors [L46]. The two main types of non-melanoma skin cancer are squamous cell carcinoma and basal cell carcinoma, with the sensitive cells for cancer induction assumed to be the basal layer of cells in each case. This assumption is supported by animal data [A15, H28].

136. In calculating dose to the skin, ICRP has recommended that skin dose should be evaluated at an average depth of 70 μm [I2]. However, when assessing dose in cases of non-uniform exposure, it may be necessary to use skin thickness values appropriate to the area of interest.

137. ***Skeleton.*** Biokinetic and dosimetric models for the skeleton take account of the two main types of bone, cortical and trabecular, and the behaviour of different bone-seeking radionuclides as well as the location of sensitive cells for the induction of bone sarcoma and leukaemia [I9, I10]. Cortical bone is the hard, dense bone that forms the outer wall of bones and the whole of the shaft of long bones. Trabecular bone is a soft, spongy bone with a lattice-work structure that is found within flat bones and in the ends of the long bones. The endosteal layer of cells on the inner bone surfaces in cortical and trabecular bone is taken to be the sensitive cells for bone sarcoma and the red bone marrow is taken to be the sensitive cells for leukaemia. It is assumed that all haemopoietically active red marrow is confined to the spaces in trabecular bone in adults, with cortical bone containing inactive yellow marrow. In children, a proportion of cortical marrow is assumed to be haemopoietically active and therefore a target for leukaemia induction. In its 1979 Report, ICRP classified bone-seeking radionuclides into two groups: bone-surface seekers, including the actinide elements, and bone-volume seekers, including the alkaline earth elements [I9]. Thus, radionuclides were assumed to be retained either on endosteal bone surfaces or uniformly distributed throughout the volume of bone mineral. Absorbed fractions were calculated for the proportions of alpha and beta energy emitted in each case that would be deposited in the sensitive regions of the endosteal layer, taken to lie within 10 μm of bone surfaces and red marrow. More realistic biokinetic models have since been developed for the main bone-seeking radionuclides, isotopes of the actinides, alkaline earths and similar elements, which allow for initial deposition on bone surfaces, movement into bone owing to bone remodelling and chemical exchange, and loss from bone, principally owing to bone resorption [I10]. For the actinides, transfer from bone to marrow is also included.

138. An increased incidence of bone sarcomas has been observed in populations exposed to alpha-emitting radium isotopes, particularly in painters of luminuous dials, but also radium chemists and people treated with radium salts for the supposed therapeutic effect [M18]. Although the ICRP assumption [I8] that the sensitive cells constitute a 10-μm-thick layer on endosteal surfaces gives reasonable dose estimates, it has been suggested that all bone surfaces may not be equally sensitive [P19] and that the sensitive region may include cells at a greater depth into the marrow [G15]. Priest [P19] argued that the observed difference in toxicity between ^{226}Ra (half-life = 1,600 years) and ^{224}Ra (3.6 days) in animals and humans cannot be explained simply in terms of a greater wastage of alpha dose from the longer-lived ^{226}Ra within bone mineral. He suggests that a

greater proportion of alpha dose from ^{224}Ra may be delivered to active trabecular surfaces and that these regions have a greater than average sensitivity.

139. Gössner et al. [G15] have reviewed the histopathology of radiation-induced bone sarcomas, showing that there are of two main types, bone-producing osteosarcomas, and non-bone-producing sarcomas of the fibrous-histiocytic type. A trend to a greater proportion of fibrous-histiocytic tumours was identified at lower doses and shorter latency periods. The data suggest that cells at risk are not only those committed to bone formation on the bone surfaces but multipotent marrow stromal cells located at some distance from the bone surface.

140. Excess leukaemia has been recorded in patients exposed to the alpha-emitting contrast medium thorotrast and in the atomic bomb survivors, but it is not a feature of exposure to isotopes of radium [I9, M46]. Comparison of leukaemia induction by thorotrast and external low-LET irradiation suggests a low RBE for alpha-induced leukaemia. The inability of ^{226}Ra to induce leukaemia [R16] may be explained by a low alpha RBE, but the distribution of sensitive cells in the marrow may also be a contributory factor. While the colloidal thorium oxide preparation thorotrast was retained in macrophages throughout the marrow, radium on bone surfaces delivers a dose only to peripheral marrow, and it may be that sensitive cells are concentrated more towards the centre of marrow spaces. Some evidence for this was provided by studies using mice [L47]. It may be, therefore, that the ICRP assumption that sensitive cells for leukaemia induction are uniformly distributed throughout red marrow [I9] may overestimate the risk of leukaemia from bone-seeking radionuclides.

141. ***Tumour induction.*** A number of reviews and papers have examined dose-response relationships for tumour induction in animals exposed to either alpha emitters or beta/gamma emitters (see, for example, [I5, L27, M11, N6, Y6]). Most information is available on the induction of bone tumours following the entry of radionuclides into the blood or lung tumours after inhalation of radioactive materials in various chemical forms, although more limited data on other organs and tissues are also available. A wide range of dose-response relationships has been obtained. These encompass data that can be fitted with simple linear models up to intermediate levels of dose and other responses with clear evidence of a threshold. The results of studies on tumour induction from intakes of radionuclides are illustrated by data on tumour induction in the lungs and skeleton.

142. ***Bone tumours.*** The incidence of bone tumours in mice, rats, dogs, and pigs given graded doses of ^{90}Sr was examined by Mays and Lloyd [M11]. Although limited data were available at low doses, and the various species had different sensitivities to tumour induction, in all cases the incidence of bone tumours at the lowest doses examined was less than would have been predicted on the basis of a linear dose response. Thus, in beagle dogs with average skeletal doses from ^{90}Sr at 1 year before death of between 0.27 Gy and 111 Gy, no tumours were found in the three lowest dose groups (1, 3.35, and 5.97 Gy), with an 8% incidence occurring at 21.7 Gy [N6]. The numbers of dogs in each group was, however, only about 12, and a small increase in incidence could not have been detected. Similar data have been reported for osteosarcoma induction by ^{90}Sr in female CF_1 mice. In groups of about 100 animals with average bone doses ranging from 0.26 to 120 Gy, no significant increase in tumour incidence was found in animals with average doses below about 10 Gy (1.3, 4.5, and 8.9 Gy) [M11, N6].

143. An extensive series of studies has examined tumour induction in animals given various alpha emitters. Lloyd et al. [L27] examined the occurrence of skeletal tumours in young adult beagle dogs given single intravenous injections of monomeric ^{239}Pu citrate. The relationship between the incidence of osteosarcoma and average dose to bone at the presumed time of tumour initiation, taken to be at 1 year before death, appeared to be linear below about 1.3 Gy (26 Sv, assuming an RBE for alpha radiation of 20) (Figure XIII). The observed tumour incidence, I (%), could be approximated by the expression I = 0.76 + 75D, where D is the average dose to bone in gray. Similar analyses of data from dogs given ^{226}Ra also gave a linear response with the expression I = 0.76 + 4.7D (for doses up to 20 Gy). The ratio of the coefficients (75/4.7 = 16±5) shows that ^{239}Pu is more effective in inducing osteosarcoma than ^{226}Ra. This is thought to be due to the tendency of plutonium to remain longer on bone surfaces and to more effectively irradiate the sensitive cells for tumour induction.

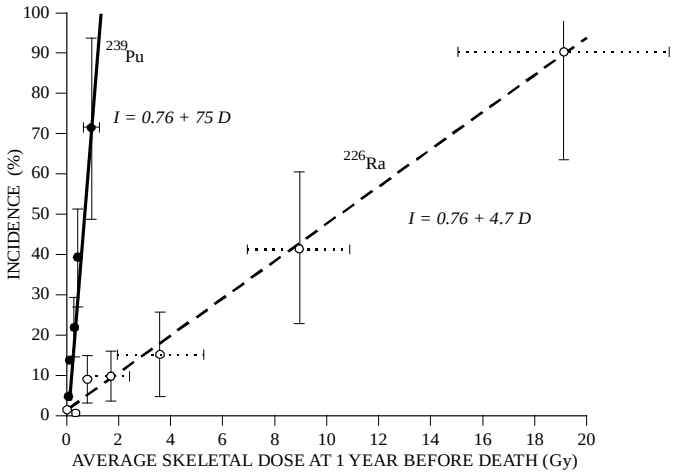

Figure XIII. Bone cancer incidence in beagle dogs following a single injection of plutonium-239 or radium-226 citrate at about one year of age [L14].
The ratio of plutonium to radium dose coefficients (75:4.7) is 16.

144. Further data on bone tumour induction in animals given alpha emitters were analysed by Mays and Lloyd [M17]. They found that although the induction of bone tumours appeared to increase linearly with dose in some cases, in others it followed threshold or sigmoid relationships. In CF_1 female mice injected intravenously at 70 days of age with ^{239}Pu [F11], no tumours were observed in groups of mice with average bone doses of 0.01 Gy (N=99) and 0.22 Gy (N=96), whereas a linear response would have predicted some 5.3 cases. The

probability of observing zero cases, if 5.3 cases is the true number, is only 5%. At 0.4 Gy and above, bone tumour incidence increased linearly with dose.

145. A linear dose response was found for osteosarcoma induction in female CF_1 mice given ^{226}Ra by intravenous injection at 70 days of age. In 1,436 mice with average bone doses below 3 Gy (high-LET), 115 cases of osteosarcoma were observed, in good agreement with 92 cases predicted using a linear dose response [F12, M17]. In contrast, in beagle dogs given ^{228}Ra and ^{228}Th, the dose-response data suggested the presence of thresholds at about 2 Gy and 0.5 Gy (high-LET), respectively [M17].

146. More complex models have also been developed to interpret dose-response relationships for bone tumour induction. Raabe [R1] has described an example for predicting risks associated with protracted exposure to ionizing radiation from internally deposited radionuclides. For long-lived radionuclides such as ^{90}Sr, ^{226}Ra, or ^{239}Pu, the radiation dose will be delivered over the lifespan of the animal. Raabe et al. [R1, R14] have interpreted the data from various lifetime studies with beagle dogs exposed by injection, ingestion, and inhalation to either beta emitters or alpha emitters. The cumulative absorbed dose required to give a specified level of cancer risk was found to be less at lower dose rates than at the higher dose rates, and the induction time required for tumours to manifest themselves tended to be longer at lower dose rates and could exceed the normal lifespan of the animal. The authors interpreted the data to suggest that at the lowest dose rates there is an effective threshold for the induction of fatal radiation-induced cancer.

147. For example, beagle dogs given eight fortnightly injections of ^{226}Ra in amounts from 0.099 kBq kg^{-1} to 46.3 kBq kg^{-1} received average lifetime skeletal doses from 0.9 ± 0.2 Gy to 167 ± 44 Gy (±1 SD). Death in these dogs was considered to be a function of three effects: (a) spontaneous death arising from causes associated with the natural lifespan, (b) death associated with radiation-induced bone tumours, and (c) death from radiation-induced skeletal injury such as radiation osteodystrophy and bone fractions occurring at high doses (Figure XIV). Mathematical three-dimensional dose-rate/time response models with log-normal probability distributions were fitted to the lifespan data for the dogs. The data plots indicated that bone cancer predominates as a cause of death at intermediate doses and is infrequent at low dose rates (because of death associated with natural lifespan) and at high dose rates (because of deaths from acute radiation injury). The cumulative dose required to cause bone cancer is smaller at the low dose rates; however at lower dose rates it takes longer to reach any specified level of risk, perhaps longer than the natural lifespan of the animal. This results in a lifespan effective threshold for cancer induction similar to the "practical" threshold described by Rowland [R17] at a cumulative lifespan alpha dose of about 1 Gy in man (see Chapter III). In practice, the lack of a significant effect during the lifespan of the animals could also be taken to indicate a risk of cancer with a very low probability of occurrence at low doses.

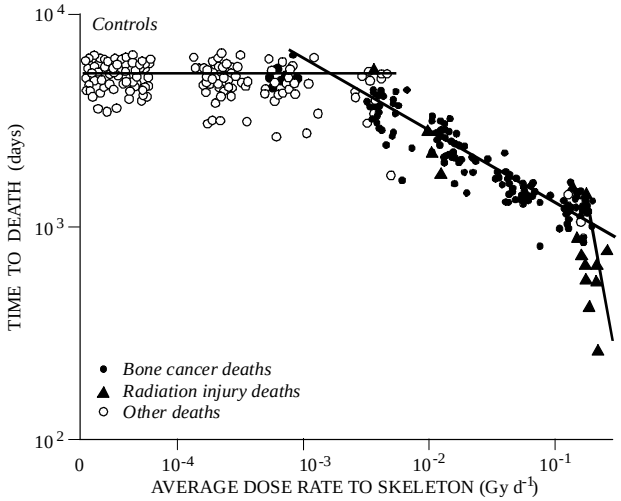

Figure XIV. Deaths from non-neoplastic radiation injury, bone cancer and other causes in beagle dogs injected with ^{226}Ra. Initial intake occurred at 435 days of age [R1].

148. Raabe et al. [R14] have also compared data on bone tumour induction in humans and CF_1 mice with data obtained in beagle dogs. When time was normalized with respect to lifespan, the three species were found to have bone cancer dose-rate/time risk functions that were almost identical and could be represented by one median regression line.

149. ***Lung tumours.*** Extensive data have also been published on lung tumour induction in rodents and beagle dogs exposed to internally incorporated alpha and beta/gamma emitters. Studies conducted in the 1960s and 1970s were considered by a Task Group of Committee 1 of ICRP [I5]. The data reviewed were from laboratories around the world and from studies using a range of different protocols and methodologies. One specific aim of the analyses was to determine the relative effectiveness of alpha emitters and beta/gamma emitters in causing lung damage, including neoplastic development.

150. The Task Group commented on some of the difficulties in ascertaining the dose response for lung tumour induction. In studies with inhaled radionuclides it is impossible to deposit the same amount of activity in the pulmonary region of different animals in a group. As a consequence, authors have commonly shown dose ranges rather than a single value. Further, researchers do not agree on how to express dose to the types of tissue found in the lung. Cumulative doses may be estimated for individual animals at death, at time of the first tumour, or for the average lifespan of the group of animals. Other variants have also been used. In all cases considered by the Task Group, average lung dose was calculated.

151. The analyses of pooled data from studies with different species generally used a probit model, as had commonly been used in dose-response analyses, and the linear dose response, which the Task Group considered to reflect conservatism. Both linear and probit models gave an adequate description of the incidence data for alpha emitters over the range of

observed doses. For beta/gamma emitters, however, neither model gave a good fit to the incidence data, which were rather variable for similar doses. The linear dose response considerably overestimated incidence at low doses. In general the pooling of data from numerous species, although it is a comprehensive approach, does not readily permit detailed comparison of any of the individual studies.

152. The results of a number of separate studies, mainly of rodents exposed to both alpha and beta/gamma emitters have been published. Sanders and Lundgren [S11] compared lung cancer induction in F344 and Wistar rats exposed to $^{239}PuO_2$. In the F344 strain, significantly increased lung tumour incidences were found at lung doses of both 0.98 Gy (20%) and 37 Gy (34%) compared with 1.7% in controls. There were insufficient data to define a dose-response function, but there was no evidence for a threshold in the response. In contrast for the Wistar rats, there was no significant increase in lung tumour incidence in animals with an average lung dose of 0.75 Gy (0%) compared with controls (0.1%), but for animals with a lung dose of 34 Gy the incidence was 68%. These data suggested the presence of a threshold at doses somewhat above 0.75 Gy.

153. The data on Wistar rats [S11] were similar to those found in a more comprehensive lifespan study [S12]. In 3,157 female Wistar rats that had inhaled $^{239}PuO_2$ only three adenomas were found in 1,877 rats at lung doses <1.5 Gy, for an incidence of 0.16%; tumour incidence increased to 41% in 228 rats with lung doses >1.5 Gy. Pulmonary squamous metaplasia was not seen in controls and was first noted in exposed rats at lung doses >1 Gy. All tumour types induced by inhaled $^{239}PuO_2$ exhibited a threshold at lung doses >1 Gy. It was concluded that for lung tumours in Wistar rats resulting from inhaled $^{239}PuO_2$, plutonium particle aggregation is required to cause proliferation of initiated cells and to promote the formation of premalignant and malignant lesions.

154. Similar results in Wistar rats were obtained by Oghiso et al. [O5], although the study was not as extensive as that by Saunders et al. [S12]. Dose-response relationships were compared among primary tumours, classified by histological type, following a single inhalation exposure to $^{239}PuO_2$. In this study there were 130 controls and 310 animals, separated into seven groups, exposed to $^{239}PuO_2$. Initial lung contents in the different groups varied between about 97 and 1,670 Bq, giving average lung doses from 0.7 to 8.5 Gy. A differential tumour response was obtained. In general, metaplasia and benign adenomas were induced at lower doses (<1 Gy), whereas malignant carcinomas were induced at relatively high doses (>1.5 Gy) (Figure XV). The peak incidence of adenomas occurred at a dose of 0.7 Gy, of adenocarcinomas at 2.9 Gy, and adenosquamous and squamous cell carcinomas at 5.4–8.5 Gy. These results were considered by the authors to indicate a differential dose response for pulmonary carcinogenesis, in which metaplasia and benign adenomas were induced at lower doses (<1 Gy) and malignant carcinomas were induced at higher doses (>1.5 Gy). It was also noteworthy that the lifespan of the 0.7 Gy group (871 ± 105 days, ±1 SD) was significantly longer than that of the control group (790 ± 144 days, $p<0.01$). In the higher exposure dose groups, lifespan was reduced.

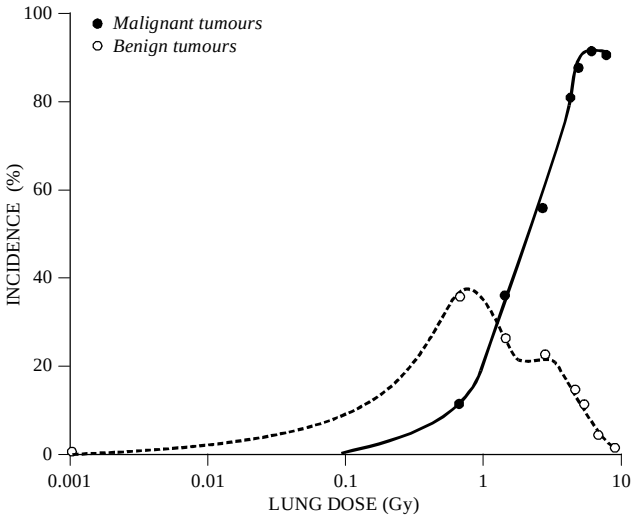

Figure XV. Benign and malignant lung tumours in rats after inhalation of $^{239}PuO_2$ aerosols [O5].

155. This threshold type of response did not seem to be found in Fisher 344 rats exposed to ^{244}Cm as the oxide. Groups of 100–200 male and female rats received average lung doses from $^{244}Cm_2O_3$ between 0.2 and 36 Gy [L28]. In general the prevalence of benign and malignant lung neoplasms increased with increasing average lung dose. For lung tumours, a linear dose-response function adequately fitted the data ($I = 0.38 \pm 0.04\ Gy^{-1}$). The response in rats exposed to $^{239}PuO_2$ ($I = 0.70 \pm 0.07\ Gy^{-1}$) was about twice the response following exposure to $^{244}Cm_2O_3$.

156. Some information is available on tumour induction in rats exposed to the beta/gamma emitter ^{144}Ce as the oxide [L39]. A total of 1,059 F344/N male and female rats (about 12 weeks of age) were exposed to graded levels of $^{144}CeO_2$, and a further 1,064 rats were maintained as controls (exposed to stable CeO_2). Groups of rats received mean lung doses of 3.6, 12, and 37 Gy. The incidence, I (%), of lung tumours increased with increasing lung dose and could be represented by a linear function of the form $I = 0.13 + 0.51D\ Gy^{-1}$, where D is the dose in gray. Because the data are limited in extent, more complex functions such as the linear-quadratic, exponential linear-quadratic, and Weibull functions also described the dose response adequately over the dose range of the study.

157. An extensive series of studies has been carried out in rodents exposed to radon and its decay products. They have demonstrated that exposures at high doses can give rise to radiation-induced lung cancers. Experimental animal studies have been valuable for understanding the consequences of exposure at varying dose rates and the influence of other environmental factors on the lung tumour response as the animals can be exposed to a variety of agents under carefully controlled conditions. Much of the information available on

experimental animals was reviewed by Cross [C22]. In animals, exposure to radon has resulted in respiratory tract tumours (adenomas, bronchiolar carcinomas or adenocarcinomas, epidermoid carcinomas, adenosquamous carcinomas, and sarcomas). In addition, pulmonary fibrosis, pulmonary emphysema, and life shortening have occurred at exposures above about 1,000 WLM^{-1} (3.5 J m^{-3}). Excess respiratory tract tumours have occurred in rats at exposure levels well below 100 WLM^{-1} (0.35 J m^{-3}), even at levels comparable to those found for typical lifespan exposures in homes. Further, tumours occurred in animals exposed to radon decay products alone; thus indicating that exposure to other environmental agents (uranium ore dust, cigarette smoke) is not necessary for carcinoma development. Most (~80%) radon-induced lung tumours in rats are considered to originate peripherally and to occur at the bronchiolar-alveolar junction. The remaining 20% are centrally located in association with the bronchi, the actual percent depending on exposure rate and possibly on exposure level. [*Note: Working level (WL) is defined as any combination of short-lived radon decay products in 1 litre of air resulting in the ultimate emission of 1.3 10^5 MeV of potential alpha energy (1 WL = 2.08 10^{-5} J m^{-3}). Working-level month: exposure equivalent to 170 hours at 1 WL concentration (1 WLM = 3.5 10^{-3} J h m^{-3}).*]

158. A notable finding in these animal studies has been that longer duration of exposure at a lower dose rate induces more lung cancers than exposures for a shorter duration at a higher dose rate. Table 9 compares the incidence of lung tumours in rats exposed at either 50 or 500 WLM per week. With exposure levels between 320 and 5,120 WLM, in all except the lowest exposure group there is a higher incidence of tumours in the groups exposed at the lower dose rate. The decrease in exposure rate not only increased the tumour incidence but specifically increased the incidence of epidermoid carcinomas.

Table 9
Percentage incidence of primary and fatal lung tumours in rats exposed to radon and decay products [a]
[C22]

Cancer type	*Exposure (WLM)*				
	320	*640*	*1 280*	*2 560*	*5 120*
At 500 WLM per week					
Number of animals examined	131	70	38	38	41
Adenoma	5	3	0	3	2
Adenocarcinoma	8	7	26	24	44
Epidermoid carcinoma	1	0	0	3	2
Adenosquamous carcinoma	0	0	3	0	0
Sarcoma	0	0	0	3	2
Fatal lung tumours	2	1	5	11	15
Animals with lung tumours (%)	15	10	29	32	49
At 50 WLM per week					
Number of animals examined	127	64	32	32	32
Adenoma	5	3	(22)	9	(22)
Adenocarcinoma	5	(20)	41	41	53
Epidermoid carcinoma	1	3	(13)	(47)	(44)
Adenosquamous carcinoma	1	3	9	(9)	3
Sarcoma	1	2	3	0	0
Fatal lung tumours	2	6	(22)	(50)	(44)
Animals with lung tumours (%)	10	(28)	(66)	(69)	(75)

a 15 mg m^3 ore dust exposures accompanied radon and radon progeny exposures; data in parentheses at 50 WLM per week are significantly ($p<0.05$) higher than corresponding data at 500 WLM per week.

159. A series of studies has also been conducted in France on the effects of radon exposure [G18, M48]. In these experiments more than 2,000 rats were exposed to cumulative doses of up to 28,000 WLM of radon gas. There was an excess of lung cancer at exposures down to 25 WLM (80 mJ h m^{-3}). These exposures were carried out at relatively high concentrations of radon and its decay products (2 J m^3). Above 6,000 WLM, rats suffered increasingly from life shortening due to radiation-induced non-neoplastic causes, thus limiting tumour development. When the dose-response data were adjusted for these competing causes of death, the hazard function for the excess risk of developing pulmonary tumours was approximately linearly related to dose. This suggests that the apparent reductions in tumour induction found at high doses may have been chiefly the result of acute damage. Later experiments, however, found that chronic exposure protracted over 18 months at an alpha energy of 2 WL (0.0042 mJ m^3) resulted in fewer lung tumours in rats (0.6%, 3/500 animals, 95% CI: 0.32–2.33) than similar exposures at a potential alpha energy of 100 WL (2 mJ m^{-3}) protracted over 4 months (2.2%, 11/500 animals, CI: 0.91–3.49) or over 6 months (2.4%, 12/500, CI: 1.06–3.74). The incidence of lung tumours in controls was 0.6% (5/800, CI: 0.20–1.49) [M48]. The confidence intervals are, however, wide, and the longer period of exposure (18 months) would in itself have been expected to result in fewer lung tumours.

160. It has been suggested by Moolgavkar et al. [M13, M29], based on a two-stage initiation-progression model for carcinogenesis, that extended duration of exposure allows time for proliferation of initiated cells and thus for a higher disease incidence. The findings are that the first mutation rate is very strongly dependent on the rate of exposure to radon progeny and consistent with *in vitro* rates measured experimentally. The second mutation rate is much less so, suggesting that the nature of the two mutational events is different. Furthermore, by incorporating cell killing into such a model, Luebeck et al. [L12] proposed that the model that gave the best fit to the data indicated that the initial increase in the proliferation rate of initiated cells depended on a second promotional step, which may be due principally to the presence of ore dust and not to radon decay products. The inverse exposure rate effect may thus be reduced in the absence of ore dust.

161. The main factors found to influence the tumorigenic potential of radon exposure in laboratory rats include cumulative exposure to radon progeny, exposure rate, unattached fraction, and associated cigarette smoke exposure. The respiratory tract cancer risk increases with the increase in cumulative exposure to radon progeny and in the magnitude of the unattached fraction. The increased risk with a high unattached fraction of radon progeny is particularly relevant to indoor radon exposure, where the unattached levels are generally much higher than in mines. The influence of cigarette smoke has been variable, depending in part on the temporal sequence of radon and cigarette smoke exposure.

162. Overall, the data on lung cancer risk resulting from exposure to radon and its decay products show an increasing risk with increasing exposure, although there are strong indications of an inverse dose-rate effect that is influenced by the presence of ore dust in the atmosphere. The data are broadly similar to those obtained from follow-up studies on uranium miners (see Chapter III).

2. Cancer risks at low doses

163. An essential input to the analysis of dose-response relationships is not only the shape of the dose response but the extent to which a statistically significant effect of radiation can be detected at low doses. It is informative to examine a number of studies that have been concerned with assessing risks at low doses.

(a) Studies

164. Laboratory animal studies that are most suitable for determining the lowest doses at which effects of radiation on tumour induction can be detected have been carried out predominantly with mice. Comprehensive data are, however, rather limited. Some of the more significant studies are briefly summarized below and analysed in the following Section.

165. Mole and Major [M3] and Mole et al. [M4] reported myeloid leukaemia incidence in male CBA-H mice acutely exposed to x rays (0.5 Gy min^{-1}) and ^{60}Co gamma rays (0.25 Gy min^{-1}) and chronically exposed to gamma rays over a period of 28 days (0.4–0.11 mGy min^{-1}). This strain of mice is exceptional, in that no case of myeloid leukaemia has been observed in more than 1,400 unirradiated male mice, so that every case occurring in irradiated animals can be regarded as radiation-induced. Total acute doses were from 0.25 to 1.0 Gy for x rays and 1.5 to 3.0 Gy for gamma rays.

166. Upton et al. [U21] used RFM mice of both sexes. For acute exposures of female mice, a dose rate of 67 mGy min^{-1} from ^{60}Co gamma rays was used, giving doses between 0.25 and 4 Gy. For male mice, x rays at 800 mGy min^{-1} were used, with doses from 0.25 to 3 Gy. Male and female mice were also exposed chronically. Data are available on the induction of myeloid leukaemia, thymic lymphoma, and ovarian tumours.

167. Ullrich [U14] and Ullrich et al. [U15, U16, U17, U18, U19] carried out experiments similar to those of Upton et al. [U21] using RFM male and female mice acutely exposed (450 mGy min^{-1}) to ^{137}Cs gamma rays. Data were reported on the tumours examined by Upton et al. [U21], together with data on Harderian gland and pituitary tumours.

168. Ullrich [U14], Ullrich and Storer [U16], and Ullrich and Preston [U20] also used BALB-C female mice to obtain further data on dose-response relationships. Acute (450 mGy min^{-1}) and chronic (0.06 mGy min^{-1}) exposures from ^{137}Cs were given. Acute doses were between 0.01 and 1 Gy, and chronic doses were between 0.25 and 2 Gy. Tumours showing a positive increase with dose were ovarian tumours as well as mammary and lung adenocarcinomas.

169. Coggle [C6] reported data on the induction of lung adenocarcinomas in male and female SAS/4 mice acutely exposed to x rays at 0.6 Gy min^{-1}. The dose range used was 0.25–3.0 Gy.

170. Covelli et al. [C7, C8] reported tumour induction in male and female $BC3F_1$ mice. They observed various types of radiation-induced tumours following acute exposure of 113 mGy min^{-1} (dose range males, 0.04–2.5 Gy; females, 0.5–5.0 Gy). The authors gave age-adjusted incidences of tumours and described tests showing which doses gave significant increases in cancer yield.

(b) Analysis

171. To determine the lowest dose at which a significant increase in tumour yield occurred in the various studies, the following method was used. The tumour yield in control animals was tested against the yield at the lowest dose used in the study. If the difference in tumour incidence is statistically significant, then that dose is taken as the lowest dose at which a significant effect is found. If the difference is not significant, the data point with the next lowest dose is included and a weighted linear regression performed, either by weighted least squares or, where possible, by iteratively re-weighted least squares. This process is continued at progressively higher doses until the linear regression coefficient becomes signifi-

cantly different from zero (p=0.05). The last dose added is then taken to be the lowest dose to give a significant effect. When calculating statistical significance, any lack of fit to a straight line is taken into account in computing uncertainties.

172. The exposure levels at which significant increases in risks of leukaemia and solid cancers could be observed are given in Table 10. The lowest dose at which a significant effect on tumour incidence could be determined is very different from study to study. It depends on factors that influence statistical power, such as the number of mice used and the spontaneous cancer rate, the cancer type, the level of radiation risk, the dose range used, and the period of follow-up.

Table 10
Lowest acute doses at which significant increases in cancers have been observed in mice

Cancer	*Mouse strain*	*Sex*	*Irradiation*	*Dose (Gy)*	*Ref.*
Myeloid leukaemia	RFM	Male	x rays	0.25	[U21]
		Male	Gamma rays	1	[U16, U17, U20]
		Female	Gamma rays	1	[U15, U16, U17, U18]
		Female	Gamma rays	2	[U21]
	CBA-H	Male	x rays	0.5	[M4]
	$BC3F_1$	Male	Gamma rays	1.5	[M3]
		Female	x rays	1	[C7, C8]
Thymic lymphoma	RFM	Male	Gamma rays	1	[U16, U17, U20]
		Male	x rays	3	[U21]
		Female	Gamma rays	1	[U15, U16, U17, U18]
		Female		2	[U21]
Lung adenocarcinoma	BALB-c	Female	Gamma rays	0.5	[U16]
	SAS/4	Both	x rays	2.5	[C6]
Mammary adenocarcinoma	BALB-c	Female	Gamma rays	0.2	[U14]
Ovarian tumour	$BC3F_1$	Female	x rays	0.16	[C7, C8]
	BALB-c	Female	Gamma rays	0.25	[U18]
	RFM	Female	Gamma rays	0.5	[U21]
Harderian gland tumour	RFM	Male	Gamma rays	3	[U16, U17, U20]
All solid tumours	$BC3F_1$	Female	x rays	1.3 [a] [b]	[C7, C8]
				4	[C7, C8]

a Excluding ovarian tumour.
b p=0.05.

173. For leukaemia induction in mice there was little evidence for an increase in risk below 1 Gy, although two studies indicated statistically significant increases at 0.25 Gy [U21] and 0.5 Gy [M3]. Most of the dose-response data for acute exposures showed no significant departure from linearity. An exception was the study by Mole and Major [M3], which showed a reduced effectiveness of radiation, per unit dose, at 1 Gy. There was also a suggestion of a departure from linearity at high doses in the results reported by Ullrich et al. in RFM mice [U16, U17, U20].

174. For solid cancers the overall results (Table 10) are similar to those for leukaemias, with significant increases in tumour incidence occurring principally at acute doses of 1 Gy and above. Increases in risk were, however, seen at lower doses for ovarian tumours (0.16 and 0.25 Gy), mammary adenocarcinomas (0.2 Gy), and lung adenocarcinomas (0.5 Gy). Although data are not given here, the use of a lower dose rate consistently resulted in a lower risk per unit of dose.

B. HEREDITARY DISEASE

175. In addition to inducing neoplastic changes in somatic tissues, ionizing radiation may produce transmissible (heritable) effects in irradiated populations by inducing mutations in the DNA of male or female germ cells. These mutations, while having no direct consequences for the exposed individual, may be expressed in subsequent generations as genetic disorders of widely differing types and severity.

176. Studies of germ-cell mutations *in vivo* are not only relevant for assessing dose-response relationships for hereditary effects but they also have value for assessing effects on the primary lesion in DNA likely to be involved in tumour initiation. As described in Chapter IV, subsequent tumour expression will depend on the influence of many other factors.

177. The evaluation of genetic hazards associated with the exposure of human populations to ionizing radiation is an important area in which the Committee has been active since its inception. To date, however, there has been a lack

of direct data that give quantitative information on genetic effects leading to disease states in humans. The substantial amount of data from other species indicates that radiation can give rise to mutations in humans that will be manifested as disease. So far there has been no alternative but to use data from experimental animals as the main basis for predicting quantitative effects in humans.

178. In the UNSCEAR 1988 Report [U4], the Committee summarized the principal assumptions thought to be necessary for extrapolating data on hereditary damage in mice and other animals to humans. The main considerations are the following:

(a) the amount of genetic damage induced by a given type of radiation under a given set of conditions is the same in human germ cells and in those of the test species used as a model;
(b) the various biological and physical factors affect the magnitude of the damage in similar ways and to a similar extent in the experimental species from which extrapolations are made and in humans; and
(c) at low doses and low dose rates of low-LET radiation there is a linear relationship between dose and the frequency of genetic effects studied.

179. Studies in mice have provided the main basis for assessing the risks of hereditary disease in humans. The doubling dose for hereditary disease that has been adopted by most national and international organizations for chronic exposure is 1 Gy (e.g. [C1, M18, U4]). Reviews of experimental data from mice generally give values in the range from 1 to 4 Gy and would therefore suggest that the value of 1 Gy adopted for humans is conservative [M18, S13].

180. A series of studies have been reported on dose-response relationships for the induction of germ-cell mutations in mice. The most comprehensive information comes from studies in male mice in which specific locus mutations were measured. Russell et al. [R5, R6], for example, presented data on dose-response relationships for male mice exposed to 0.72–0.9 Gy min^{-1} for doses between 3 Gy and 6.7 Gy and $\leq$8 mGy $min^{-1}$for doses between 0.38 and 8.61 Gy. In both cases the data could be fitted by a linear dose-response relationship over the whole dose range examined. For chronic exposure, $I = (8.04\ 10^{-6} \pm 1.19\ 10^{-6}) + (7.34\ 10^{-6} \pm 0.83\ 10^{-6})D$; for acute exposure, $I = (8.12\ 10^{-6} \pm 1.19\ 10^{-6}) + (2.19\ 10^{-5} \pm 0.19\ 10^{-5})D$, where I is the mutation frequency per locus and D is the dose in gray. The difference in slope for the two exposure conditions, by a factor of about 3, reflects the difference in the dose rates and opportunity for repair of damage at lower dose rates (Figure XVI). It might be expected that if lower doses had been used in the high-dose-rate study (0.72–0.9 Gy min^{-1}), the slope of the response at lower total doses would approach that found for low-dose-rate exposures.

181. It was notable that although the incidence of mutations fell by a factor of about 3 for a reduction in dose rate from 800–900 mGy min^{-1} to 8 mGy min^{-1}, further reduction in the dose rate to 0.007 mGy min^{-1} failed to further reduce the yield of mutations. This independence of dose rate was shown over a range of doses differing by rather more than a factor of 1,000, and it was concluded that it was unlikely that a further reduction in mutation frequency would be obtained at even lower dose rates [R6]. This suggests that a substantial fraction of the damage to DNA that results in the induction of heritable mutations is not amenable to effective repair.

182. Similar results for specific-locus mutations in male mice were obtained in studies by Lyon et al. [L40]. For a gamma ray dose from ^{60}Co of 6.3 Gy given in 60 equal daily fractions at 0.17 Gy min^{-1}, the mutation frequency ($4.17\ 10^{-5}$ per locus) was very similar to that obtained in mice chronically exposed at 0.08 mGy min^{-1} to a total dose of 6.2 Gy ($3.15\ 10^{-5}$ per locus). The mutation rate with fractionation was, however, about a third of that obtained for a single exposure to 6.4 Gy at 0.17 Gy min^{-1} ($13.1\ 10^{-5}$ per locus).

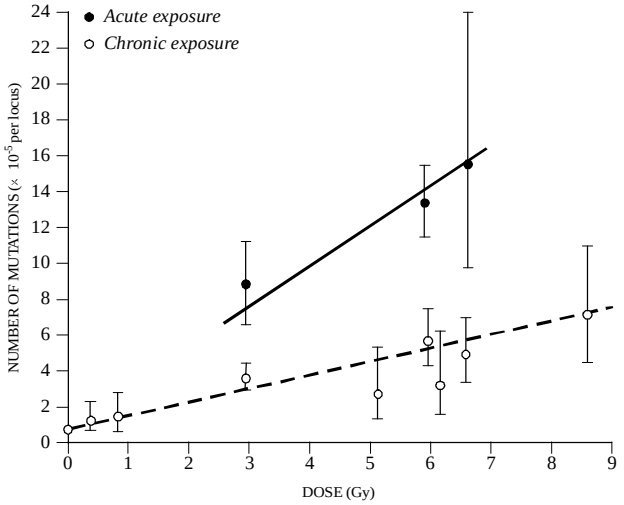

Figure XVI. Specific locus mutations in mouse spermatogonia following radiation exposure [R6].

183. Searle [S10] reviewed data from a number of publications on specific-locus mutations in spermatogonia of mice after chronic exposure to gamma rays. Data points from a number of authors, including those by Russell et al. [R5, R6], were obtained for doses in the range 0.38–8.6 Gy. A linear relationship gave a good fit to the data on mutation frequency: $I = 8.34\ 10^{-6} + 6.59\ 10^{-6}D$ (assuming 100 roentgens $\approx$ 1 Gy). This fit does not differ significantly from that obtained by Russell and Kelly [R6]. With acute exposures, a peak in the incidence of mutations was obtained with a decline in the incidence at doses between 6.7 and 10 Gy. The reduction at high dose rates may be attributed to more extensive killing of spermatogonia at doses above 6.7 Gy and to a lower mutational response in those more resistant spermatogonia that survive.

184. Searle [S10] also reviewed data on specific locus mutations in mice both acutely and chronically exposed to neutron irradiation with dose rates varying between 0.79 Gy min^{-1} and 0.01 Gy min^{-1}. A linear function fitted essentially all the data points between 0.5 Gy and 2.1 Gy, with the exception of a single value at 1.9 Gy. The fit had the form $I = 8.30\ 10^{-6} + 1.25\ 10^{-4}D$. There was no evidence

of a dose-rate effect for neutrons. The ratio between the slopes for male mice chronically exposed to low-and high-LET radiations gives an RBE of 19.

185. In female mice irradiated just before birth, there is a more pronounced dose-rate effect for mutational damage to oocytes than in those irradiated later. Using the specific-locus method, Selby et al. [S24] examined the effect of dose rate on mutation induction in mouse oocytes. Female mice were given 3 Gy of whole-body x-irradiation at dose rates of 0.73–0.93 Gy min^{-1} and 7.9 mGy min^{-1} at 18.5 days after conception. The frequency of specific-locus mutations measured in the offspring decreased from 8.7 10^{-6} to 6 10^{-7} mutations per gray per locus (a factor of about 14) between the high-dose-rate and low-dose-rate exposure. In practice, the mutation rate at the low dose rate (7.9 mGy min^{-1}) did not differ significantly from that in controls, indicating very effective repair. Similar calculations based on the results of irradiating mature and maturing oocytes at the same dose rates [R19, R20] suggest an approximately fourfold drop in the induced mutation frequency in the adult. These results suggested that females irradiated just before birth have a more pronounced dose-rate effect, although the confidence limits of magnitude of the dose-rate effect are too wide for firm conclusions to be drawn.

C. SUMMARY

186. The results of animal studies contribute to the database of information available for assessing the biological effects of low doses of ionizing radiation and dose-response relationship. Because of differences in radiosensitivity between animals and humans, the results obtained from animals cannot be used directly to obtain quantitative estimates of cancer risks for human populations. Animal studies are, however, valuable for determining the shape of dose-response relationships as well as for examining the biological and physical factors that may influence radiation responses. They are also of use for examining how factors such as age at exposure, radiation quality, and dose protraction or fractionation can influence the tumour response. Laboratory animals have the advantage that they are a homogeneous population with minimal biological variability, and the influence of confounding factors can be eliminated. Although studies with laboratory animals generally involve fewer individuals than epidemiological studies, they have the advantage that they are carried out under controlled conditions with good estimates of the radiation dose and with a known spontaneous cancer rate. In the case of radiation-induced hereditary disease animal studies provide the principal source of quantitative information.

187. Dose-response relationships for many tumour types in various animal models following exposure to both low- or high-LET radiation can be reasonably well represented by a linear or linear-quadratic function for the dose ranges analysed. In many cases, however, alternative fits to the data are also possible. Other model fits include the possibility of a threshold dose below which tumours will not occur, as well as more complex functions in which the time for the tumour to appear is much later at low dose rates than at high dose rates and thus can also suggest the presence of a threshold for a response. For some lung tumours it has been demonstrated that high local doses from alpha irradiation are required to cause proliferation of initiated cells and to promote the development of malignant lesions.

188. Analysis of a series of studies in mice has shown that the lowest dose at which a statistically significant ($p=0.05$) increase in tumour yield is observed varies from study to study. It depends on the number of animals used in each experiment, the radiation sensitivity of the species to specific cancers, and the spontaneous cancer rate. It also depends on the range of doses used. From the animal data reviewed, the lowest single (acute) dose to give a significant ($p=0.05$) effect on tumour yield is of the order of 100–200 mGy (low-LET). The higher values obtained in other studies can be attributed to lack of sensitivity, high control incidence, or to small numbers of animals. Values for the lowest dose to give a significant increase in risk following continuous (chronic) irradiation are generally higher than those for acute irradiation. It may be concluded that animal studies provide quantitative information on risks of radiation-induced tumour induction at low to intermediate doses but do not, and probably cannot, provide direct information at doses much less than about 100 mGy.

189. For radiation-induced hereditary disease, the most comprehensive information comes from measurements of specific-locus mutations in mouse spermatogonia. Data from a number of laboratories have demonstrated a dose-response relationship for low-dose exposures from low-LET radiation that is well fitted by a linear response. The lowest dose tested in these studies was 380 mGy. Data from both male and female mice indicate a significant effect of dose rate. It was notable that although the incidence of mutations in male mice fell by a factor of about 3 for a reduction in dose rate from 800–900 mGy min^{-1} to 8 mGy min^{-1}, a further reduction in the dose rate to 0.007 mGy min^{-1} failed to further reduce the yield of mutations. This independence of dose rate occurred over a range of doses differing by rather more than a factor of 1,000, and it was concluded that it was unlikely that a further reduction in mutation frequency would be obtained at even lower dose rates. This suggests that a substantial fraction of the damage to DNA that results in the induction of heritable mutations is not amenable to effective repair.

III. EPIDEMIOLOGY

190. The extent to which epidemiological studies can provide information on the effect of ionizing radiation on the induction of cancer at low doses is considered in this Chapter. Although the role of radiation in inducing cancer was recognized soon after the discovery of x rays by Röntgen in 1895, up to the early 1950s only high doses causing acute tissue damage were considered to be important. This view was reflected in the early recommendations of the International Commission on Radiological Protection (ICRP) and by national organizations. By 1959, however, the stated aim of ICRP in setting dose limits was to "prevent or minimize somatic injuries and to minimize the deterioration of the genetic constitution of the population" [I6]. These recommendations reflected an increasing awareness of the effect of radiation in inducing cancer, particularly leukaemia, at low doses and was largely the result of information becoming available from the follow-up of the survivors of the atomic bombings and groups exposed for medical reasons (see, for example, [L29]).

191. By the early 1970s it was known that radiation is capable of causing tumours in many tissues of the body, although the frequency of appearance following a unit dose varied markedly from one organ or tissue to another. Information on the dose-related frequency of tumour induction by radiation had become available from a number of epidemiological studies of persons exposed to external radiation or internally incorporated radionuclides. In the UNSCEAR 1972 Report [U8], the Committee gave preliminary estimates of the risk of leukaemia and some solid cancers based on the survivors of the atomic bombings and other groups exposed at high dose rates. It also pointed out that animal studies suggested that risks per unit dose at lower dose rates could be lower and that risk estimates based on groups exposed at high dose rates would be overestimates for doses and dose rates received from environmental sources.

192. The chief sources of information on the risks of radiation-induced cancer were the survivors of the atomic bombings exposed to whole-body irradiation at Hiroshima and Nagasaki; patients with ankylosing spondylitis and other patients who were exposed to partial body irradiation therapeutically, either from external radiation or from internally incorporated radionuclides; and various occupationally exposed populations, in particular uranium miners and radium dial painters. Follow-up of these populations had shown that there is a minimum period of time between irradiation and the appearance of a radiation-induced tumour, although this "latency period" varies with age and from one tumour type to another. Some types of leukaemia and bone cancer have latency periods of only a few years, with most of the risk being expressed within about 25 years of exposure. Many tumours of solid tissues (e.g. liver or lung) have latency periods of 10 years or more, and it was not clear whether their incidence passes through a maximum and subsequently declines with time following exposure or whether the risk levels out or even increases indefinitely during the remainder of life.

193. To project the overall cancer risk for an exposed population, it is therefore necessary to use empirical models that extrapolate over time data based on only a limited portion of the lives of the individuals. Two such projection models have been generally considered:

(a) the additive (absolute) risk model, which postulates that radiation will induce cancer independently of the spontaneous rate after a period of latency and that variations in risk due to gender and age at exposure may occur; and
(b) the multiplicative (relative) risk model, in which the excess cancer risk (after latency) is given by a constant factor applied to the age-dependent incidence of natural cancers in the population.

Both models imply an increasing risk of cancer with increasing radiation dose. In addition to these two models, alternative fits to the epidemiological data to assess lifetime risks have also been used such as a model expressing excess relative risk as a function of attained age [K27]. Further information is given in Annex I, "*Epidemiological evaluation of radiation-induced cancer*".

194. Most organizations assessing risks in the 1970s, including UNSCEAR in its 1977 Report [U7], adopted the additive model for the assessment of cancer risks, although the Committee on the Biological Effects of Ionizing Radiation (BEIR I) [C17] of the United States National Academy of Sciences used both models for risk assessment. In a major revision of its recommendations in 1977, ICRP, in its System of Dose Limitation, considered it necessary to limit the incidence of radiation-induced fatal cancers and severe hereditary disease to a level accepted by society [I8]. Implicit in this approach for stochastic effects was the necessity to use quantified risks of radiation-induced disease in setting limits on exposure. The risks of cancer and hereditary disease adopted by ICRP were derived mainly from reviews by UNSCEAR [U7]. Organ-specific risks were given for the red bone marrow, the lungs, cells on bone surfaces, and the thyroid and breast. No specific risks were given for the other organs and tissues of the body, which were pooled in a risk factor for all the "remainder"organs and tissues.

195. During the 1980s new information progressively became available from the Life Span Study in Japan, and this necessitated a revision of the earlier risk estimates by UNSCEAR. The data available from the extended follow-up of the survivors of the atomic bombings indicated that a multiplicative risk model now gave a best fit to data for most solid cancers [U4]. These new risk estimates, which also allowed for improvements in dosimetry, were taken into account by ICRP in its 1990 recommendations [I2].

196. Overall, the lifetime risks calculated in recent years by various national and international organizations are not too different (e.g. [C1, I2, M18, U4]). The estimates of risk have, however, been obtained by direct extrapolation from epidemiological studies. They are, therefore, appropriate for populations exposed at high doses and dose rates. To allow for a reduced effect of radiation in inducing cancer when exposures are at low doses or low dose rates, most organizations have recommended the use of a reduction factor to obtain risks for application in radiation protection. ICRP [I2] applied a reduction factor of 2 (it called the factor a dose and dose-rate effectiveness factor (DDREF)) to give a risk coefficient for radiation protection purposes. In the UNSCEAR 1993 Report [U3], the Committee reviewed epidemiological and experimental data relevant to the choice of a reduction factor. It recommended that for tumour induction, the DDREF adopted should, to be on the safe side, "have a low value, probably no more than 3". Insufficient data were available to make recommendations for specific tissues.

197. Epidemiological studies were recently reviewed in the UNSCEAR 1994 Report [U2], and further studies and results are reviewed in an accompanying Annex I, "*Epidemiological evaluation of radiation-induced cancer*". In this Chapter the statistical difficulties associated with obtaining quantitative estimates of the risk of radiation-induced cancer from epidemiological studies at low doses are first examined. The available data from groups exposed at high dose rates, from which dose-response relationships and quantitative risk estimates are generally obtained, and from groups exposed at low doses and dose rates are then reviewed. Also considered is the choice of an appropriate value of the reduction factor for assessing risks at low doses and doses rates from studies of groups exposed at high doses and high dose rates.

198. There are no human data so far that can be applied in determining quantitative dose-response relationships or risk estimates for hereditary disease. Risk factors for hereditary disease have been considered in previous UNSCEAR reports [U3, U4].

A. STATISTICAL CONSIDERATIONS

199. Making quantitative estimates of the risk of cancer associated with low doses of ionizing radiation is complicated. Small epidemiological studies often have insufficient statistical power to detect any increase in risks. If bias has arisen in a study through, for example, failure to follow up a large percentage of a cohort of persons or to allow for confounding factors, then spurious positive or negative findings could occur. In low-dose studies where the excess risks are predicted to be small, it is particularly important to ensure that the potential for bias and confounding is kept as low as possible, as this can create spurious results.

200. It is not, at present, possible to distinguish cancers induced by ionizing radiation from those due to other causes. A particular result of an epidemiological study is normally considered to be "statistically significant" if, in the absence of an effect, the probability of its occurrence is less than 1 in 20. If a large number of disease outcomes (e.g. different cancer types) are examined, however, possibly for each of several age groups and time periods, it is quite likely that a "statistically significant" finding will arise simply by chance. It is therefore important to examine the results of any epidemiological study in the context of possible dose-response relationships, other epidemiological studies, and supporting experimental evidence.

201. As with animal studies, the statistical power of an epidemiological study to detect an excess risk associated with ionizing radiation exposure depends on a number of factors. In Annex I, "*Epidemiological evaluation of radiation-induced cancer*", a procedure is described for assessing the power of a study to detect an elevated risk of a disease before a study is conducted. The statistical precision of completed studies is also examined. An example illustrates how the power of a cohort study to detect an elevated risk depends on the relative sizes of both the exposed and control populations, their absolute numbers, and the total numbers of cancers. These, in turn, depend on the baseline cancer rates, the length of follow-up, the radiation dose, and the specific radiation sensitivity of the organ(s) or tissue(s). Thus a study based on a very large cohort may not be particularly informative if a rare cancer is under investigation and the follow-up is short. Conversely, a study based on a fairly small cohort may be quite informative if a common cancer is being investigated and the follow-up is long. The distribution of the population and the number of cancer cases between various exposure dose groups will also influence the ability of a study to define a dose-response relationship. More detailed information on statistical considerations is given in the above-mentioned Annex.

202. The limitations of statistical power and the possibility of bias or confounding will constrain not only the ability to detect small increases in the risk of cancer but also the determination of whether or not there is the potential for a dose threshold for radiation carcinogenesis in specific tissues. Some examples of dose-response relationships obtained from epidemiological studies and the ability to detect risks at low dose are illustrated below.

B. HIGH-DOSE AND HIGH-DOSE-RATE EXPOSURES

203. The primary basis for evaluating risks of cancer associated with radiation exposure is the epidemiological study of human health in populations that include groups exposed at high doses and generally at high dose rates. The main features of the major high-dose-rate epidemiological studies were considered in the UNSCEAR 1994 Report [U2] and are reviewed in Annex I, "*Epidemiological evaluation of radiation-induced cancer*". The Life Span Study of the survivors of the atomic bombings at Hiroshima and Nagasaki by the Radiation Effects Research Foundation (RERF) is of particular importance in risk estimation. As well as involving a population of all ages and both sexes, the Life Span Study is based on large numbers of persons with a wide range of

whole-body doses. Consequently, it has high statistical power to examine any variation in cancer risk with dose. The interpretation of the dose-response data is, however, complicated by the fact that exposure was to both gamma rays and neutrons. An RBE of 10 has generally been assumed when fitting the dose-response data.

204. Other high-dose, high-dose-rate epidemiological studies are more limited in terms of the sex and age structure of the exposed population or in terms of the organs irradiated. However, they do provide additional information on risks for particular organs or for exposures at particular ages.

205. As discussed in Annex I, "*Epidemiological evaluation of radiation-induced cancer*", the statistical power of epidemiological studies to assess risks depends on the range of doses received by the study population and the spontaneous cancer rate. Analyses based on a restricted set of data for exposures in the low-dose region would have much reduced statistical power to detect risks. However, it may still be possible to detect raised risks in some circumstances. Furthermore, analysis of the dose-response relationship over the whole range of doses can be informative in making inferences about risks at low doses, when interpreted in conjunction with the mechanistic and computational modelling approaches described in Chapters IV and V.

1. Dose-response relationships

(a) Survivors of atomic bombings

206. Various analyses of the dose-response data for the Life Span Study have been reported, and with increasing length of follow-up the quality of the information available has improved considerably. Pierce and Vaeth [P1] examined mortality data from the follow-up of the Life Span Study to 1985, based on the most recent published DS86 dosimetry. In their analyses, those persons with shielded kerma estimates in excess of 4 Gy were excluded, in view of an apparent levelling-off in the dose response that may be associated with errors in the estimates of such high doses or with cell killing. The authors concluded that for all cancers other than leukaemia the data could be well fitted by a linear dose-response model, although a linear-quadratic model would not be inconsistent with the data.

207. Shimizu et al. [S1] assessed the slopes of the dose-response curves for the survivors of the atomic bombings in various low-dose regions. Over the lowest dose range (0–0.49 Gy) with a statistically significant trend ($p<0.05$), the value of the excess relative risk per gray for all cancers, other than leukaemia, was 0.38. This is similar to the value obtained for the whole dose range (0.41), in line with the analysis by Pierce and Vaeth [P1], suggesting a linear dose-response relationship.

208. For leukaemia mortality, the data up to 1985 on survivors of the atomic bombings suggested that a linear dose-response model did not provide a good fit and that a linear-quadratic model would be preferred [P1]. In the analysis of Shimizu et al. [S1], the excess relative risk per gray of leukaemia mortality in the dose region 0–0.49 Gy was 2.40 ($p<0.05$), which is about half of the value over the whole dose region (0–6 Gy) of 5.21 ($p<0.001$). This supported the conclusions of Pierce and Vaeth [P1] that a linear-quadratic dose-response model better fits the data.

209. Errors in the estimates of dose in the Life Span Study can substantially alter the shape of dose-response relationships. The problem of random dosimetry errors for the RERF data on the Life Span Study has been investigated by a number of authors [G2, J3, P1, P7]. Pierce and Vaeth [P1] found that after adjustment for dosimetric errors there were non-significant indications of upward curvature in the dose-response function for mortality from all solid cancers, while for leukaemia the evidence for curvilinearity became stronger.

210. The evidence for possible curvilinearity in the dose response for leukaemia and for solid cancers in the most recent cancer incidence data [P3] has been examined [L7, M19]. A variety of relative risk models have been fitted to the data, including those that allow for a possible dose threshold. Errors in estimates of doses were also allowed for, as these can substantially alter the shape of the dose-response relationship.

211. For solid cancers taken together, a variety of models provided little evidence for curvilinearity. A significant positive dose response was found for all survivors receiving doses less than 0.5 Sv but not for doses less than 0.2 Sv (assuming an RBE of 10 for neutrons). A threshold-linear relative risk model fitted to the data gives no support for a threshold above about 0.2 Sv, and the data are consistent with the absence of a threshold. For most solid cancers taken separately, the data on cancer incidence are also consistent with a linear dose-response relationship [T4] (also see Annex I, "*Epidemiological evaluation of radiation-induced cancer*"). These findings are in accord with previous analyses of the dose response for all solid cancers taken together.

212. In contrast, the latest data on non-melanoma skin cancer incidence indicate substantial curvilinearity, consistent with a possible dose threshold of about 1 Sv to the skin or with a dose response in which the excess relative risk (ERR) is proportional to the fourth power of dose, with a decrease in the response at high doses (>3 Sv) [L30] (Figure XVII). Supporting epidemiological data on the shape of the dose response for non-melanoma skin cancer are, however, limited. For example, Ron et al. [R15] found no evidence for curvilinearity in the dose response in a group of children in Israel who had been treated with large therapeutic doses of radiation for tinea capitis (ringworm of the scalp). However, the doses in this study were generally much higher than those received by survivors of the atomic bombings. Thus there are no patients with doses less than 5 Gy (low-LET) in the Israeli data set. The only other information on the shape of the dose response for skin cancer comes from animal experiments. Some evidence of a threshold has been obtained in studies with mice and rats [A5, B23, O3, P8], although a linear-exponential form of induction curve was obtained for beta-irradiated male SAS/4 mice [W8].

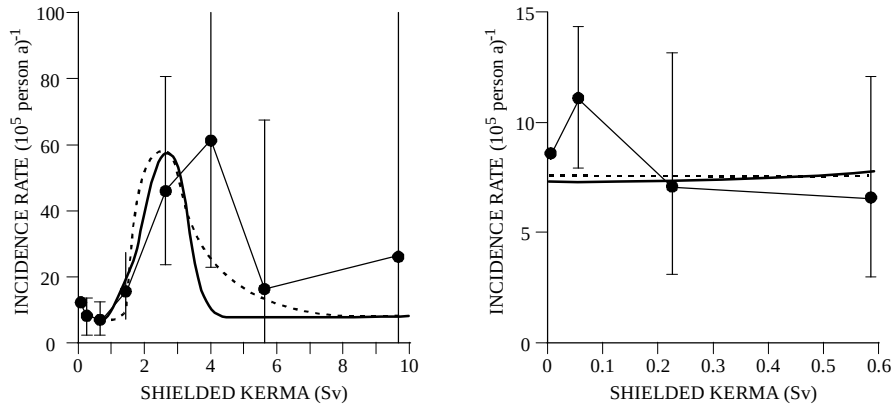

Figure XVII. Observed incidence of non-melanoma skin cancer in survivors of atomic bombings (CI: 90%) compared with fourth-power exponential (solid line) and linear-exponential threshold (dotted line) models of dose response [L30].

The diagram on the right shows the low-dose region in detail.

213. In contrast with solid cancers, the analysis by RERF and other groups of the dose-response relationship for leukaemia incidence in the Life Span Study cohort found quite a marked upward quadratic component, i.e. significant upward curvature [P2, P3], with the evidence for non-linearity being strongest for acute myeloid leukaemia [P3]. For the three main radiation-inducible leukaemia subtypes analysed together (acute lymphatic leukaemia, acute myeloid leukaemia, and chronic myeloid leukaemia), there is a significant increase in the risk of leukaemia if the dose responses for all survivors with doses less than 0.5 Sv are considered together [L7, M19]. This significance vanishes, however, if doses less than 0.2 Sv are considered.

214. Analysis of leukaemia incidence among the Japanese atomic bomb survivors by Little and Muirhead [L7] showed that incorporation of a threshold in the linear-quadratic dose response yielded an improvement in fit at borderline levels of statistical significance [best estimate of threshold for a linear-quadratic-threshold model was 0.12 Sv (95% CI: 0.01–0.28; two-sided p=0.04)]. This analysis takes account of random dosimetric errors, but not possible systematic errors in dose estimates. The fits of a linear-quadratic-threshold dose response to the recently released leukaemia mortality data [P2] and that takes account of random dosimetric errors, demonstrated that the threshold was not significantly different from zero [best estimate of threshold for a linear-quadratic-thresh-old model was 0.09 Sv (95% CI: <0.00–0.29; two-sided p=0.16)] [L44]. Similar findings have been reported by Hoel and Li [H26] in analyses that do not take account of dosimetric error. Comparison of the incidence and mortality data by Little and Muirhead [L44] and Little [L49] demonstrates the essential similarity of the leukaemia incidence and mortality data. Little and Muirhead [L44] concluded that the most probable reason for the difference between the findings in the incidence and mortality data sets was the finer subdivision of dose groups in the mortality data set. (There are 14 dose groups in the mortality data sets in their publicly available form, compared with 10 dose groups in the incidence data sets.)

215. Recent analyses by Kellerer and Nekolla [K25] and Little and Muirhead [L52] of the tumour incidence and mortality data demonstrate that if account is taken of possible systematic errors in the Hiroshima DS86 neutron dose estimates, then there is evidence of appreciable upward curvature in the dose response for solid tumours in the Life Span Study data. This is particularly marked if analysis is restricted to the 0–2 Gy dose range rather than the 0–4 Gy dose range that has been used for most analyses of dose response in the Life Span Study. Over the 0–2 Gy dose range, the low-dose extrapolation factor (LDEF) for all solid tumour incidence is 1.43 (95% CI: 0.97–2.72), and so is comparable with the LDEF for leukaemia incidence, 1.58 (95% CI: 0.90–10.58) [L52].

216. Recent data on the mortality of the atomic bomb survivors was reported by Pierce et al. [P2]. The follow-up covers the period to 1990 and includes an extra 10,500 survivors for whom DS86 dose estimates have been calculated. The total cohort comprises approximately 86,500 persons, 60% of whom received doses in excess of 5 mSv. Of the total population, 44% had died by 1990, including 8,827 who died of cancer. The shape of the dose-response curve for all solid cancers is essentially linear up to 3 Sv, beyond which there is an apparent decrease in risk. This may be attributed both to cell killing and to imprecision in the estimates of high doses (Figure XVIII).

217. As discussed in Annex I, "*Epidemiological evaluation of radiation-induced cancer*", the dose-response relationships for mortality from many specific tumour types (stomach, colon, lung) are consistent with a linear response although generally based upon the analysis of a restricted number of cases. For leukaemia, the dose response over the range 0–3 Sv can be fitted with a linear-quadratic dose-response relationship (Figure XVIII).

218. While the Life Span Study provides information on cancer risks in a number of tissues, there are others for which there is either very little or no evidence for an effect. These include, for example, the bone, cervix, prostate, testes and rectum.

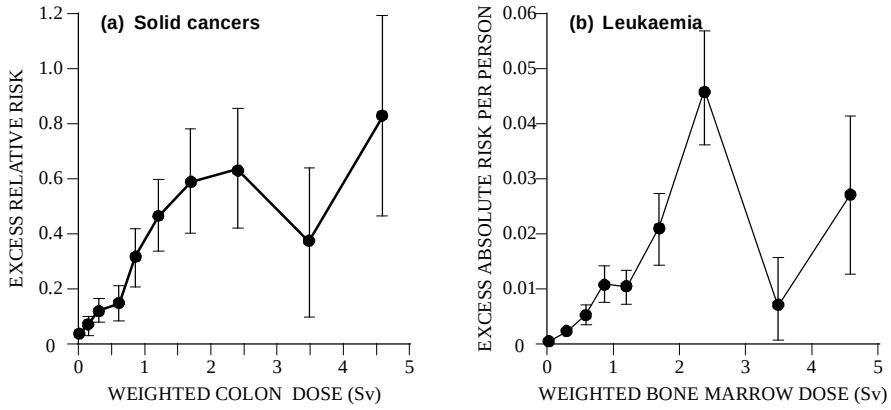

Figure XVIII. Dose response for mortality from solid cancer (males of 30 years of age at exposure) and leukaemia in survivors of atomic bombings in Japan [P2].

(b) Other groups exposed to low-LET radiation

219. Additional data on dose-response relationships for groups exposed to low-LET radiation are available from a number of other studies. For Canadian tuberculosis patients given fluoroscopies, Miller et al. [M2] showed that a linear dose-response relationship gave a good fit to the data on breast cancer among patients in Canadian provinces other than Nova Scotia. For patients from Nova Scotia, who generally received higher doses, the dose-response relationship was also consistent with linearity, but it had a steeper slope than for other Canadian provinces. Howe and McLaughlin [H31] have given further results from an extended follow-up of this population. The data on breast cancer mortality could again be fitted with a linear dose-response relationship. As before, the slope of the dose trend was greater for patients in Nova Scotia than for patients in other provinces.

220. Dose-response analyses have also been performed for some other groups with medical exposures. Boice et al. [B6] studied the relationship between the risk of breast cancer and dose for women in Massachusetts (United States) given multiple chest x-ray fluoroscopies. For this study, doses were mostly in the range 0–3 Gy. A linear dose-response model was found to provide as good a fit to these data as a linear-quadratic model, whereas a purely quadratic model did not fit well. Among women given radiotherapy for cervical cancer, the risk of leukaemia increased with dose up to 4 Gy, in a manner consistent with linearity, although the data were also consistent with a quadratic dose response; beyond 4 Gy the risk decreased, probably as a result of cell killing [B7]. At lower doses, Ron et al. [R8] found that the risk of thyroid cancer among children in Israel irradiated for tinea capitis was consistent with a linear dose-response relationship, based on doses that were mostly less than 0.15 Gy.

(c) Groups exposed to high-LET radiation

221. Information on dose-response relationships that depart from the conventional linear or linear-quadratic response has been obtained for bone tumours arising from alpha particle irradiation of bone following the deposition of isotopes of radium. Extensive epidemiological information is available on groups of persons exposed, principally by ingestion, to ^{226}Ra and ^{228}Ra in the 1920s and 1930s. The most comprehensive data relate to female radium dial painters. The data on tumour induction in this population have been the subject of extensive analysis over the last 40 years (e.g. [E1, F13, H21, R4]). After the radium programme at the Argonne National Laboratory finished in the early 1990s, Rowland brought together all the data collected in this long-term study [R16]. His most recent analysis considered all female radium dial painters with body content measurements and who had entered the study prior to 1950, a total of 1,530 women [R17]. In this cohort, 46 women had bone sarcomas and 19 had head sinus carcinomas; 3 women had both a bone sarcoma and a head sinus carcinoma. The analysis incorporated revised estimates of systemic intake, which took into account the magnitude of the original intake. This has been shown to influence the retention kinetics and hence the cumulative doses [K15, R7]. The intakes by the various members of the cohort covered several orders of magnitude. The 46 bone sarcomas had appearance times ranging from 7 to 63 years. The lowest systemic intake associated with a bone sarcoma was 3.7 MBq (100 μCi). This malignancy, diagnosed in 1981 and resulting from an intake in 1918, was thus detected 63 years later.

222. Various forms of a general incidence–systemic intake expression

$$I = (\alpha SI + \beta SI^2)\, e^{-\gamma SI} \qquad (3)$$

were fitted to the data and tested with a χ^2 statistic. In the equation, I is the incidence of bone tumours, α, β, and γ are constants, and SI is the systemic intake. No acceptable fit to the equation was found. However, when a constant, C, was included in a general function of the form

$$I = C + kSI^{\beta}\, e^{-\gamma SI} \qquad (4)$$

in which k and β are constants, a good fit to the data could be obtained with $C = -1.44\ 10^{-4}$, $k = 2.14\ 10^{-15}$, $\beta = 3.15$, and $\gamma = 7.06\ 10^{-5}$. With the incidence I equal to zero, this gives an intercept at 2,920 kBq. This fit to the data, shown

in Figure XIX, gives good evidence for a threshold dose for the induction of bone tumours. The dose-response data were further analysed by Thomas [T12], who suggested the data were consistent with a threshold for tumour induction in the range 3.9–6.2 Gy high-LET (average bone dose). He proposed a rounded value of 10 Gy (average bone dose) as a "practical threshold" below which there should be little cause for concern.

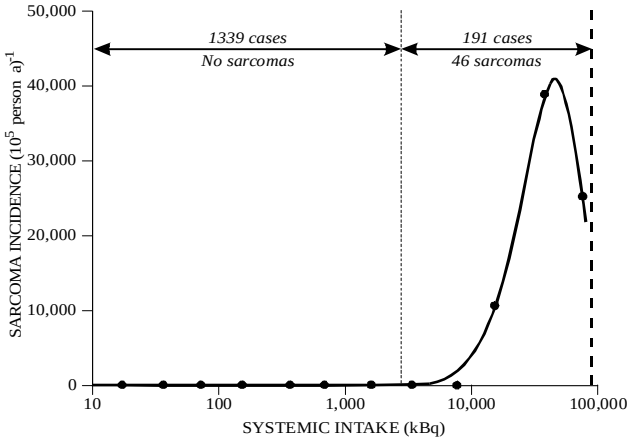

Figure XIX. Bone sarcoma incidence in female radium dial painters [R17].
Systemic intake is kBq ^{226}Ra plus 2.5 × ^{228}Ra activities.

223. Various forms of the general dose-response expression were also fitted to the data on head sinus carcinoma. In contrast to the data on bone sarcomas, linear, linear-exponential, and dose-squared-exponential functions all provided acceptable fits. Models that included a threshold would also fit the data, but the threshold value was not statistically significant.

224. It was concluded that the tumour induction data for osteosarcoma induction show a very steep dose response [R16]. Whether this actually demonstrated a threshold or simply showed a very low probability of osteosarcoma induction at intakes below about 3,000 kBq could not be determined. For head sinus carcinoma, the data did not suggest the presence of a threshold, although various model fits to the data were possible, reflecting the paucity of data, which prevented discrimination between alternative functions.

225. Further information on bone tumour pathology in persons exposed to external radiation or internally incorporated radionuclides may explain some of these observations. A review of bone tumour pathology in patients treated with ^{224}Ra revealed an unexpectedly high proportion of bone sarcomas of the fibrous connective tissue type, including the first case of malignant fibrous histiocytoma (MFH) of bone described after internal irradiation [G17]. Out of 46 bone tumours in the ^{224}Ra patients, osteosarcoma was the most common histological type (48% of cases), but 30% of these were fibrosarcoma-MFHs and the remainder were chondrosarcomas, malignant lymphomas, myelomas, and malignant chordomas. The 30% of fibrosarcoma-MFHs substantially exceeds the usual prevalence of this disease, which is 8%–11% in spontaneous bone tumours. In a follow-up study, a similar spectrum of tumours was obtained in persons occupationally exposed to $^{226/228}$Ra, patients given external irradiation and other so-called secondary bone tumours arising at sites of pre-existing bone lesions as had been obtained in the ^{224}Ra patients [G17].

226. The authors of the review [G17] concluded that disturbance of the local cellular system caused by deterministic radiation damage and repair resulted in the unexpectedly high proportion of fibrosarcoma-MFHs. It was considered that the development of the tumours reflected the cell types involved in a disturbed remodelling process in the skeleton. The reactive proliferation of the predominantly fibroblastic tissue at the site of tissue damage could be the presumptive origin of this special type of radiation-induced bone sarcoma. As a fibrotic response would be likely to arise as a consequence of deterministic radiation damage, the fibrosarcoma-MFH type of tumour might well arise only at doses above a limiting threshold.

(d) Groups exposed to radon

227. Radon has been extensively studied as a human carcinogen. Epidemiological studies are reviewed in Annex I, "*Epidemiological evaluation of radiation-induced cancer*" and are summarized here only briefly. The results of a series of cohort studies of miners in countries throughout the world have provided the basis for estimates of the risk of lung cancer associated with exposure to radon and its decay products. These data, although subject to some uncertainties, have allowed characterization of exposure response relationships [L51]. The exposure response relationship in the various studies of radon exposed miners is consistent with linearity, but the slope appears to be higher at lower exposure rates (Figure XX). As discussed in Annex I, this apparent inverse exposure-rate effect does not imply that low exposures carry a greater risk than higher exposures; rather it suggests that for a given total exposure, the risk is higher if the exposure is received over a long rather than a shorter period of time. This

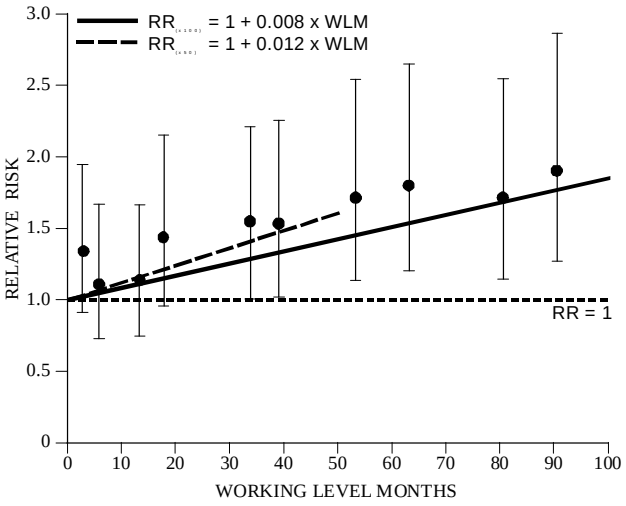

Figure XX. Relative risks of lung cancer from pooled data for miners, restricted to <100 WLM exposure and also to <50 WLM exposures [L53].

could reflect some cell killing at high exposure rates. Case-control studies of residential radon exposure and lung cancer have also been conducted in various countries. Although these have also been informative, the generally lower exposures of people and methodological difficulties have meant the power of these studies is less than that of the occupational studies. However, the estimates of lung cancer risk based upon a recent meta-analysis of these eight studies are in close agreement with the risk predicted on the basis of miner data [L53].

228. The Committee on Biological Effects of Ionizing Radiations (BEIR VI) considered the data on 11 miner cohorts exposed to radon previously analysed by Lubin et al. [L48]. The most recent data available were used in developing the Committee's risk models for radon exposure [C26]. The Committee recognized that care is needed in combining data from different cohorts of underground miners around the world. The levels of exposure to radon and other relevant covariates, such as arsenic and tobacco smoke, differed appreciably among groups of miners. The completeness and quality of the data available on relevant exposures also differed notably among the cohorts. Information on tobacco consumption was available for only 6 of the 11 cohorts; of these 6, only 3 had information on duration and intensity of exposure to tobacco smoke. Lifestyle and genetic factors that influence susceptibility to cancer might also account for heterogeneity among cohorts.

229. Despite those differences, the Committee concluded that the best possible estimate of lung cancer risk associated with exposure to radon and its decay products would be obtained by combining, in a judicious manner, the available information from all 11 cohorts. The Committee used statistical methods for combining data that both allowed for heterogeneity among cohorts and provided an overall summary estimate of the lung cancer risk. Confidence limits for the overall estimate of risk allowed for such heterogeneity.

230. The Committee's risk models described the excess relative risk as a simple linear function of cumulative exposure to radon, allowing for differential effects of exposure during the periods 5–14 years, 15–24 years, and 25 years or more before death from lung cancer. The most weight was given to exposures occurring 5–14 years before death from lung cancer. The Committee examined two types of risk models in which the excess relative risk was modified either by attained age and duration of exposure or by attained age and exposure rate. The excess relative risk decreased with both attained age and exposure rate and increased with duration of exposure. For cumulative exposures below 0.175 J h m^{-3} (50 WLM), a constant-relative-risk model without these modifying factors appeared to fit the data as well as the two models that allow for effect modification.

2. Minimum doses for a detectable increase in cancer risk

231. It is important to examine the lowest levels of dose at which a significantly elevated level of radiation-induced cancer has been observed in human populations. Relevant information from epidemiological studies is available from the follow-up of the atomic bomb survivors, from other studies of thyroid cancer in infants, children, and adults, and from studies of the risk of cancer in children following radiation exposure *in utero*.

(a) Survivors of atomic bombings

232. The analysis by Pierce et al. [P2] of the atomic bomb survivor mortality data set finds a statistically significant (two-sided $p<0.05$) trend in mortality risks in the 0–50 mSv range for all solid cancers combined, based upon follow-up to 1990 (assuming an RBE for neutrons of 10). This finding is based on the fitting of a linear relative risk model to the 0–50 mSv data, but using fixed adjustments for sex and age at exposure based on fits of a model to the full data set. Pierce et al. [P2] pointed out that without these adjustments for sex and age, the significance of the trend in dose in the 0–50 mSv group would be lost.

233. As discussed by Little [L11], this procedure is statistically problematic. Little [L11] and Pierce et al. [P11] proposed modified forms of the one-degree-of-freedom test for trend in the low-dose region, using nested models that incorporate sex and age adjustments but that do not rely on fixed modifications fitted to the whole dose range. When either of these modified tests is used, the finding of a significant increasing (two-sided $p<0.05$) trend with dose in the low-dose region (0–50 mSv) remains valid [P11].

234. Notwithstanding these statistical considerations, Pierce et al. [P2, P11] were cautious in their interpretation of this finding, which is at variance with the findings in the latest atomic bomb survivor solid tumour incidence data in which a significant excess risk of solid cancers is only seen down to doses of 200–500 mSv [T4]; they indicated that the finding in the 0–50 mSv group might be artefactual, resulting from the differential misclassification of cause of death in the lowest dose groups. Further information on recent data from the atomic bomb survivors is given in Annex I, "*Epidemiological evaluation of radiation-induced cancer*".

(b) Thyroid cancer incidence

235. Information on the risks of radiation-induced thyroid cancer is described in Annex I, "*Epidemiological evaluation of radiation-induced cancer*". Studies of thyroid cancer incidence following radiation exposure were reviewed by Shore [S6], and a combined analysis of seven studies was performed by Ron et al. [R9]. Among various cohorts with external low-LET exposures, the excess relative risk per gray tends to be higher for thyroid cancer than for most other solid cancers. Furthermore, the excess relative risk is higher for those irradiated at young ages than for adults. Studies of cohorts with low-dose, external irradiation of the thyroid in childhood are therefore of value for examining risks at low doses. The risks of thyroid cancer following exposure to ^{131}I are less well understood, as discussed in Annex I, "*Epidemiological evaluation of radiation-induced cancer*".

236. A study of about 10,800 children in Israel given x-ray treatment for tinea capitis was reported by Ron et al. [R8]. The total dose was given in five daily fractions to five treatment fields on the scalp. While the dose to the scalp was of the order of several gray, the average total thyroid dose was calculated to be only about 100 mGy. An analysis over the range 0–0.5 Gy showed a statistically significant trend of increasing risk of thyroid cancer with increasing thyroid dose. In addition, the trend in relative risk per unit dose was greater for those irradiated at ages under five years than for those irradiated at older ages, in line with the general observation of an increasing relative risk with decreasing age at exposure, as well as being consistent with a study of thyroid cancer in young persons irradiated for enlarged tonsils [P4]. Among the tinea capitis patients less than five years old at exposure, the relative risk at about 0.1 Gy (100 mGy) was approximately 5 and was significantly greater than 1. This finding was, however, based on a fairly small number of cases, although it arose among those persons for whom the risk would be predicted to be greatest.

237. Thyroid cancer in a cohort of 2,657 infants in New York State given x-ray treatment for a purported enlarged thymus gland and followed for an average of 37 years has been reported by Shore et al. [S2]. Estimated thyroid doses ranged from 0.03 to more than 10 Gy, with 62% receiving less than 0.5 Gy. The dose-response relationship for thyroid cancer was fitted by a linear dose-response relationship, with no evidence of a quadratic dose component. An analysis restricted to the range 0–0.3 Gy showed a statistically significant trend with dose (p=0.002), although based on just four thyroid cancer cases with non-zero doses. The estimate of absolute excess risk per unit dose over this dose range was similar to that from the Israeli tinea capitis study [R8].

238. Ron et al. [R9] conducted a combined analysis of data from seven studies of thyroid cancer after exposure to external radiation. The range of doses varied considerably between the different studies. For exposure before age 15 years, linearity was considered to best describe the dose response, even down to 0.1 Gy. The estimated excess relative risk per gray of 7.7 (95% CI: 2.1–28.7) is one of the highest values found for any organ.

239. Thyroid cancer in a cohort of 4,404 children of whom 2,827 were given x-ray treatment for cancer in childhood and followed for an average of 15 years has been reported by de Vathaire [D14]. Estimated thyroid doses ranged from 0.001 to 75 Gy, with 41% receiving less than 0.5 Gy. The dose-response relationship for thyroid cancer was best fitted by a linear dose-response relationship, with no evidence for a quadratic component. A standardized incidence ratio of 35 (90% CI: 10–87, p<0.01) was found to be associated with a dose of 0.5 Gy to the thyroid.

(c) Exposures *in utero*

240. A number of studies have been published that have examined the risks of cancer in childhood following exposures *in utero*. These studies have particular advantages for detecting risks of cancer at low doses because of the low spontaneous cancer rate in childhood.

241. Information on cancer risks following radiation exposure *in utero* are available from studies of those with prenatal diagnostic x-ray exposures, as well as those irradiated as a consequence of the atomic bombings of Hiroshima and Nagasaki. The largest study of childhood cancer following prenatal x-ray exposure is the Oxford Survey of Childhood Cancers (OSCC), which is a national case-control study of childhood cancer mortality carried out in the United Kingdom. Information is also available from other studies of prenatal x-ray exposure that have been carried out in North America and elsewhere [B28].

242. The Oxford Survey of Childhood Cancers was started in the mid-1950s. Up to 1981, mothers of 15,276 cases and the same number of matched controls had been interviewed [K10]. During the late 1950s, the study investigators reported a doubling in the risk of childhood cancer associated with prenatal x-ray exposure [S7]. Later analysis covering a longer period indicated a falling risk with time and an average raised risk of about 40% (95% CI: 31–50) [B8, K10].

243. Data on doses to the embryo and fetus are available for the Oxford Survey of Childhood Cancers, although there is some uncertainty in these values. Table 11 shows the mean number of films per x-ray examination in the

Table 11
Doses from prenatal x rays in the Oxford Study of Childhood Cancers

Birth year	*Mean number of films per examination*	*Mean dose per film according to reference (mGy)*	
		[U8]	*[S21]*
1943-1949	1.9	18	4.6
1950-1954	2.2	10	4.0
1955-1959	1.9	5	2.5
1960-1965	1.5	2	2.0

Survey according to calendar period, together with estimates of the average dose per film made by the Committee in the UNSCEAR 1972 Report [U8] and by Stewart and Kneale [S21]. UNSCEAR estimated that the average dose per examination was 10–20 mGy (low-LET) during the 1950s and decreased over time. Stewart and Kneale's dose estimates

were about half of the UNSCEAR values. Based on the UNSCEAR dose estimates, Muirhead and Kneale [M8] estimated the absolute radiation-induced risk for the incidence of all cancers up to age 15 years to be about 0.06 Gy^{-1} (low-LET) (95% CI: 0.04–0.10). A similar risk estimate was calculated by Mole [M6] based on a national survey in the United Kingdom of doses from obstetric radiography performed in 1958, for which the average dose was about 6 mGy.

244. There has been concern that owing to the retrospective nature of the Oxford Survey of Childhood Cancers, which relied at least partially on mothers' memories, some bias may have been introduced. The results of the follow-up were supported by a study in the United States [M9, M10] of contemporary records of x-ray exposures of children born in hospitals in the north-eastern United States. In an initial study [M9] of 734,243 children born between 1947 and 1954, 556 children were identified as having died from cancer between 1947 and 1960. Prenatal x-ray exposure was associated with an increased risk of cancer, with relative risks for leukaemia of 1.58 and for solid cancers of 1.45. These increases were very similar to those in the Oxford Survey. In an extension of the study, however, with a further 695,157 children born up to 1960 and having 786 additional cases of cancer, the relative risk for leukaemia was similar to the first phase (1.48) but that for solid cancers was appreciably lower (1.06). The overall values of relative risks for leukaemia (1.52) and solid cancers (1.27) do not differ significantly when compared directly (p=0.4).

245. Bithell [B28] reviewed a number of studies that examined the risk of childhood cancer following *in utero* radiation exposure. None of the studies on their own had the statistical power of the Oxford Survey of Childhood Cancers, but a total of 12 studies, when taken together, gave a weighted average of an increase in relative risk of 1.37 (95% CI: 1.26–1.49). Including the Oxford Survey of Childhood Cancers data gave a relative risk of 1.39 (CI: 1.33–1.45). While the individual study designs and methods of analysis were very different, the overall finding lends support to the results of Childhood Cancers.

246. Doll and Wakeford [D3] reviewed the evidence from epidemiological studies on the risk of cancer in childhood from exposure of the fetus *in utero* from diagnostic radiology. They also considered the limited studies in experimental animals. They concluded that while information is available from a number of epidemiological studies, the most significant comes from the Oxford Survey. It was concluded that there is strong evidence for a causal relationship, with radiation doses to the fetus of the order of 10–20 mGy giving increases in the risk of childhood leukaemia and solid cancers of about 40%. Because of the low risk of cancer in childhood, the calculated absolute risk coefficient was approximately 6% Gy^{-1}. The analysis supports the view that small doses of radiation are potentially carcinogenic. The possibility still exists that there may be some as yet unidentified confounding factor in the Oxford Survey affecting both the probability of the fetus being irradiated *in utero* and the risk of subsequent cancer. A feature of the data from the Oxford Survey that remains unexplained is that the increase in risk for both leukaemia and solid cancers following exposure *in utero* is essentially the same, with a relative risk of about 1.4. Most other human and animal studies consistently indicate different sensitivities of leukaemia and solid cancers [B45].

247. Several cohort studies of *in utero* exposures have not shown evidence of excess risk. Those studies, however, were small in size. Among those exposed to atomic bomb radiation *in utero* [J1], no childhood leukaemia cases have been observed. For 1,263 children irradiated *in utero* and followed from birth, two cases of cancer arose up to 15 years of age, compared with 0.73 expected from Japanese national rates [Y2]. The resulting upper limit on the 95% confidence interval for the absolute radiation-induced risk is 2.8 10^{-2} Gy^{-1} (low-LET). Continued follow-up showed an excess of adult cancers among those exposed to atomic bomb radiation *in utero*. Based on the follow-up to 1988, the relative risk at 1 Gy was estimated to be 3.77 [Y2], which is similar to that seen among survivors of the atomic bombings irradiated in the first 10 years of life [S1]. Further follow-up to the end of 1989 suggested a subsequent decrease in the relative risk [Y1], in line with the pattern indicated by the earlier follow-up of those exposed post-natally at ages under 10 years.

248. More recently, Delongchamp et al. [D2] reported cancer mortality data in atomic bomb survivors exposed *in utero* for the period October 1950 to May 1992. Only 10 cancer deaths were reported among persons exposed *in utero*. Although there were only two leukaemia deaths, this was higher than in a control group (p=0.054). Mortality from solid cancers at ages over 16 years was in excess of expected (ERR = 2.4 Sv^{-1}; 90% CI: 0.3–6.7); all the deaths occurred in females.

249. Thus, although there is some consistency in case-control studies in showing a raised risk of childhood cancer, the absence of clear confirmation in cohort studies leaves some uncertainty in establishing a risk estimate. For the Oxford Survey of Childhood Cancers, however, an increase in childhood cancer risk by about 40% is associated with doses of about 10–20 mGy (low-LET). A number of other studies, taken together, support this finding.

3. Effect of dose and dose rate

250. As explained above, quantitative information on the risk of cancer in human populations comes largely from epidemiological studies of population groups exposed at intermediate and high doses and dose rates. For the assessment of the risk of cancer from environmental and occupational exposure to radiation, a reduction factor, frequently termed a dose and dose-rate effectiveness factor (DDREF), has normally been used to assess risks at low doses and low dose rates. The choice of reduction factors was reviewed most recently in the UNSCEAR 1993 Report [U3] and has also been reviewed by ICRP [I2] and by a number of other international bodies. In the UNSCEAR 1993 Report [U3], the Committee examined cellular studies, data from experimental animal studies, and

information from epidemiological studies that would allow judgements to be made on an appropriate reduction factor. The judgements made in that report remain valid and are summarized here only briefly.

251. The dose-response information on cancer induction in the survivors of the atomic bombings in Japan provided, for solid tumours, no clear evidence for a reduction factor much in excess of 1 for low-LET radiation. For leukaemia, the dose response fits a linear-quadratic relationship, and a best estimate of the reduction factor is about 2. Analyses by Little and Muirhead [L52] of the latest cancer incidence data that take account of possible random errors and possible systematic errors in DS86 dose estimates show that there is little indication of upward curvature in the dose response for solid tumours over the 0–4 Gy dose range, although over the 0–2 Gy dose range and after adjustment of Hiroshima DS86 neutron dose estimates the upward curvature is more pronounced. There is marked upward curvature in the dose response for leukaemia over the 0–4 Gy dose range, which becomes less pronounced if attention is restricted to those receiving less than 2 Gy [L52]. If adjustments are made to the Hiroshima DS86 neutron dose estimates, then over the 0–2 Gy dose range the LDEF for all solid tumours is 1.43 (95% CI: 0.97–2.72), and so is comparable with the LDEF for leukaemia, 1.58 (95% CI: 0.90–10.58) [L52]. There is only limited support for the use of a reduction factor from other epidemiological studies of groups exposed at high dose rates, although for both thyroid cancer and female breast cancer some data suggest a value of about 3 may be appropriate.

252. The results of studies in experimental animals conducted over a dose range that was similar to, although generally somewhat higher than, the dose range to which the survivors of the atomic bombings in Japan were exposed, and at dose rates that varied by factors between about 100 and 1,000 or more, give reduction factors from about 1 to 10 or more, with a central value of about 4. Some of the tumour types for which information is available have a human counterpart (e.g. myeloid leukaemia and tumours of the breast and lung) while others do not (e.g. Harderian gland in the mouse) or require for their development substantial cell killing and/or changes in hormonal status (ovarian tumour, thymic lymphoma). Similar results to those obtained with animal tumour models have been obtained for somatic mutations and for transformation of cells in culture, although the reduction factors obtained have not been as large. In a number of the experimental studies on tumour induction, linear functions would give a good fit to both the high- and low-dose-rate data in the range from low to intermediate doses. This indicates that even if the cellular response can, in principle, be fitted by a linear-quadratic dose response, in practice it is not always possible to resolve a common linear term for exposures at different dose rates.

253. If human response is similar to that in experimental animals, then it can be envisaged that at lower dose rates than were experienced in Hiroshima and Nagasaki, a reduction factor greater than the value of about 1.5 that is suggested by analysis of the dose-response data could be obtained. However, information from human populations exposed at low dose rates suggests risk coefficients that are not very different from those obtained for the atomic bomb survivors, although the risk estimates have wide confidence intervals.

254. In the UNSCEAR 1993 Report [U3], the Committee concluded that, when taken together, the available epidemiological and experimental data suggested that for tumour induction, the reduction factor adopted should, to be on the safe side, have a low value, probably no more than 3. Insufficient data were available to make recommendations for specific tissues [U3]. For high-LET radiation, a reduction factor of 1 was indicated on the basis of experimental data that suggested little effect of dose rate or dose fractionation on tumour response at low to intermediate doses. It was noted that a value of somewhat less than 1 is suggested by some studies, but the results are equivocal, and cell killing may be a factor in the tissue response [U3].

255. In the case of hereditary disease, the adoption of a reduction factor of 3 was supported by experimental data in male mice, although a somewhat higher value has been found with one study of female mice.

C. LOW-DOSE-RATE EXPOSURES

256. Information from studies of groups exposed to low dose rates is potentially of more direct relevance to risk estimates. However, studies of low-dose-rate exposure generally involve low doses and, because of the probably low excess risks, are likely to be hampered by a lack of statistical power and possibly also by confounding factors. Examination of the results of low-dose-rate studies can, however, provide a check on the risks derived by extrapolation from high-dose-rate studies.

1. Occupational exposures

257. Several studies have been conducted of nuclear industry workers. In the United States, Gilbert et al. [G3] performed a joint analysis of data for about 36,000 workers at the Hanford, Oak Ridge, and Rocky Flats weapons plants. Neither for the grouping “all cancers” nor for leukaemia was there any indication of an increasing trend in risk with dose. However, the upper limit of the 90% confidence interval for the excess relative risk per unit dose was several times greater than the corresponding value for the survivors of the atomic bombings in Japan in the case of all cancers other than leukaemia and slightly greater than the value from Japan in the case of leukaemia.

258. The first analysis of the National Registry for Radiation Workers (NRRW) in the United Kingdom examined cancer mortality in relation to dose in a cohort of over 95,000 workers [K3]. The mean lifetime dose received was 33.6 mSv; however, over 8,000 workers had a lifetime dose in excess of 100 mSv. For all malignant neoplasms, the trend in the relative risk with dose was positive but was not statistically significant (p=0.10). Based on a relative risk projection model,

the central estimate of the lifetime risk based on these data was 10% Sv^{-1}, which is 2½ times the value of 4% Sv^{-1} cited by ICRP [I2] for risks associated with the exposure of workers (based on applying a DDREF of 2 to the Japanese data). The 90% confidence interval for the NRRW-derived risk ranged from a negative value up to about six times the ICRP value. For leukaemia (excluding chronic lymphatic leukaemia, which does not appear to be radiation-inducible), the trend in risk with dose was statistically significant (p=0.03). Based on a projection model as used by BEIR V [C1], the central estimate of the corresponding lifetime leukaemia risk was 0.76% Sv^{-1}, which is 1.9 times the ICRP [I2] value for a working population (0.4% Sv^{-1}), with 90% confidence limits ranging from just above zero up to about six times the ICRP value.

259. A second analysis of the NRRW cohort was published in 1999 [M47] and covered a total of 124,743 workers. For leukaemia, excluding chronic lymphatic leukaemia, there was a marginally significant increasing risk with dose. The central estimate of excess relative risk per sievert, 2.55 (90% CI: −0.03–7.16), is similar to that estimated for the Japanese atomic bomb survivors at low doses (2.15 Sv^{-1}, 90% CI : 0.43–4.68); the corresponding 90% confidence limits were tighter than in the first analysis, ranging from just under four times the risk estimated at low doses from the Japanese atomic bomb survivors to about zero. For all malignancies other than leukaemia, the central estimate of the trend with dose, 0.09 Sv^{-1} (90% CI: 0.28–0.52), was closer to zero than in the first analysis and smaller than the Japanese atomic bomb estimate of 0.24 Sv^{-1} (90% CI: 0.12–0.37) (without the incorporation of a dose-rate reduction factor). Also, the 90% confidence intervals were tighter than before and include zero. Overall, the second NRRW analysis provides stronger evidence than the first on occupational radiation exposure and cancer mortality; the 90% confidence interval for the risk per unit dose now excludes values that are more than four times those seen in the atomic bomb survivors, although they are also consistent with there being no risk at all.

260. The NRRW therefore provides some evidence of an elevated risk of leukaemia associated with occupational exposure to radiation and, like the combined study of workers in the United States, is consistent with the risk estimates for low-dose/low-dose-rate exposures derived by ICRP [I2] from the data on the survivors of the atomic bombings in Japan.

261. A cohort study of occupational radiation exposure has been conducted using the records of the National Dose Registry of Canada [A17]. The cohort consisted of 206,620 individuals monitored for radiation exposure between 1951 and 1983, with mortality followed up to the end of 1987. A total of 5,425 deaths were identified by computerized record linkage with the Canadian Mortality Database. A trend of increasing mortality with increasing cumulative radiation exposure was found for all causes of death in both males and females. In males, cancer mortality appeared to increase with radiation exposure without any relationship to specific types. Unexplained trends of increasing mortality due to cardiovascular diseases (males and females) and accidents (males) were also noted. The excess relative risk for radiation-induced cancer was calculated to be 3.0% per 10 mSv (90% CI: 1.1–4.8) for all cancers combined and was significantly higher than the comparable risk estimate for survivors of the atomic bombings. However, the very low SMR for all-cause mortality suggests that record linkage procedures between the Canadian National Dose Registry and the Canadian Mortality Database may have been imperfect and that there could have been some confounding of the dose response.

262. In the UNSCEAR 1994 Report [U2] information was given on the association of leukaemia and radiation exposure among workers at the Mayak facility in the Russian Federation, some of whom received substantial exposures several decades ago [K26]. Risk coefficients for radiation-induced leukaemia were similar to those given by the ICRP [I2] for workers, although no confidence interval was provided. Limitations in the study were that 15% of the original cohort had been lost to follow-up, and bone marrow doses from plutonium remained to be evaluated.

263. An international study of cancer risk among radiation workers in the nuclear industry was coordinated by the International Agency for Research on Cancer (IARC) [C20, I1]. It consisted of a combined analysis of mortality data for nearly 96,000 workers in Canada, the United Kingdom, and the United States. The groups of workers studied were the subject of individual analyses that had been published in 1988 or earlier. The United Kingdom component of this study was the Nuclear Industry Combined Epidemiological Analysis (NICEA) [C3], based on workers at BNFL Sellafield, the United Kingdom Atomic Energy Establishment, and the Atomic Weapons Establishment. The other groups studied were workers at the three United States Department of Energy plants referred to earlier (Hanford, Oak Ridge, and Rocky Flats) [G3] and workers at Atomic Energy Canada Ltd. [G4].

264. Analysis of the combined cohort of radiation workers showed a statistically significant trend in the risk of leukaemia (excluding chronic lymphatic leukaemia) with external dose. This finding is similar to that reported in the first analysis of the NRRW [K3], although the results are not independent, since many of the workers in the NRRW were also in the IARC study. The central estimate of risk per unit dose corresponded to 0.59 times the value estimated from the atomic bomb survivors based on a linear dose-response model and 1.59 times the value based on a linear-quadratic model fitted to the atomic bomb survivor data; the corresponding 90% confidence interval ranged from about zero up to four times the value from the linear-quadratic atomic bomb survivor model. The evidence for a trend with dose was particularly strong for chronic myeloid leukaemia, as was also reported in the large study of workers in the United Kingdom [M47], some of whom were included in the international study.

265. For all cancers other than leukaemia, the central estimate of the trend in risks with dose was negative, but the upper 90% confidence limit corresponded to about twice the value arising from a linear extrapolation to low doses of

results for the survivors of the atomic bombings in Japan, i.e. about four times the estimate for low-dose-rate exposures based on a reduction factor of 2.

266. The authors of the IARC study concluded that their analysis provides little evidence that the risk estimates that form the basis of current radiation protection standards are appreciably in error. Since most of the workers studied are still alive, however, they recommended further follow-up of these and other workers to increase the precision of risk estimates. To further address the issue of effects at low doses, IARC is now coordinating an enlarged International Collaborative Study of Cancer Risk among Radiation Workers in the Nuclear Industry [C2]. This study will contain additional workers from countries such as France and Japan, and the combined cohort should number several hundred thousand.

2. Environmental exposures

267. Studies of exposures to natural background radiation (other than radon) or to environmental contamination from man-made sources have generally involved examining geographical correlations in cancer rates. Such studies can be difficult to interpret, owing to the effect of confounding factors such as sociodemographic variables and other factors that vary geographically, together with the lack of information on doses.

268. Sources of natural background radiation include terrestrial gamma rays and cosmic radiation, which vary considerably with geographical location. Many attempts have been made to correlate radiation exposure with cancer mortality or incidence in different populations. While this would in principle give information on exposures at relatively low radiation doses, such attempts are subject to considerable difficulties, as was described in the UNSCEAR 1994 Report [U2]. Interpreting the data is made difficult by uncertainties in the doses actually received, geographical variation in the accuracy of cancer diagnoses, and confounding with the numerous other environmental factors. Furthermore, when different geographical areas are compared, exact matching of control groups or groups exposed at different levels can be difficult. As a consequence, studies that have tried to compare cancer risks from natural background radiation in different geographical locations are subject to considerable uncertainty and must be interpreted with care [C1].

269. Darby [D10] has made some estimates of the proportion of deaths from various cancers that might be caused by exposure to natural background radiation based on models developed by the BEIR V Committee [C1]. These models were based on the data from the survivors of the atomic bombings in Japan. They predict that about 11% of deaths from leukaemia might be caused by post-natal exposure to natural background sources, excluding radon. For other cancers the estimate was 4% or less. The interval between exposure and the development of the disease is shorter for leukaemia than for most other tumours. There is a higher relative risk for leukaemia, and the influence of other environmental factors on leukaemia risk is less than for many other types of cancer. It might be expected, therefore, that any effect of variations in natural background would be more readily detectable for leukaemia than for other cancer types. As described in the UNSCEAR 1994 Report [U2], however, well designed studies conducted in a number of countries find no significant association between natural background radiation and leukaemia (excluding chronic lymphatic leukaemia) (e.g. [I7, T10, U2, W11]).

270. Few of the studies examining cancer incidence in relation to exposure to natural background radiation have tried to obtain realistic dose estimates that take into account differences between indoor and outdoor exposure and the effects of population movement. One exception is a Chinese study [W12] that compares leukaemia mortality in two neighbouring regions having quite different levels of exposure as a result of the high thorium content in monazite sands. Yangjiang is a high-background-radiation area and Taishan/Enping is a control area. In both regions there was a highly stable population, and considerable effort went into measuring radiation exposure both indoors and outdoors. In the high-background area the radiation dose calculated to the red bone marrow by age 50 years would have been about 60 mSv greater than that for someone living in the low-background area. During 1970–1985, the age-adjusted mortality rates for leukaemia in females were 2.21 and 3.56 10^{-5} PY^{-1} in the high- and low-background areas, respectively, while in males the rates were 3.32 and 3.82 10^{-5} PY^{-1}, where PY stands for person-years. The differences were not significant; if anything, they suggested a lower risk in the more highly exposed population [W12]. The study had low statistical power to detect an effect, if one existed, as the relative risk expected was about 1.2 in the highly exposed group, and effects of this magnitude are very difficult to detect epidemiologically.

271. An extension to this study covering 1987 to 1990 has also been reported [T9]. The later study covered a fixed cohort with 78,614 persons in the high-background-radiation area and 27,903 in the control area at the start of 1987. Dose estimates were obtained by measurements using environmental gamma-ray dose-rate measurements and individual TLDs. The cohort was added to that monitored previously to give a total population of 64,070 subjects in the high-background-radiation area and 24,876 in the control area at the beginning of 1979. In total, the study covered 949,018 person-years (PY) during 1979–1990 (696,181 in the high-background-radiation area and 252,837 in the control area). The relative risks (the high-background-radiation area compared with the control area) for all cancers and for all cancers except leukaemia in each of three dose subgroups in the high-background-radiation area did not differ significantly from 1. The relative risks for site-specific cancers of the lungs, liver, and stomach were generally less than 1, while for nasopharyngeal cancer and leukaemia they were greater than 1. It is noteworthy that the result for leukaemia was the reverse of that found in the earlier study [W12]. The authors concluded that even for the combined data the sample size in each group was not large enough to come to any definite conclusions.

272. A further extension to the study has also been reported [T17] covering a total of 125,079 subjects with 1,698,350 PY (10,415 cancer deaths) followed from 1979 to 1995. The population was separated into controls and high, medium and low dose groups. Despite higher death rates in the males than in the females no significant difference was found between the persons from the high-background-radiation area and the controls; if anything, the death rates in the high-background-radiation area were lower.

273. It may be concluded from this and other studies reviewed in the UNSCEAR 1994 Report [U2] that comparative studies of groups exposed to differing levels of natural background gamma radiation have not demonstrated any significant effects on cancer incidence.

274. Some studies of environmental exposures have examined the temporal trends in cancer rates. For example, Darby et al. [D1] examined temporal trends in childhood leukaemia in the Nordic countries in relation to fallout from atmospheric nuclear weapons testing during the 1950s and the 1960s. They concluded that there was some evidence of a raised risk associated with the "high" exposure period, when children would have received a dose from fallout of about 1.5 mSv, compared with the adjacent "medium" exposure period, when the dose received would have been about 0.5 mSv (relative risk for ages 0–14 years is 1.07; 95% CI: 1.00–1.14). These data are consistent with a relative risk of 1.03 predicted with the BEIR V leukaemia model [C1], although the central estimate from this study is larger than the BEIR V value, a difference that may be explained by the different follow-up times on which the two values are based (0–7 years and 5–15 years, respectively).

275. Studies have been reported of a population in the East-Urals that was exposed to radioactive materials following an accident at the Mayak reprocessing plant in September 1957 [K29]. A total of 7,854 persons who received radiation doses estimated to be between 40 and 500 mSv have been followed. No statistically significant changes in causes of death, mortality or reproductive function have been found compared with control values from the province and USSR data. Although this study is to be continued, it illustrated the difficulties in conducting carefully controlled epidemiological studies, which require a defined control group and accurate dose estimates.

D. SUMMARY

276. Epidemiological studies provide direct quantitative data on the risks of cancer in humans following radiation exposure. The main source of information is the Life Span Study of survivors of the atomic bombings in Hiroshima and Nagasaki in 1945. Substantial information is also available from studies of people occupationally or medically exposed either to external radiation or to internally incorporated radionuclides.

277. The Life Span Study is important, as it gives information on the effects of whole-body irradiation following exposure at different ages. The interpretation of the dose-response data is, however, complicated by the fact that exposure was to both gamma rays and to neutrons. An RBE of 10 has generally been assumed when fitting the dose-response data. The data show a pattern of increasing risk with increasing dose for both leukaemia and most solid cancers. The most recent analyses of the data suggest that the numbers of solid cancers induced in the population depends on the spontaneous cancer rate, and that at least for those exposed in adulthood the absolute level of the radiation-induced risk increases with age over the period of follow up. The follow-up study indicates a significant ($p=0.05$) increase in the risk of radiation-induced fatal solid cancers over the dose range of 0–50 mSv (assuming an RBE for neutrons of 10). Caution is needed in interpreting this finding, however, as an increased incidence of solid cancers is seen only at doses down to 200–500 mSv, suggesting the possibility of bias at the lower dose range.

278. The data on mortality from leukaemia are best fitted by a linear-quadratic dose response, while for all solid cancers taken together, a linear dose response provides a best fit for dose-response data up to doses of about 3 Sv. However, while a linear dose response can also be fitted to the data for a number of individual tumour types, in the case of non-melanoma skin cancer there is substantial curvilinearity in the dose response, consistent with a possible dose threshold of about 1 Sv or with a dose response in which the excess relative risk is proportional to the fourth power of dose. It is notable that if analyses are restricted to the dose range up to 2 Gy and account taken of possible systematic errors in the Hiroshima DS86 data, then there is evidence of appreciable upward curvature of the dose response for solid tumours. It has become clear that further follow-up and improved information on the doses received will be needed before the shape of the dose response at low doses for both morbidity and mortality can be determined with confidence at doses below about 100–200 mSv. While the Life Span Study has shown elevated cancer risks in a number of tissues, there are others for which there is either very little or no evidence for an effect. These include, for example, the bone, cervix, prostate, testes and rectum.

279. Information on cancer risks is also available from a number of studies of patients irradiated for medical reasons. Many of the patients in these studies received high doses to particular organs, often 1 Gy or more, although some received much lower doses. Patients were generally given acute exposures, although women treated with fluoroscopy for tuberculosis were given highly fractionated doses. As with solid cancers in the Life Span Study, the dose-response data from many of these studies are generally consistent with a linear dose-response relationship at low to intermediate doses. Results from several studies have suggested a statistically significant increase in the risk of thyroid cancer at doses of about 100–300 mGy received in childhood.

280. In contrast, the best fit to the data on bone tumour induction in radium dial painters exposed to $^{226/228}$Ra can be obtained with a model indicating a "practical threshold" for a response at an average bone dose in the range 3.9–6.2 Gy (high-LET). This observation might also reflect the extent of the data available at low doses. For head sinus carcinomas in the radium dial painters, linear, linear-exponential, or dose-squared exponential functions all provided acceptable fits to the data. Data are also available on the risk of bone sarcomas in patients given ^{224}Ra. Recent analysis of the pathology of these tumours has shown that a high proportion of them (30%) are malignant fibrous histiosarcomas, which is higher than would have been expected in sarcomas occurring spontaneously (8%–11%). It has been proposed that these tumours can only be expected to arise in tissue with deterministic radiation damage and so would be expected to appear only above a threshold dose. Similar conclusions have been drawn for the bone tumours arising in the radium workers.

281. Extensive data are available on cohorts of miners occupationally exposed to radon and its decay products. These studies have provided information on the risk of radiation-induced lung cancer. The most recent analyses of the data examined a range of risk models. However, for cumulative exposures below 0.175 J h m^{-3} (50 WLM), a constant-relative-risk model without any modifying factors, such as attained age and exposure rate, appeared to fit the data well.

282. A number of studies have provided information on the risk of childhood cancer following obstetric radiography. In the Oxford Survey of Childhood Cancers, a statistically significant 40% increase in the childhood leukaemia rate (up to 15 years of age) has been seen following doses of 10–20 mGy (low-LET). Similar results have been obtained in a number of other, smaller studies of the effects of obstetric radiography. Although there may be some increase in sensitivity to radiation at this early stage of development, there is no reason to believe the mechanisms involved in tumour induction will be fundamentally different from those in adults. The number of cells at risk would, however, be different. The principal reasons for being able to determine this increase in risk, which in absolute terms is small, is the low background incidence of leukaemia in childhood and greater sensitivity to radiation. A feature of the data from the Oxford Survey that remains unexplained is that the increase in risk for both leukaemia and solid cancers following exposure *in utero* is essentially the same, with a relative risk of about 1.4. Most other human and animal studies consistently indicate different sensitivities of leukaemia and solid cancers.

283. More recently, direct information on the effects of low-dose, chronic exposure has become available from studies of radiation workers. The estimation of cancer risks associated with exposure to low doses poses particular problems. The predicted level of excess risk associated with such exposures is lower than that for high-dose exposures, and consequently the size of the study population required to detect a raised risk is usually much larger than that required for the high-dose studies. The information available to date is generally consistent with information on the risks of cancer obtained from the high-dose-rate studies, although having wide confidence intervals, and would also be consistent with there being no risk at all. A long period of follow-up and pooling of data from different studies will be necessary if statistically useful data are to be obtained.

284. A number of studies have been published that have examined the risks of cancer in areas of high natural background. Comparative studies on groups exposed to different levels of natural background radiation do not, however, have the statistical power to detect significant effects on cancer incidence. There are difficulties in interpreting the data as a result of uncertainties in the doses actually received, geographical variation in the accuracy of cancer diagnoses, and confounding by other environmental factors.

IV. MECHANISMS AND UNCERTAINTIES IN MULTI-STAGE TUMORIGENESIS

285. The development and application of modern molecular methods has, in recent years, substantially increased the understanding of the mechanisms of tumorigenesis. At the same time, there has been an equivalent increase in the understanding of the action of radiation on cellular DNA, control of the reproductive cell cycle, and the mechanisms of DNA repair and mutagenesis.

286. Mechanisms of radiation oncogenesis were reviewed by the Committee in the UNSCEAR 1993 Report [U3] and are considered further in Annex F, "*DNA repair and mutagenesis*"; and Annex H, "*Combined effects of radiation and other agents*". Accordingly, the aim of this Chapter is to provide an updated view of the mechanisms of tumorigenesis in order to relate them to data on dose-effect relationships. Emphasis will be placed on current uncertainties surrounding the mechanisms of radiation tumorigenesis, with a view to exploring their importance for the development of biologically based computational models that seek to describe radiation cancer risk at low doses and low dose rates (Chapter V).

A. MULTI-STAGE PROCESSES IN TUMORIGENESIS

287. In accord with earlier proposals on spontaneously arising neoplasia [F1, F5, V1], UNSCEAR supports a multi-stage model as a conceptual framework for describing radiation tumorigenesis [U3]. A generalized model of this form is illustrated in Figure XXI. In this model, radiation tumorigenesis is imprecisely subdivided into four phases: neoplastic initiation, promotion, conversion, and progression. This operational framework, while subject to considerable uncertainty, may be used to illustrate the critical cellular and molecular processes that direct neoplastic change.

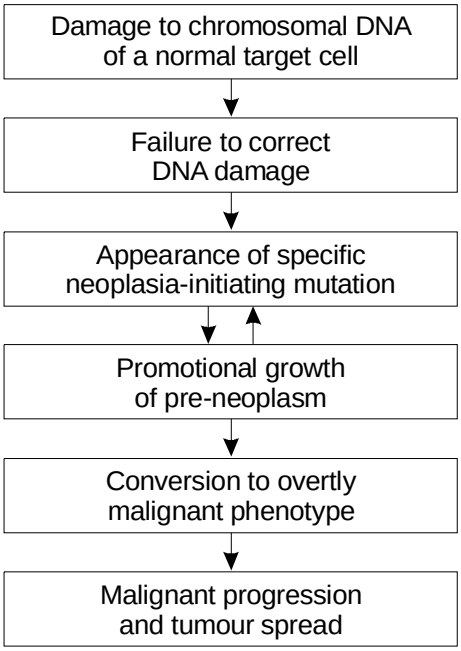

Figure XXI. A simple generalized scheme for multi-stage oncogenesis.

1. Initiation of neoplasia

288. Neoplastic initiation may be broadly defined as essentially irreversible changes to appropriate target somatic cells, driven principally by gene mutations that create the potential for neoplastic development [C9, U3]. Such tumour gene mutations can have profound effects on cellular behaviour and response, e.g. dysregulation of genes involved in biochemical signalling pathways associated with the control of cell proliferation and/or disruption of the natural processes of cellular communication, development, and differentiation. Although the full expression of such neoplasia-initiating mutations invariably requires interaction with other later-arising gene mutations and/or changes to the cellular environment, the initiating mutation creates the stable potential for pre-neoplastic cellular development in cells with proliferative capacity.

2. Promotion of neoplasia

289. Neoplastic development is believed to be highly influenced by the intra- and extracellular environment, with the expression of the initial mutation being dependent not only on interaction with other endogenous mutations but also on factors that may transiently change the patterns of specific gene expression, e.g. cytokines, lipid metabolites, and certain phorbol esters. As a consequence, there may be an enhancement of cellular growth potential and/or an uncoupling of the intercellular communication processes that act to restrict cellular autonomy and thereby coordinate tissue maintenance and development [T5, U3]. In this way, tumour-initiated cells can receive a supranormal growth stimulus and begin to proliferate in a semi-autonomous manner, allowing for the clonal development of pre-neoplastic lesions in tissues, e.g. benign papillomas, adenomas, or haemopoietic dysplasias.

3. Neoplastic conversion

290. Neoplastic conversion of pre-neoplastic cells to a state in which they are more committed to malignant development is believed to be driven by further gene mutations accumulating within the expanding pre-neoplastic cell clone. Evidence is accumulating that the dynamic cellular heterogeneity that is a feature of malignant development may in many instances be a consequence of the early acquisition of gene-specific mutations that destabilize the genome. Mutations of the *TP53* gene or one of a set of DNA mismatch repair genes provide examples of such destabilizing events in neoplasia [F3, H6, H17, L10]. There is also evidence that mutations resulting in enhanced chromosomal non-disjunction may also contribute to oncogenic change [L3].

291. An elevated mutation rate established relatively early in tumour development may, therefore, provide for the high-frequency generation of variant cells within a pre-malignant cell population. Such variant cells having the capacity to evade the constraints that act to restrict proliferation of aberrant cells will tend to be selected during tumorigenesis.

4. Progression of neoplasia

292. The progression of neoplastic disease may be dependent on metastatic changes that facilitate (a) the invasion of local normal tissues, (b) the entry and transit of neoplastic cells in the blood and lymphatic systems, and (c) the subsequent establishment of secondary tumour growth at distant sites [H4, T1]. It is the metastatic process and tumour spreading that are mainly responsible for the lethal effects of many common human tumours. Again, it is believed that in many cases gene mutations are the driving force for tumour metastasis, with the development of tumour vasculature an important element in disease progression [F4].

B. MUTATIONAL EVENTS MEDIATING THE TUMORIGENESIS PROCESS

293. Although models such as that shown in Figure XXI are most useful in placing clinical, histopathological, and cellular/molecular experimental data in the context of a generalized mechanism of tumour development, important

uncertainties remain. Identification of these uncertainties should help to guard against over-interpretation of data relating to radiation tumorigenesis, particularly with respect to biological modelling.

294. The gene-specific determinants of the initiation process that is believed to operate to allow entry of normal somatic cells into a given neoplastic pathway are incompletely understood, although for some organs there are strong associations with specific tumour gene mutations [U3, V1]. A similar degree of uncertainty attaches to the molecular events that determine the other cellular transitions noted in Figure XXI. While it is accepted that, in general, target cells for tumorigenic initiation will reside in the stem cell compartment of most tissues, the specific identity and location of these cells is poorly understood. As noted earlier in the Annex, this represents a significant uncertainty in some areas of radiation tumorigenesis, particularly with respect to alpha particle irradiation.

295. In general, the concept of stepwise interaction between loss-of-function mutation of tumour-suppressor genes and gain-of-function mutations of proto-oncogenes [U3] is still believed to apply. Further tumour-specific gene mutations have been identified, and there is much new information on the biochemical interactions between tumour gene mutations, which may destabilize the genome, compromise control of cell signalling, proliferation, and differentiation, and interfere with the normal interaction of cells in tissues (see [K1, S3]).

296. In the UNSCEAR 1993 Report [U3], the Committee concluded that the somatic genetic changes to cells that intermediate multi-stage tumour development potentially involve sequential mutation of different classes of genes, i.e. proto-oncogenes, tumour-suppressor genes, genes involved in cell-cycle regulation, and genes that play roles in maintaining normal genomic stability. It should be recognized, however, that the above classification serves principally as a framework for discussion and that there is substantial functional overlap between these classes.

1. Proto-oncogenes

297. Proto-oncogenes may be broadly defined as tumour-associated genes that can sustain productive gain-of-function mutations that result in over-expression or more subtle functional abnormalities in a wide range of cellular proteins. These proteins normally serve to control or effect cellular signalling and the temporal maintenance of growth and development [H9, L14, M20, W3]. Indeed, the known proto-oncogene proteins perform an extraordinary range of specific cellular functions, many of them interacting with each other in biochemical signalling cascades that, for example, target mitogenic processes, apoptotic activity, cell-to-cell interactions, and cytoskeletal functions. The capacity to effect transcriptional or post-translational activation of such pathways is a common theme for many such genes.

298. Thus, mutations resulting in altered proto-oncogene activity/specificity can lead to profound and constitutively expressed cellular effects; the close linkage between many cellular signalling pathways means that these effects are frequently pleiotropic. Table 12 gives a convenient scheme for classifying proto-oncogenes, along with a few examples.

Table 12
Classification scheme for proto-oncogene products

Designation	*Product*	*Examples*
Class 1	Growth factors	PDDGF-β chain (*sis*) and FGF-related growth factor (*hst*)
Class 2	Receptor and non-receptor protein tyrosine kinases (RPTK and NRPTK)	*src* (NRPTK) and *erbB* (RPTK); also *ret*
Class 3	Receptors lacking protein kinase activity	Angiotensin receptor (*mas*)
Class 4	Membrane-associated G proteins	*Ras* family
Class 5	Cytoplasmic protein-serine kinases	*raf-1* and *mos*
Class 6	Cytoplasmic regulators	SH2/SH3 protein (*crk*)
Class 7	Nuclear transcription factors	*myc, myb, jun, fos*
Class 8	Cell survival factors	*bcl-2*
Class 9	Cell cycle genes	*PRAD1* (cyclin D1)

299. Numerous and often multiple proto-oncogene activation events characterize different tumours; some of these were discussed in the UNSCEAR 1993 Report [U3]; others are noted in a series of reviews [B9, L14, M20, M21, V1] and are also mentioned later in this Annex.

300. In the context of this Annex, a very important issue is the nature of the mutational events that characterize proto-oncogene activation. Although there has been a large gain in the biochemical understanding of proto-oncogene action, little has changed since 1993 in respect of mutational activation mechanisms [U3]. In essence, human proto-oncogenes may be activated by point mutation (e.g. *RAS*), by gene amplification (e.g. *MYC*), or by chromosomal rearrangement (e.g. *ABL*) [W3].

301. There has, however, been a rapid increase in knowledge of the range of proto-oncogene activation events via chromosomal rearrangement and their often early role in tumorigenesis. These advances have been reviewed [R2], and cytogenetic data relevant to human tumorigenesis have been subject to detailed analyses [M23].

302. In brief, an increasingly wide range of chromosomal rearrangements associated with many subtypes of human

lympho-haemopoietic neoplasms have been characterized at the molecular and biochemical levels. More than 30 gene activation events resulting from proto-oncogene juxtaposition with T-cell receptors and immunoglobulin loci are known in T- and B-cell neoplasms, respectively. In the case of specific gene fusion by chromosome translocation/inversion, more than 25 examples have been characterized, with myeloid neoplasms the predominant carriers. Table 13 provides examples of chromosome translocations in human lympho-haemopoietic neoplasms.

Table 13
Examples of human tumour-suppressor genes

Gene	*Chromosome map location*	*Cancer type*	*Product location*	*Mode of action*
APC	5q21	Colon carcinoma	Cytoplasm	Transcription regulator
DCC	18q21	Colon carcinoma	Membrane	Cell adhesion/signalling
NF1	17q21	Neurofibromas	Cytoplasm	GTPase-activator
NF2	22q12	Schwannomas and meningiomas	Inner membrane	Links membrane to cytoskeleton?
p53	17p13	Multiple	Nucleus	Transcription factor
RB1	13q14	Multiple	Nucleus	Transcription factor
VHL	3p25	Kidney carcinoma	Membrane	Transcription factor
WT-1	11p13	Nephroblastoma	Nucleus	Transcription factor
p16	9p21	Multiple	Nucleus	CDK inhibitor
BRCA-1	17q21	Breast carcinoma	Nucleus	Transcription factor/DNA repair
PTCH	9q	Skin (basal cell)	?	Signalling protein
TSC2	16p13	Multiple	?	?

303. It has also become apparent that although proto-oncogene juxtaposition/fusion is most commonly observed in lympho-haemopoietic tumours, such events are also charac-teristic of certain solid tumours. For example, Ewing's sarcoma frequently carries a chromosomally mediated *FLI/EWS* gene fusion [R2], and in some papillary thyroid cancers the *RET* proto-oncogene can be activated by a set of specific chromosome rearrangements [Z1].

304. Since the cytogenetics of solid tumours are often complex and difficult to resolve accurately, it may be that proto-oncogene activation via chromosomal rearrangement is being underestimated. New methods of cytogenetic analysis by FISH are now available to approach this problem [S14].

2. Tumour-suppressor genes

305. Tumour-suppressor genes are defined as genes that can act as negative regulators of cellular processes such as signal transduction, gene transcription, mitogenesis, and cell development/ differentiation [H10, H11, L15, W3]. As noted later, some genes that act to regulate cell-cycle progression, apoptosis, and various aspects of DNA processing may also be included in this category. Consequently, all cancer-associated genes that act via a loss-of-function mechanism may be described as tumour suppressors even though, universally, they may not have true tumour-suppressing activity [H11]. The loss of function of tumour-suppressor genes characterizes a broad range of human neoplasms; some examples discussed in this Annex are listed in Table 14.

Table 14
Examples of chromosome translocations in lympho-haemopoietic neoplasia

Chromosome translocation	*Disease*	*Translocation*	*Genes involved*
Involving T-cell receptors	T-cell acute lymphatic leukaemia	t(1;7)(p32;q34) t(1;14)(p32;q11) t(1;7)(p34;q34) t(7;9)(q34;q32) t(7;9)(q34;q34)	*TCRβ-TCL5* *TCRδ-TCL5* *TCRβ-LCK* *TCRβ-TAL2* *TCRβ-TAN1*
Involving immunoglobulin	Burkitts lymphoma/B-cell acute lymphatic leukaemia B-cell chronic lymphatic leukaemia Pre-B-cell acute lymphoma	t(8;14)(q24;q32) t(2;8)(p11;q24) t(8;22)(q24;q11) t(2;14)(p13;q32) t(5;14)(q31;q32)	*IgH-MYC* *Igk-MYC* *Igl-MYC* *IgH-REL* *IgH-IL-3*
Involving fusion gene sequences	Pre-B-cell acute lymphoma Acute myeloid leukaemia Chronic myeloid leukaemia /B-cell acute lymphatic leukaemia	t(1;19)(q23;p13) t(6;9)(p23;q34) t(9;9)(q34;q34) t(8;21)(q22;q22) t(9;22)(q34;q11)	*E2A-PBXσσ* *DEK-CAN* *SET-CAN* *AML1-ETO* *BCR-ABL*

306. Unlike activated proto-oncogenes, which are functionally dominant, most tumour-suppressor genes require mutation of both autosomal copies to occur, often via intragenic point mutation of one copy and complete deletion of the other [U3]. Thus, the genomic location of potential tumour-suppressor genes is often revealed by the presence of consistent, region-specific DNA losses in a given tumour type. As noted in the UNSCEAR 1993 Report [U3], there are, however, examples where the mutation of one copy of such a gene can result in a change in cellular phenotype via intragenic mutations that result in so-called dominant negative effects. There are other examples where it seems that one gene copy is mutated conventionally and the other silenced by DNA methylation; there are also cases where effects from gene copy number have been found [H11]. Changes in chromosome complement (ploidy) are common during the development of many tumours, and it seems likely that some specific numerical chromosome changes in neoplasia relate to the loss of tumour-suppressor functions.

307. Overall, the loss of function that is characteristic of the role of tumour-suppressor genes means that the responsible mutational events can vary greatly, i.e. there can be intragenic point mutation/deletion, interstitial chromosome segment deletion, whole chromosome loss, or epigenetic silencing. Much will depend on the capacity of the target cell to remain viable, particularly with the deletion of large segments of DNA. This position contrasts with proto-oncogene activation, which demands relatively high DNA sequence specificity with respect to both intragenic point mutation and gene-specific juxtaposition or fusion. Few such gain-of-function mutations are expected to involve large DNA losses.

3. Genes involved in cell-cycle control and genomic stability

308. Abrogation of normal control of the cell cycle and maintenance of genomic stability is frequently observed in neoplasia. These phenotypes are sometimes closely linked, and recent advances have led to the consensus view that mutations leading to cell-cycle defects and mutator phenotypes can be critical for neoplastic development [H12, L10].

309. An example of the effect of tumour-suppressor mutations on cell-cycle control and genomic stability is provided by the *TP53* gene, which is mutated in a high proportion of tumours of various types [G8, L41]. The p53 protein is known to bind DNA and can act on a transcriptional regulator with potential effects on cell-cycle progression, DNA repair/recombination, and apoptosis [B43, H13, O1].

310. The half-life of the p53 protein in cells is short but increases in response to cellular stress, including DNA damage. Through mechanisms that remain uncertain, the increase in p53 protein serves to check the cell cycle in G_1/S or sometimes in G_2/M. It is believed that such cell-cycle checkpoints promote cellular recovery from stress, including the facilitation of DNA repair [H6, H14].

311. According to these proposals, when *TP53* is appropriately mutated, cell-cycle control and its checkpoints for repair are compromised, and during subsequent cellular development, errors of DNA replication and damage repair accumulate. Failure to adequately effect apoptotic death in damaged cells is also believed to be a feature of *TP53*-deficiency that contributes to neoplastic development [O1]. Other protein products of tumour-suppressor genes that impinge on cell-cycle control include pRb, p16, p27, and p85, and a complex series of cascade interactions involving tumour-suppressor and proto-oncogene proteins, together with cytokines, is believed to maintain close control of cell replication and apoptosis (see [K11, M22, N2]). It can be seen that many of the mutations that accumulate during neoplastic development do so because of the need for cooperation in order to fully compromise normal proliferative control.

312. In this context it has been argued for many years that the accumulation of the series of gene-specific clonal mutations that are believed to drive tumorigenesis would be improbable if normal genomic stability was maintained. Thus, recent findings regarding the spontaneous development of genomic instability in tumours has come as no great surprise. In addition to the *TP53*-mediated effects noted above, other aspects of somatically acquired genomic instability have been debated widely [H6, L10, L17]. The most important of these is the role of defects in DNA mismatch repair.

313. Mismatches in DNA base pairing occur at a relatively high spontaneous rate through replication errors (RER) and spontaneous oxidative/hydrolytic damage; these mismatches are corrected at high fidelity by a repair system that is highly conserved across species [F3]. Following the finding of a high frequency of replication errors in short microsatellite repeat sequences (the RER^+ phenotype) in a variety of tumours, some form of DNA mismatch repair defect was suspected.

314. Subsequently, many RER^+ human tumours, particularly those of the gastrointestinal (GI) tract, were shown to harbour mutations in DNA mismatch repair genes, principally *hMSH2* and *hMLH1* [A2, F3, H17, S15]. It is clear, however, that "instability" genes other than *TP53* and those associated with DNA mismatch repair are somatically mutated in human tumours. For example, the onset of aneuploidy is often a feature of the transition from pre-neoplastic to malignant phenotype, but the genes participating in the control of ploidy remain poorly understood. Recently, however, a dominantly expressing gene in this category has been revealed by a combination of FISH cytogenetics and somatic cell fusion techniques applied to a panel of colorectal cell lines [L3]; this gene appears to be functionally independent of *TP53* status.

315. Some progress is also being made with respect to somatic tumour genes that have a known or suspected role in DNA damage recognition and processing. The 11q22-encoded *ATM* gene of human ataxia-telangiectasia (A-T) and its role in the cellular and biochemical response to radiation damage are described in Annex F, "*DNA repair and mutagenesis*". With the knowledge that ataxia-telangiectasia patients are genetically predisposed to the development of neoplasms of the T-lymphoid lineage, a

search has been conducted for somatic *ATM* mutation in sporadic T-prolymphocytic leukaemia (T-PLL) [S16]. This investigation revealed that 11q22 losses and biallelic *ATM* mutations were present in a high proportion of sporadic T-PLL, suggesting a tumour-suppressor-like role for this gene in target T-cell precursors. It may be speculated that this is associated with its role in controlling genomic stability, particularly with respect to T-cell receptor sequences. Also noted in Annex F, *"DNA repair and mutagenesis"*, is the growing recognition that the breast cancer suppressor genes *BRCA1* and *BRCA2* play a role in the recognition/repair of damage to cellular DNA. Although the specific functions of these genes with respect to genomic stability remain to be resolved, their importance to heritable and sporadic breast cancer is well established.

4. Early events in multi-stage tumorigenesis

316. In the UNSCEAR 1993 Report [U3], the Committee recognized the difficulties of identifying the specific genes that, in mutant form, act at the initiation phase of tumorigenesis. For some lympho-haemopoietic neoplasms, specific chromosomally mediated proto-oncogene events were suggested to occur early in neoplastic development, and the *Mll1* gene data outlined later strengthen this view. Equally, however, many human myeloid neoplasms are characterized by region-specific chromosome deletions [M23], some of which are believed to arise early.

317. In the case of human solid tumours of certain tissues, there is growing evidence that those genes that act early are also represented as rare germ-line determinants of heritable cancer; the principal examples of this association are the *RET* gene in thyroid cancer, the *APC* gene in colorectal cancer, the *VHL* gene in renal cancer, the *PTCH(patched)* gene in basal cell carcinoma, and the *RB1* gene in retinoblastoma/ osteosarcoma [H11, S17, W3].

318. Although the *BRCA1* and *BRCA2* genes of breast cancer may be exceptions, a concept of early tumour development is evolving from the above associations. The concept requires a relatively tissue-specific "gatekeeper" gene to be mutated in order for stem-like cells to enter a phase of inappropriate clonal expansion [K12, S17]; this expansion then allows for the accumulation of further mutations. According to the concept, the accumulation of other mutations in the neoplastic pathway in the absence of gatekeeper defects will only infrequently result in the clonal development of recognizable tissue lesions. In essence, the temporal order of mutational events is likely to be important for productive neoplastic growth with loss of specific gatekeeper genes as critical early events.

319. In the UNSCEAR 1993 Report [U3], the Committee drew heavily on evolving models of colorectal carcinogenesis to support its views on the mechanisms that drive the genesis of solid tumours. In the same way, further data relating to this tumour type, while not necessarily fully representative of all solid cancers, may also be used to support the gatekeeper hypothesis.

320. A key element in this hypothesis as it relates to colorectal cancer is that the first consistent mutation in tissue lesions should be monoclonal mutation of the *APC* gatekeeper gene, which acts as a transcriptional regulator [N4]. In the main, the data to be discussed later [K12] support this, but a recent investigation of the temporal sequence of gene mutations adds considerable weight to the argument.

321. Using tumour microdissection and allelotyping methods, the sequence and tempo of allelic losses in a series of colorectal cancers at different stages of development was followed [B10]. The principal losses that were tracked were those associated with deletion of *APC* (5q21), *TP53* (17p13), and *DCC* (18q21). In brief, loss of heterozygosity (LOH) via allelic loss was not recorded in normal tissue surrounding colorectal tumours. However, 5q but not 17p losses arose abruptly and consistently at the transition from normal tissue to benign adenoma; a proportion of adenomas also showed 18q losses. Losses to 17p occurred equally abruptly and consistently at the adenoma to carcinoma transition border, and in highly advanced and invasive carcinomas, there was a high level of allelic variation indicative of clonal heterogeneity due to genomic instability.

322. Thus, commencing with *APC* loss from cells in normal tissue, the development of colonic tumours is characterized by abrupt waves of clonal expansion, with *TP53* loss and chaotic allelic variation being critical watersheds in the evolution of the fully malignant phenotype. Considering these and other molecular genetic observations with colorectal cancer, a temporal model of neoplastic initiation and malignant development has been proposed [B10]. This is illustrated in Figure XXII.

323. Although critical evidence in support of the gatekeeper gene hypothesis remains to be gathered, the hypothesis does account for many key observations made with respect to tumour genetics. Assuming for the moment that the hypothesis realistically reflects the processes of tumour initiation and subsequent development, then the spontaneous or induced mutation of rate-limiting and tissue-specific genes will be critical. Albeit less forcefully, the data discussed in this Chapter also imply that induced mutation of other genes in the neoplastic pathway for a given tissue will tend to be of less importance. This would be particularly true if a mutator phenotype, as described earlier, were to arise relatively early in the development of a malignancy; evidence for such early development of genomic instability is accumulating [S34]. With such mutator phenotypes, secondary mutations might be expected to arise in a developing neoplastic clone at a sufficiently elevated spontaneous rate for exogenous DNA-damaging agents at low doses to have no great effect on subsequent tumour development. Some caution is needed, however, before concluding firmly that the majority of induced neoplasms will spontaneously acquire genomic instability during malignant development. In this context it has been argued that current models of tumorigenesis place too much emphasis on the elevation of mutation rates and that cellular selection of evolving clones is more critical [T8].

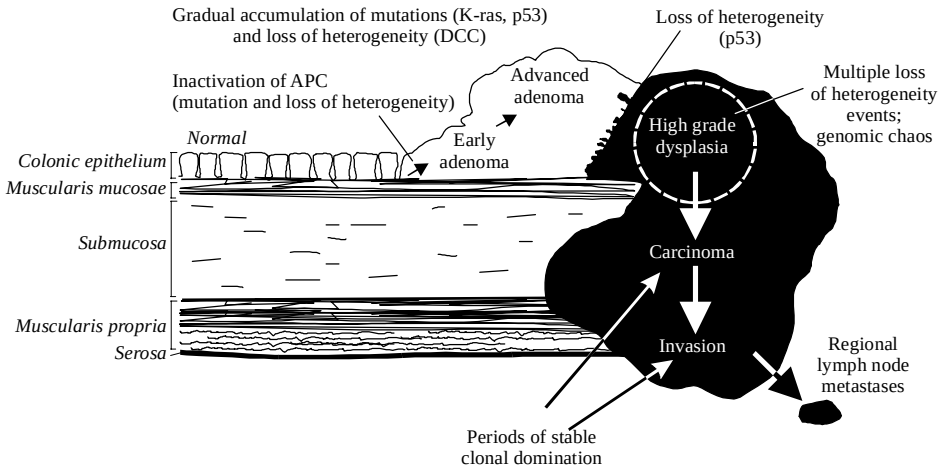

Figure XXII. A model of the sequence of genetic events in neoplastic development in the human colon [B10].

Initial mutations of loss of heterogeneity at the APC locus of a colonic epithelial cell is followed by adenoma development involving k-ras mutation and DCC loss.
Loss of p53 from advanced adenoma marks the transition between benign and malignant disease characterized in turn by the development of genomic instability, multiple gene losses and invasive behaviour/metastasis to regional lymph nodes.

5. Non-mutational stable changes in tumorigenesis

324. It has been recognized for some years that non-mutational but stable changes to cellular genomes can contribute to neoplastic development [C10, F2, U3]. In the light of knowledge of the role of specific genes in the tumorigenic process, the central questions are whether the activation/silencing of such genes can be identified in neoplasms and what mechanisms are involved. Such non-mutational mechanisms are broadly termed epigenetic and are believed to involve DNA methylation, genomic imprinting, and changes in DNA-nucleoprotein structure. As will be seen, these mechanisms are not mutually exclusive.

325. The DNA methylation status is believed to be one of the principal determinants of gene expression, and numerous studies have revealed widespread changes in the methylation patterns of the genomes of neoplastic cells [B11]. According to one theory, these changes contribute to the epigenetic modulation of gene expression, while another theory states that increased abundance of 5-methylcytosine serves to elevate spontaneous mutation rates in affected genomic domains. There is some evidence that both processes can occur, but attention will be given here to gene expression effects.

326. The promotor regions of genes are often rich in islands of CpG dinucleotides. These islands are normally free of methylation, irrespective of the state of expression of the genes in question [C10]. Studies with a wide range of neoplastic cells have revealed that *de novo* methylation of CpG islands is frequently acquired, e.g. [D4, J2, J3, M24]. Such effects have been recorded, for example, in a significant fraction of sporadic retinoblastoma and renal tumours for the *RB1* and *VHL* genes, respectively. Recently the *p16* gene (various cancers) and oestrogen receptor gene (colonic cancers) have been shown to be similarly methylated, sometimes at an early stage of tumorigenesis [I3, I4, M1]. In essence, methylation-mediated epigenetic changes in somatic gene expression appear to be an alternative route to mutation for the inactivation of tumour-suppressor genes.

327. Cytosine methylation of CpG dinucleotides is also known to be involved in the process of genomic imprinting, whereby specific genes are marked during gametogenesis for subsequent differential somatic expression [B12, C10]. These imprints, which inactivate one gene copy of sets of autosomal genes throughout the genome, are retained throughout development in spite of a wave of genome-wide demethylation during embryogenesis. Since certain genes involved in neoplastic development are believed to lie within imprinted genomic regions, the remaining active copy will be exposed, and a single somatic mutation can therefore result in the full expression of a mutant cellular phenotype [F2, U3].

328. Evidence of the involvement of this gametic form of imprinting on tumorigenesis was outlined in the UNSCEAR 1993 Report [U3] and has been discussed in depth elsewhere [F2]. Although the original gene-inactivating role ascribed to gametic imprinting with respect to the *RB1*, *IGF2*, and *H19* genes may be correct in certain instances, an alternative process may also operate. According to this second hypothesis, genomic imprinting serves principally to repress the expression of one somatic copy of growth-promoting genes. Loss of imprinting (LOI) during tumorigenesis acts to de-repress this normally silent copy, thereby increasing gene dosage and contributing to the deregulation of cellular growth and development. The data that support this second hypothesis include *N-MYC* gene amplification in neuroblastoma, loss of imprinting in colorectal cancer, and certain aspects of *H19* gene activation and *BCR-ABL* gene fusion, together with the overall picture of DNA demethylation in neoplasia [F2, R10, M31].

329. However, a considerable degree of uncertainty attaches to the contribution of genomic imprinting in tumorigenesis. Overall it appears that classical region-specific genomic imprinting established during gametogenesis may not play a large role in the development of common tumours. On the other hand, somatic changes in gene expression that do involve changes in the methylation status of critical genes may be widespread in common tumours and contribute significantly to their development.

330. The third stream of knowledge concerning epigenetic changes in gene expression derives from relatively recent findings in yeast concerning the nature of DNA-nucleoprotein interactions and its relationship to chromatin structure. So-called mating-type switches in yeast depend on the silencing of *HM* mating loci by trans-acting factors [R11]. The silencing of *HM* loci has been shown to occur via the action of silent information regulator (Sir) proteins; these also act on silent genes close to chromosome termini. Current evidence favours a role for a complex of Sir and other regulators in sequence-specific binding to silent target genes, with the acetylation of neighbouring histone proteins as a critical factor; CpG island methylation may also be important.

331. Overall it seems that gene silencing demands the formation of tightly folded nucleoprotein configurations in chromatin (heterochromatization), with the Sir proteins playing a role in establishing the necessary pattern of histone deacetylation. In subsequent studies, a mutant form of the yeast gene *SAS2* was found to enhance the loss of gene silencing; the yeast and mammalian forms of this gene have been shown to have sequence homology with several known acetyltransferase genes. Again, a role in the formation of heterochromatin structure is implied [R11]. These and other mechanisms that link gene expression with chromatin structure have been considered in depth [E4]. Knowledge of the biochemistry and genetics of Sir proteins also provides evidence that their diverse functions include regulation of the cell cycle and repair of DNA double-strand breaks. It has been suggested that such regulation may involve the provision of heterochromatic sites for the storage of DNA repair and replication proteins [G19]. DNA strand break repair is considered in depth in Annex F, "*DNA repair and mutagenesis*".

332. An association between these gene-silencing observations in yeast and tumorigenic processes in man was established by the finding that *MOZ*, a human homologue of yeast *SAS2*, was a partner in a fusion gene (*MOZ-CBP*) generated by the primary t(8;16) chromosome translocation in human myeloid leukaemia [B13]. Although critical evidence is lacking, it seems feasible that the fusion protein could act to redirect MOZ acetylation function to an inappropriate set of genomic domains. In this way normal patterns of heterochromatization and gene activation/silencing would be compromised. Further to this, there is now evidence of a synergy between DNA demethylation and inhibition of histone deacetylase in the re-expression of genes silenced during tumorigenesis [C18].

333. Thus it is becoming clearer that region-specific changes in the heterochromatic state of chromosome regions can have profound effects on gene expression. Given the accepted role of gene expression changes in neoplasia, it would be surprising if the acetylation-related *MOZ-CBP* fusion noted above proved to be an isolated example of oncoprotein involvement, and recent studies point towards a more general role in neoplasia of the genes involved in modifying chromatin [K18].

6. Summary

334. Proto-oncogenes and tumour-suppressor genes control a complex array of biochemical pathways involved in cellular signalling and interaction, growth, mitogenesis, apoptosis, genomic stability, and differentiation. Mutation of these genes can, in an often pleiotropic fashion, compromise these controls and contribute to the multi-stage development of neoplasia. Mutant proto-oncogenes disturb cellular homeostasis in a dominant gain-of-function manner, whereas for tumour-suppressor genes sequential loss-of-function mutation of both autosomal copies is usually, but not always, required. Thus, proto-oncogene mutations are invariably subtle, while the mutations of tumour-suppressor genes can range up to gross DNA deletion.

335. On the basis of accumulating knowledge it is argued that early proto-oncogene activation by chromosomal translocation is often associated with the development of lympho-haemopoietic neoplasia. In contrast, for many solid tumours there is a requirement that tissue-specific tumour-suppressor genes that act as gatekeepers to the neoplastic pathway must undergo mutation; some of these mutations directly or indirectly affect control of the cell cycle and apoptosis. On the basis that solid tumour initiation is most frequently associated with tumour-suppressor gene mutation, it has also been proposed that the subsequent onset of spontaneous genomic instability via further clonal mutation is a critical event in neoplastic conversion from a benign to a malignant phenotype. Loss of apoptotic control is believed to be an important feature of neoplastic development and is described in more detail later in this Annex. Apoptosis as a response to radiation is also discussed in Annex F, "*DNA repair and mutagenesis*".

336. In spite of continuing gains in knowledge, it is important to recognize that much of the information available on multi-stage tumorigenesis remains incomplete, thus limiting the predictive power of mechanistic models that seek to describe these complex cellular processes. Although the concept of sequential and interacting gene mutations as the driving force is more firmly established, there is a lack of understanding of the complex physiological interplay between these events and its consequences for cellular behaviour and tissue homeostasis. It is also important to stress that the concepts outlined in this Section derive from detailed studies in a somewhat limited set of tumour types; there is an inherent danger in applying a single mechanistic concept to all or many tumour types.

337. Uncertainty also surrounds the degree to which non-mutational (epigenetic) changes to the genomes of neoplastic cells contribute to tumorigenesis. Increases in the methylation status of critical tumour-suppressor genes is known to be an alternative to mutational inactivation in a range of neoplasms, and loss of methylation imprints may also serve to increase the activity of some growth-promoting genes. DNA methylation is also believed to be involved in the genomic imprinting processes occurring during gametogenesis, but these may not make a major contribution to tumorigenesis. New evidence also implicates histone acetylation in genomic heterochromatization and gene silencing. It is suggested that such gene silencing may make a potentially important contribution to epigenetic change.

338. An important feature of recent studies has been the clarification of the role of specific gene mutations in tumours that serve to destabilize the genome, thereby allowing for the rapid spontaneous development of clonal heterogeneity and tumour progression. Although critical evidence is lacking, it is possible to envisage that after this transition point is reached, tumour development may be relatively independent of exogenously induced DNA damage.

C. CELLULAR AND MOLECULAR TARGETS FOR TUMOUR INITIATION

339. In the UNSCEAR 1993 Report [U3], the Committee reviewed data for appraising the cellular targets that are or might be involved in tumour initiation. The critical question posed was whether the mutation of single genes in a single normal target cell in tissue could, in principle, divert that mutant cell into a potentially neoplastic pathway. At the time of the UNSCEAR 1993 Report, the evidence available broadly supported this view. Uncertainties on this issue were, however, recognized, and since that time there have been further developments, which are summarized in the following paragraphs.

1. Monoclonal origin of tumours

340. The critical features of human and animal tumours that lend support to the single-cell (monoclonal) origin of tumours are that they exhibit (a) consistent and characteristic chromosomal and/or gene mutations in all neoplastic cells, (b) clonality with respect to the expression of X-chromosome-encoded genes in tumours of females, and (c) characteristic monoclonal restriction enzyme polymorphism of known and anonymous DNA sequences. It has also been noted that molecular analysis of human tumours associated with exposure to chemical carcinogens and ultraviolet radiation, together with that of tumours arising in genetically predisposed individuals, adds weight to the concept that the majority of tumours are of single-cell origin [U3].

341. It was recognized, however, that because such analyses are performed on macroscopic neoplasms, monoclonality might, in some circumstances, be due to cellular selection via proliferative advantage, i.e. initially neoplasms are predominantly polyclonal but become increasingly monoclonal during early growth. Although this issue remains somewhat problematical, a number of recent observations allow further comment.

342. The first observation concerns tumours that are believed to have their origins *in utero*. Some leukaemias arising in monozygotic twin children have, in the past, been shown to share the same primary chromosomal anomaly, implying, but not proving, that they arose in a monoclonal fashion from an early precursor cell population present when the two fetuses shared a common (*in utero*) blood supply. This interpretation has been greatly strengthened by the finding that such leukaemia in monozygotic twins can have identical molecular rearrangements of a proto-oncogene termed *Mll1* [F6].

343. The monoclonality of childhood solid cancers is also strongly supported by the finding that a specific tumour-suppressor-gene-associated chromosome loss/reduplication event in early embryogenesis can lead not only to mosaicism in normal tissue but also to the development of monoclonal Wilms' tumour [C11].

344. A second line of evidence concerns further developments in the understanding of multi-stage colon carcinogenesis [U3]. Mutation/loss of the tumour-suppressor gene *APC* has for some time been believed to be a critical early event in the development of human colon cancer. Up to about 70% of early colonic adenomas show apparently monoclonal structural/functional loss of this gene [P5], and a critical role in tumour initiation seems likely [B10].

345. With use of a mouse (Min) model of intestinal carcinogenesis, this view of monoclonal tumour initiation has been strengthened. In essence, aberrant crypts, the earliest intestinal lesions detectable microscopically, have been microdissected from Min mice and shown to be monoclonal with respect to *Apc* loss [L18, L19] (but see also para. 284]. This and another mouse model of myeloid leukaemia, described below, have also been used to provide evidence of early monoclonal events associated with radiation tumorigenesis. There are also data describing early events in thymic lymphomagenesis [M33].

346. Given the recent evidence outlined in this Annex and that previously reviewed by the Committee [U3], it seems likely that the vast majority of tumours in humans and animals arise from mutation of single target stem-like cells in tissues. This view continues to find the support of most commentators but has been debated widely [A3, F7, F8, R12].

347. The Committee also noted publications where tumour monoclonality has been questioned [U3], and a striking contribution to the debate was recently published [N3]. The basic finding was that a large proportion of intestinal adenomas in the gastrointestinal tracts of human familial adenomatous polyposis patients, who were also XO/XY in genotype and therefore mosaic for the Y-chromosome, was apparently polyclonal. In this study polyclonality was judged by the presence within single adenomas of a mixed

population of cells with respect to the Y-chromosome sequence. By this measure, up to 76% of adenomas were polyclonal. However, early Y-chromosome loss and field effects creating tumour clustering and collision [U3] might contribute to this finding.

348. Tumour clustering may also explain new data on the apparent polyclonality of a proportion of spontaneously arising intestinal adenomas in Min mice as assessed by genetic features other than *Apc* loss [D9]. Thus, polyclonality may be acquired during adenoma development rather than arising *de novo* at the time of initiation. For example, fusion of independent *Apc*-deficient microclones may allow for cooperative growth. These data illustrate some of the problems that remain in resolving the early molecular events and complex cellular interactions of tumour development. In spite of these uncertainties there remain experimental data on intestinal tumorigenesis that forcibly support monoclonality for induced neoplasms [G11].

2. Molecular targets for radiation tumorigenesis

349. Following its review of the mechanisms of mutagenesis, oncogenesis and the data available on molecular targets, the Committee suggested that loss of critical tumour-suppressor genes via DNA deletion might be the principal mechanism by which radiation damage might contribute to tumour development [U3]. It was suggested that proto-oncogene activation via point mutation or chromosomal rearrangement played a less critical role overall but might be important for certain tumours.

350. Direct human data relating to these issues remain, however, fragmentary. As noted in Annex F, *"DNA repair and mutagenesis"*, more data have emerged on *TP53* gene mutations in radiation-associated human tumours, particularly lung tumours. Unfortunately the interpretation of these data remains highly problematical, and at present it is not possible to judge whether intragenic mutation of this gene is an early radiation-associated event in any human tumour type. *TP5*3 gene mutations have also been studied in liver tumours arising in excess in patients treated with the radiographic contrast agent thorotrast, which contains alpha-emitting thorium oxide [I14]. These studies comment more on secondary *TP53* mutation than on early radiation-associated events in liver tumorigenesis.

351. In the case of human thyroid cancer, chromosomally mediated rearrangement of the *RET* proto-oncogene is a common but not invariate feature; such events are believed to occur early in the genesis of the papillary form of this tumour [Z1]. *RET* proto-oncogene rearrangements have been found in some cases of papillary childhood thyroid cancer arising in areas contaminated by the Chernobyl accident. Since three different forms of *RET* rearrangement are present, overall, among spontaneously arising papillary thyroid cancer cases, it is possible that in radiation-associated cases one particular form will predominate. A recent commentary [W4] on one data set suggests that the spectrum of these rearrangements in Chernobyl-related papillary thyroid cancer is unremarkable, although in other studies [B14, K13] one type (*RET/PTC3*) appeared to be more frequent than expected. A causal relationship between *RET* rearrangement and radiation remains, therefore, a matter of some uncertainty. Nevertheless, the finding of *RET* rearrangement following experimental high-dose irradiation of human thyroid cells [M25] suggests that specific and rare *RET* proto-oncogene rearrangements associated with the genesis of these tumours can be induced by radiation.

352. The study of second cancers after radiotherapy [C12, C28] provides another direct approach to the problem. Investigations of gene-specific mutations in such tumours have yet to be particularly informative, and at this stage of knowledge cytogenetic approaches may prove to be more productive. Studies that include cytogenetic evaluation of therapy-related sarcoma, meningioma, and rectal carcinoma provide some evidence that chromosomally complex monoclonal tumours having hypodiploid karyotypes with multiple deletions may be most common [C13, C28]. The number of therapy-related tumours characterized in this way remains, however, too small to make these findings conclusive.

353. With respect to target DNA regions and genes for radiation tumorigenesis, more rapid progress is being made through the use of experimental models of tumorigenesis in rodents. Regarding as yet uncharacterized molecular targets, some studies with breast and thyroid clonogens provide evidence of an apparently high frequency of tumour-initiating events that, it is argued, may reflect the involvement of non-mutational processes [C19, U3]. Uncertainties attaching to the status of these events are discussed later in this Annex, and here attention will focus on mouse models that more specifically suggest genomic targets for tumorigenesis. Three examples of this work are given below.

354. In a mouse genetic model of germ-line *p53*-deficiency ($p53^{+/-}$), quantitative studies of tumorigenesis (principally lymphomas and sarcomas) showed these mice to be extremely sensitive to tumour induction by an acute dose of 4 Gy from gamma rays [K2]. Of particular note was the shortening of the tumour latency period after irradiation. Molecular studies of these tumours revealed that complete loss of wild type *p53* was a consistent event; these data, together with those from quantitative studies, provided good evidence that *p53* and surrounding sequences could act as a direct target for radiation.

355. A somewhat unexpected finding was that in almost all cases of *p53* loss from induced tumours there had been duplication of the mutant *p53* gene. A likely reason for this was provided by subsequent *in vivo* analyses of post-irradiation cytogenetic damage in the haemopoietic system of murine *p53* genotypes [B4]. These studies showed that although *p53*-deficiency had only marginal effects with respect to the frequency of structural chromosomal rearrangements after radiation, there were substantial effects on whole chromosome loss and gain (aneuploidy). This enhancement of radiation-induced aneuploidy appeared to be driven by a *p53*-associated

defect in a G_2/M cell-cycle checkpoint. Thus, it was suggested that loss of wild type *p53* occurred through loss of the whole of the encoding chromosome (chromosome 11). For the purposes of establishing genetic balance and viability, there was strong selection for those cells that had duplicated the remaining chromosome 11, which accounts for the duplication of mutant *p53*. On this basis it may be seen that in some circumstances the molecular target for radiation oncogenesis may be as large as a whole chromosome.

356. Somewhat similar studies have been undertaken using the Min mouse ($Apc^{+/-}$) model of intestinal carcinogenesis [S18]. Using F1 hybrid mice carrying the *Apc* mutation, a 2 Gy whole-body dose from x rays has been shown to double the spontaneous incidence of intestinal adenomas [E5]. In hybrid genetic backgrounds it is possible to determine through the use of polymorphic microsatellites whether spontaneous and radiation-associated adenomas arise through early loss of wild type *Apc* and, if so, the type/extent of the mutation involved. Published studies [H32, L20] reveal that complete loss of wild type *Apc* is characteristic of the majority of both spontaneously arising and radiation-associated early adenomas. These mutational events may involve whole chromosome loss or interstitial deletions, but deletion events tend to predominate in radiation-associated adenomas [H32]. Again, therefore, a tumour-suppressor gene appears to be acting as a direct target for radiation, with gene losses usually being associated with gross deletion events.

357. The third example of informative animal data concerns the induction by radiation of acute myeloid leukaemia (AML) in certain strains of mice. It has been known for some years that these acute myeloid leukaemias are consistently associated with early arising deletions from chromosome 2 [B15, H15]. More recently, however, cytogenetic studies of bone marrow cells of irradiated mice [B16] have revealed that characteristic chromosome 2 deletions are apparent within the first few days following *in vivo* irradiation. Carrier cells of stem-like origin remain, however, relatively indolent in bone marrow for many months until unknown secondary events trigger them into rapid clonal expansion prior to the development of overt monoclonal acute myeloid leukaemia. The identity of the gene loss from mouse chromosome 2 that initiates acute myeloid leukaemia development remains unknown, but the critical chromosomal region encoding an acute myeloid leukaemia suppressor gene has been narrowed to around 1 cM (~10^6 base pairs) [C5, S40]. Thus, data on the mechanisms of radiation-induced murine acute myeloid leukaemia point to tumour-initiating loss of gene function from stem-like cells in bone marrow, followed by the accumulation of spontaneous secondary events that trigger initiated cells into a pathway leading to monoclonal leukaemia development.

3. Summary

358. On the basis of a large body of data it may be judged that, in the main, tumours appear to have their origin in gene/chromosomal mutations affecting single target stem-like cells in tissues. It is recognized, however, that there are circumstances where early phases of tumour development may be bi- or even polyclonal and that monoclonal selection occurs later.

359. Direct evidence on the nature of radiation-associated initiating events in human tumours is sparse, and rapid progress in this area should not be anticipated. By contrast, good progress is being made in resolving early events in radiation-associated tumours in mouse models. In the case of tumours induced in *p53* and *Apc* heterozygously deficient mice, radiation appears to target the remaining wild type tumour-suppressor gene via gross chromosomal deletion. Radiation-induced deletion of a specific chromosomal segment also appears to act as an initiating event for mouse acute myeloid leukaemia. These molecular observations lend further support to the view expressed in the UNSCEAR 1993 Report [U3] that radiation-induced tumorigenesis will tend to proceed via gene-specific losses from target stem cells.

D. CELLULAR FACTORS THAT COUNTER ONCOGENIC DEVELOPMENT

360. Somatic cells employ a series of measures to protect against the development of abnormal and potentially neoplastic phenotypes. In essence, a certain proportion of these barriers has to be breached by the cell before it becomes committed to malignant development. Thus, the process of multi-stage oncogenesis may be viewed as the stepwise acquisition of cellular properties that allow evasion of these protective functions [U3].

1. Control of cellular proliferation and genomic stability

361. The ordered replication of DNA during the reproductive cell cycle, the equal sharing of the replicated genome to the daughter cells, and the close control of mitotic activity is an essential element of normal tissue development and maintenance [U3]. Under normal circumstances cells can respond to specific mitogenic stimuli, continue proliferation while that stimulus is maintained, and fall to a resting state when it is removed. Such normal somatic cells are also believed to have a finite lifespan, and as a consequence of an internal genetic programme, after completing a given series of reproductive cycles, they cease proliferative activity and enter a degenerative senescence phase. There is also a strong requirement for phase controls within the reproductive cycle itself, such that DNA replication is appropriately initiated and completed before genomic segregation to daughter cells and that in the event of non-optimal cellular conditions, the cell cycle is checked until the problem is rectified. Cell-cycle checkpoints may be particularly sensitive to induced DNA damage, and there is some evidence that the presence of very few DNA double-strand breaks can lead to cell-cycle arrest [H23].

362. In recent years much has been learned of the control of cellular proliferation [N2], the process of cellular senescence [H3, H5, S4], and the importance of cell-cycle checkpoint

control [H7] for maintenance of genomic stability. As this information accumulates, it has become evident that all of the above normal controls are potentially subject to mutational change during multi-stage oncogenesis and therefore require some consideration in the modelling of tumour development.

363. The information discussed previously by the Committee [U3] and in Section B of this Chapter includes a number of examples of how gene/chromosomal mutations and sometimes epigenetic events in tumours can compromise control of the cell cycle (e.g. *RB1*, *TP53*, and *p16*) and/or decrease genomic stability, e.g. *TP53*, DNA mismatch repair genes, and *ATM*. As noted earlier, the resulting abnormal patterns of cellular proliferation and the generation of clonal heterogeneity are sentinel features of neoplastic growth, representing the escape from normal cellular constraints. Thus, precise control of the cell cycle and high-fidelity DNA damage recognition/repair are clearly important protective factors against tumour development. Also associated with proliferative control and genomic stability are the DNA sequences present at chromosome termini (telomeres).

364. The characteristic hexamer repeat sequences (TTAGGG) at mammalian telomeres erode via incomplete replication during each cell cycle. Since the majority of human somatic cells lack expression of the enzyme telomerase that adds these hexamer repeats to telomeres, it has been suggested that the process of erosion acts as a "molecular clock" (see [H16, K14]). In this way the replication potential of somatic cells is limited, and there is direct evidence that the senescence of human cells is, in some part, determined by the absence of telomerase [B35, J4].

365. During the senescence process as measured *in vitro*, there is a tendency for cells to become chromosomally unstable, with many of the resulting aberrations being centred on chromosome termini [C14, K14]. Thus, telomeric erosion during senescence renders chromosomes prone to end-to-end association and subsequent cycles of breakage and fusion.

366. It is believed that telomere-sequence-mediated cellular senescence is one of the means whereby cells may be eliminated from neoplastic pathways. It follows, therefore, that in human cells the stabilization of telomeres and the generation of immortal or lifespan extended phenotypes is likely to be a critical step in tumorigenesis [G9, K14]. In accord with this proposition many, but not all, human tumours have been shown to carry stabilized telomeres via reactivation of telomerase or utilization of alternative pathways of telomere maintenance (see [B17, K14]).

367. Although good progress continues to be made in this whole area, a simple relationship between senescence, immortalization, telomerase, and tumorigenesis should not be inferred [B17, J4, L21].

2. Programmed cell death and gene expression

368. Programmed cell death, also termed apoptosis, plays an important role in restricting the growth of many normal cell lineages and is an important element in the regulation of organ development and maintenance [R3]. Apoptotic processes are believed to be controlled by the interaction of intra- and extracellular factors with the signalling machinery of the cell. These signals, or in some cases their absence, can trigger the recipient cell into a characteristic biochemical suicide pathway that usually involves genomic degradation. Importantly, apoptotic responses can also accompany exogenous insult, induced by ionizing radiation, genotoxic chemicals, and other sources of stress; in some cellular systems these responses have been associated with prior perturbation of the cell cycle. The biochemistry and genetics of apoptosis are becoming much better understood, and advances in the whole area have been reviewed extensively [C16, H22, K6] and have received comment with respect to radiation protection [S22]. A detailed description of these mechanisms is beyond the scope of this Annex, but a brief outline is appropriate.

369. The process of cellular apoptosis may be divided conveniently into three phases: initiation, effector, and degredation (nucleolytic and cellular). The initiation phase differs according to cell type and the source of stress, while the effector and degradation phases, although regulated, tend to be more uniform [K7]. As noted in Section B, a range of proto-oncogenes and tumour-suppressor genes participate in the intracellular signal cascades that can initiate apoptosis. Here, information on two apoptosis-related genes, *TP53* [E3] and *Bcl2* [K7], will be presented.

370. Biochemical studies indicate that almost all productive mutations of *TP53* compromise the ability of the protein to bind to gene-specific DNA sequences and to regulate transcription; in general, the cellular consequences are alterations in growth arrest or apoptosis. Although p53 protein response is apparent under a range of stresses, recent studies suggest a common root. A series of findings (see [K5]) imply that intracellular oxidative stress is a critical trigger for p53-mediated apoptosis. Together with p85 and perhaps Abl protein, p53 is believed to regulate the redox state of the cell [K5, Y3], and it is this state that may be a common determinant of apoptosis or survival. Other aspects of p53-dependent and developmentally regulated apoptosis including the role of ceramide are discussed in Annex F, *"DNA repair and mutagenesis"*.

371. The second example concerns the genes in the *Bcl2* family [K6, K7]. These include *Bcl* X_L, *Bcl-w*, and *Blf-1* (death antagonists) and *Bax*, *Bak*, *Bcl* X_S, and *Bad* (death promotors). The protein products of these genes participate in a complex network of biochemical reactions that differ between cell types. These pathways may be linked with *Raf*, *MEK*, and *Jun* amino terminal kinase (JNK) protein and also, via *Ras* protein, with the p85 pathway noted above [K7, P6].

372. In a broad sense it is believed that it is the balance between pro- and anti-apoptotic factors that determines cell fate [K7]. Thus, apoptotic signals via, for example, cell surface receptors, redox changes, reactive oxygen species, and Ca^{++} ion concentration (initiation phase) are sensed by

the Bcl-2 regulatory complex, resulting in changes in mitochondrial permeability (effector phase). According to current proposals, the degredative and nucleolytic phases then proceed as a consequence of the release of directly apopto-genic factors, e.g. caspases, superoxide anions, and endo-nucleases from mitochondria into the cellular cytosol [K7].

373. Overall, it may be seen that cells possess a highly developed system for detecting stress, eliciting biochemical responses, and, in essence, deciding on the basis of bio-chemical balance whether to survive or to proceed towards cell death.

374. The potential of these stress-related apoptotic pathways to reduce tumorigenic risk, although not formally established, is strongly indicated. There appear to be at least two principal points of action of apoptosis during tumorigenesis. There is evidence for at least three stress-related pathways in cells that respond to genotoxic insult, including that from ionizing radiation, i.e. those pathways centred on Abl, JNK, and p53 proteins [C4]; the p53 pathway has been judged to be the "universal sensor" of damage in normal cells. A variety of other cellular genes have also been shown to be up- or down-regulated in response to radiation. While a comprehensive review of such studies is beyond the scope of this Annex, Table 15 provides, by way of example, a summary of the data obtained after neutron or gamma-ray exposure of Syrian hamster cells [W10]. These results were obtained after exposures of 0.21–2.0 Gy of neutrons or 0.96–3.0 Gy of gamma rays at low and high dose rates. These data should not, however, be taken as representative of mammalian cells in general, and cell type dependency in induced gene expression should be expected.

Table 15
Radiation effects on gene expression in Syrian hamster cells
[W10]

Gene	*Effect on expression* [a]		*Function*
	Neutrons	*Gamma rays*	
Interleukin-1	Increase	Increase	Cytokine
β-actin	Decrease	Decrease	Cytoskeleton
γ-actin	Increase	Increase	Cytoskeleton
β-PKC	No change	Increase	Signal transduction
Rp-8	Increase	No change	Apoptosis
c-*fos*	Decrease	Increase	Transcription factor
c-*myc*	No change	No change	Nuclear protein
α-tubulin	Increase	Increase	Cytoskeleton
fibronectin	Decrease	Increase	Cellular matrix
Interleukin-6	Increase	-	Cytokine
Proliferating cell nuclear Ag (PCNA)	Increase	Increase	Transcription factor/repair
Superoxide dismutase	-	Increase	Scavenger
c-*jun*	Increase	Increase	Transcription factor
Rb	Decrease	Increase	Nuclear protein
H4-histone	Decrease	Increase	Nuclear protein
p53	No change	No change	Nuclear protein

a All changes evident within the first four hours following radiation exposure. Neutron dose rates: 1 mGy min^{-1} and 140 mGy min^{-1}; gamma ray dose rates: 10 mGy min^{-1} and 120 mGy min^{-1}.

375. The development of high-throughput screening technologies promises to greatly increase the power of resolution of studies on such radiation-associated changes in gene expression in mammalian cells. For example, using these new techniques a linear non-threshold dose response for the transcriptional induction of the stress-related genes *CIP1/WAF1* and *GADD45* has been demonstrated for gamma ray doses in the range 20–500 mGy [A18]. The consequences of such induced stress responses for low-dose tumorigenesis remain a matter for speculation. Nevertheless, an association between the *in vitro* induction of *PBP 74* gene transcription by 250 mGy radiation and human cell hypersensitivity to cell inactivation might be explained by some form of damage threshold for the enhancement of DNA repair [A18, S41]. One speculation is that if such a hypersensitive mechanism for cell inactivation were to dominate at low doses (say, up to around 100 mGy), mutation induction rates would be depressed, leading to a non-linear and, perhaps, a threshold-type relationship for radiation cancer risk [J8]. If, however, this increased sensitivity to cell inactivation were to be accompanied by increased cell mutation rates, no such threshold would be expected. The data available do not allow these two possibilities to be distinguished, although some of the data discussed in Annex F, *"DNA repair and mutagenesis"*, suggest a direct dose-effect relationship between cell inactivation and gene mutation. Stress-related cellular responses are also discussed in Annex F, *"DNA repair and mutagenesis"*, which draws attention to new work that associates the appearance of specific novel proteins with cellular stress.

376. In the absence of complete DNA repair fidelity, the whole organism gains a large advantage by promoting the death of damaged and potentially neoplastic cells. However,

the true effectiveness of apoptotic pathways in removing such aberrant cells cannot be judged at this time. The mere fact that the frequency of gene/chromosomal mutations increases in cell populations surviving genotoxic insult argues against an extremely high capacity for apoptotic surveillance of mutagenic damage in all cell types. In the context of ionizing radiation, the shape of the low dose response for the induction of apoptosis in different cell types remains very uncertain, and equal uncertainty surrounds the influence of dose rate. Accordingly, for the purposes of modelling tumorigenic risk, judgements on the balance between mutagenesis and apoptosis at low doses cannot be made with confidence. The radiobiological factors that influence the induction of apoptosis vary with cell type, and there is also some dependency on the mechanisms involved [B27, S29]. In general, doses greater than 0.5 Gy of low-LET are necessary to obtain statistically significant increases in apoptotic rates; a plateau in the dose response is frequently seen at doses >5 Gy. In the well-studied human lymphocyte system there is evidence that the induction of apoptosis is largely independent of LET and dose rate, implying that in these cells, initial DNA damage is more important than its repair [V4]. DNA double-strand lesions are believed to be one of the determinants of apoptotic response, but some have suggested that damage to plasma membranes may also act as an apoptotic signal [O4]. There is also evidence of linkage of the signalling of apoptosis and cell-cycle arrest; for example, a protein known as survivin has been implicated in the control of both apoptosis and a mitotic spindle checkpoint [L37]. Additional aspects of apoptotic response are discussed in Annex F, *"DNA repair and mutagenesis"*.

377. Apoptosis is also believed to play an important role in tumour cell survival during post-initiation clonal expansion. At a critical point during clonal expansion, the oxygen supply to the neoplasm begins to become limiting [F4, U3]. It has been proposed that under these circumstances the redox stress placed on tumour cells triggers an apoptotic response, with cell death being most pronounced in the regions most distant from vascular supply [K5, N5]. Thus, during this phase in tumorigenesis, apoptosis will be playing a crucial role in limiting *in situ* growth and invasive behaviour.

378. Given the scenarios noted above, it is not surprising that a broad array of tumour types carry a variety of mutant genes that directly or indirectly uncouple stress response and apoptosis. Resistance to apoptosis may be viewed as the means whereby cell survival is favoured over cell death, and under conditions of *in vivo* stress, this phenotype will tend to be strongly selected. The p53 pathway appears to be the universal sensor of cellular stress, and it is this feature that may make loss-of-function *TP53* mutations so prominent in human tumorigenesis.

379. Overall, it is judged that apoptotic suicide of cells provides an important protective mechanism against aberrant cell growth and neoplasia. However, via gene-specific mutation, a number of potential bypasses or mechanisms of tolerance are available.

3. Cellular differentiation and other cellular interactions

380. The stepwise accumulation of genetic/epigenetic events demands continuing growth potential in cells that have sustained a neoplasia-initiating event. Running counter to this is the normal process of terminal cellular differentiation, whereby uncommitted progenitor cells assume specialized functions in tissues and no longer retain proliferative potential. Thus, a developing subpopulation of cells may carry a tumour-initiating mutation that, for example, deregulates cellular proliferation but in the absence of further phenotypic change will complete a quasi-normal programme of terminal differentiation mediated by cellular interactions.

381. In this way neoplasia-initiated cells will, in the absence of other changes, exit the pathway to malignancy. Thus, the antiproliferative process of terminal differentiation will tend to be rate-limiting with respect to overt malignancy and may be evidenced by the accumulation of benign lesions in tissues. There are numerous examples of associations between proto-oncogene/tumour-suppressor gene functions and cellular differentiation/development [K1, L1, R2, S3]; here it will be sufficient to give only a few examples.

382. In the case of the lympho-haemopoietic system, the development of the different cell lineages is known to depend on a complex interplay between cell-cell interaction, cytokines, and intracellular signalling cascades [O2]. The genes *AML1* and *tal/SCL* have been implicated in haemopoietic stem-cell differentiation and, in mutant form, are known to contribute to the genesis of certain types of leukaemia. Other examples of leukaemia/lymphoma-associated genes with roles in normal differentiation processes include genes of the *Hox* and *Pax* families, *RBTN2*, *RARA*, and *Mll1* [O2, R2]. As noted in Section B, the functional development of the T- and B-haemopoietic cell lineages is highly dependent on the recombination of immune gene sequences, and specific mis-recombination of these sequences makes a major contribution to T- and B-cell neoplasia. Recent evidence also links downstream signals from these recombinogenic processes with subsequent clonal growth and differentiation; that is, normal growth and differentiation of cells may be blocked if recom-bination does not proceed normally [W5]. In general, it may be concluded that many mutations in lympho-haemopoietic neoplasia serve to compromise the closely controlled process of cell-lineage-dependent differentiation.

383. Similar evidence exists with respect to solid tumours [L14]. For example, via its interaction with *Ras* proteins, the protein product of the *NF1* tumour-suppressor gene plays a role in regulating the normal growth and differentiation of neural cells, and the Ras/Raf/MAP kinase intracellular signalling pathway appears to play a more general role in the regulation of cellular differentiation. In addition, there are data that support a role for tumour-associated catenin/APC, Rb1, and DCC proteins in transcriptional regulation/cellular signalling processes that control cell-lineage-dependent growth and differentiation.

384. Thus, many forms of proto-oncogene/tumour-suppressor gene mutations, often in combination and via perturbation of cellular signalling, will have dual effects on cellular growth and differentiation. The maintenance of constitutive growth of stem-like cells having differentiation defects may be viewed as an important element in the early phases of tumour development. In essence, the homeostatic imbalance created by these mutations will tend to promote the clonal expansion of cells having limited potential for terminal differentiation. Alone, however, such clonal growth may not be productive, since the cells will be potentially subject to senescence and apoptosis, both of which serve to limit the opportunity to accumulate the further mutations necessary for overtly malignant development. Nonetheless, as noted earlier, each of the processes of senescence and apoptosis may itself be compromised by gene mutation, e.g. by telomerase deregulation and *TP53* gene mutation, respectively. In principle, therefore, extended clonal growth can be achieved. Further to this, and in accord with previous discussion, the early appearance of genome-destabilizing mutations may dramatically accelerate neoplastic development.

385. Overall, the frequency with which the genes involved in normal cellular differentiation are mutated in tumours testifies to the protective function offered by terminal cellular differentiation to a non-proliferative state. It is judged, however, that alone, such aberrant differentiation is usually insufficient for full malignancy and that cooperating mutations that further extend clonal lifespan and/or destabilize the genome are likely to be required.

386. Intercellular transmission of biological signals followed by intracellular biochemical cascades is believed to be an integral component of the differentiation of cells. Not surprisingly, cell-to-cell communication has also been implicated in the expression of neoplastic phenotypes, and more recently, cellular communication has been shown to influence radiation response.

387. The UNSCEAR 1993 Report [U3] reviewed the role of cellular communication via gap junctions in neoplastic development. In summary, it is believed that the establishment of such intercellular communication can lead to the suppression of neoplastic features by neighbouring normal cells. During clonal evolution, however, many tumour cells lose the capacity to communicate with normal cells and in this way become less receptive to tissue regulation, i.e. they become increasingly autonomous. Mechanistic links between gap junction processes, tumour promotion, and cell cycle control were also discussed in the UNSCEAR 1993 Report [U3]. In respect of radiation response, the term "bystander effect" has been coined to describe a range of *in vitro* responses occurring in unirradiated cells that are close neighbours of others receiving a given radiation dose, usually from single ionizing particles.

388. The effects seen in bystander cells include changes in gene expression [A19], lethality [M49], sister chromatid exchange [D13], chromosome breakage [P21], and gene mutation [N11]. The mechanisms involved are not well understood but are believed to involve the transfer of factors from irradiated cells via the extracellular medium or via intercellular communication [A19, M50]. Such effects have yet to be demonstrated *in vivo*, and their consequences for tumour risk cannot be judged. In the context of this Annex, the most important data set concerns apparent alpha-particle-induced bystander effects on gene mutation [N11]. These studies imply that at a low fluence of alpha particles, the frequency of gene mutations arising in bystander cells exceeds by up to fivefold that in cells intersected by single particles. At higher particle fluences, the bystander contribution to mutation rates decreases, and in this way the dose response for mutation induction is supralinear, with a steep rise at doses below ~50 mGy. Assuming that there is a direct relationship between mutation rate and tumour risk, the data noted above imply that per unit dose of alpha particles, tumour risk at doses below ~50 mGy is substantially greater than that at higher doses. Whether these data represent an important source of uncertainty in high-LET radiation risk estimates must await replication of the study and the establishment of its generality, particularly in the *in vivo* situation. Other data on radiation response implicate cellular interactions in the induction of genomic instability [G20, M51] and in adaptive responses [I15]. Some studies in this general area also imply that cellular DNA may not always be the principal target for radiation effects, particularly those that may have transient epigenetic components. Although it remains difficult to integrate such data into a mechanistic framework for assessing tumour risk at low doses, the findings noted above caution against a dogmatic view in the modelling of dose-response data on the basis of DNA damage alone.

4. Cellular surveillance

389. Following review of epidemiological, animal, and cellular studies, the Committee concluded that conventional T- and B-lymphocyte-mediated immune response was not a particularly effective protective mechanism against the development of most human tumours [U3]. Although these immune responses appear to be able to target the specific non-self antigens presented by oncogenic viruses or their associated neoplasms, the common radiogenic tumours seem unable to effectively initiate timely immune responses or are capable of efficiently evading surveillance [B18, U3]. The Committee did, however, recognize some uncertainty surrounding the potential protective role of certain classes of cytotoxic T-lymphocytes (CTL), including natural killer (NK) cells [U3]. A number of novel approaches have been used to resolve some of these uncertainties.

390. One area of recent study [L23] has been to determine whether the poor immunogenicity of most tumour types is due to the lack of signals for co-stimulation of full CTL activity; this is believed to be mediated by specialized antigen-presenting cells. A study with mice using an antibody to block CTLA-4, a negative regulator of CTL activation, showed that such a blockade resulted in the rejection of transplanted and pre-existing human tumour cells and also the development of resistance to a second challenge. It seems, therefore, that with fully malignant

cells, an effective anti-tumour response can be elicited provided that specific immune regulators are manipulated.

391. Another approach to the problem has been to seek mutational signatures in tumours indicative of evasion of immune surveillance. In one such study [B19], it was argued that the RER^+ mutator phenotype of certain colorectal carcinoma cell lines might generate a sufficiently diverse array of mutant protein neo-antigens to elicit a strong CTL response. If this is the case, inefficient antigen presentation via the loss of beta-2-microglobulin (β2M) would be strongly selected in the resulting tumour cell population. Such correlation was observed in a study of 37 cell lines, where the four mutator lines were the only examples in which β2M expression was lost.

392. Beta-2-microglobulin associates with polymorphic heavy-chain glycoproteins in cell membranes for the purposes of antigen presentation by the resulting class I major histocompatibility complex (MHC). Intracellular antigens are believed to be transported to the MHC by proteins of the TAP family [E6]. Not only do a substantial fraction of human tumours lack expression of the class I MHC, but there is some evidence of the involvement of *TAP* gene mutation in tumours. Other strategies adopted by tumour cells in order to evade immune surveillance include the expression of decoy receptors, the Fas-mediated inactivation of CTL/NK cells at membrane surfaces, and the secretion of factors that inhibit or inactivate CTL [V5].

393. In general, these observations, while providing some correlative support for the view that evasion of CTL-mediated surveillance may be of some importance in tumour development, provide no information on the effectiveness of this surveillance in reducing tumour risk. It is, however, intriguing to note emerging evidence of a possible two-way interaction between genomic instability in neoplasia and CTL response. On the one hand, the RER^+ mutator phenotype may serve to generate sufficiently strong tumour antigen signals for CTL response; on the other, it can provide an enhanced mutational capacity to evade the resulting T-cell surveillance. For potentially anti-tumorigenic CTL response, there is also some evidence to support a model whereby normal cells that engulf apoptotic tumour cells can migrate to lymph nodes, where, in principle, they can invoke a response to tumour antigens [A8].

394. With respect to NK cells, it is now well established that this class of cytotoxic cells can, in principle, exert a degree of anti-tumour activity via the release of factors such as interferon γ, tumour necrosis factor, and Fas ligand. There is also some evidence of an additional antitumour mechanism involving NK attack on tumour vasculature [B24, F9]. In spite of numerous studies, there is, however, no convincing evidence of a correlation between NK abundance/function *in vivo* and tumour development or prognosis (see [F9]). In general, this area of study remains most controversial, and with current knowledge it is not possible to judge the extent to which NK cells act to protect against non-viral human cancers.

395. Overall, the role of immune surveillance in protecting against common neoplasms has yet to be adequately described, and some studies tend to argue against this proposition [B18, U3]. Gains in fundamental knowledge will probably contribute to the debate. For example, the complement protein system is an important determinant of humoral immune surveillance and is believed to target certain malignant cells. In accord with this, a novel stress-related protein has been revealed that appears to participate in the discrimination of malignant cells by homologous complement [M26]. The recent observation that the proto-oncogene *PML* of human myeloid leukaemia plays a role in the regulation of antigen presentation in cells also implies the need for some developing haemopoietic neoplasms to evade cellular surveillance [Z2]. Equally, however, the tumorigenic expression of *Apc*-deficiency in Min mice is not enhanced by a defect (*scid*) in immune function [S18]. Thus, recent findings can be used to both support and question the true role of cellular surveillance in tumour defence. Studies of low-dose stimulation of immune functions, e.g. [L58, M54] have previously been reviewed by the Committee [U2] and a few additional studies have been published (e.g. [H36, K30, S42, S43]) in more recent years. Doubts were expressed as to whether the immune system plays a significant role in any cancer-related adaptive processes at low doses.

5. Summary

396. Through a better understanding of the processes that mediate multi-stage tumorigenesis it has become evident that neoplastic development is subject to a number of cellular constraints. The main constraints are control of cellular proliferation/genomic stability, the induction of programmed cell death, tumorigenic suppression by cell-cell communication, and terminal differentiation to a non-proliferative cellular state. In addition, for at least certain tumour types there is evidence that immunosurveillance mechanisms can recognize and restrict the growth of neoplastic cells.

397. These protective mechanisms are believed to provide a high level of protection against neoplastic growth and development. In spite of this, there is growing evidence that during the evolution of tumours, resistance to or tolerance of all these countermeasures can be developed via gene-specific mutation. Thus a substantial proportion of consistent mutations in tumours may be linked directly with cellular strategies aimed at maintaining viability and growth, avoiding terminal differentiation and immune recognition, and promoting genomic instability such that a wide range of clonal variants are available for the full development of malignancy. On the basis of current molecular genetic knowledge, there seems no good reason to suppose that different modes of *in vivo* constraint apply to spontaneously arising and carcinogen-induced tumours. Evidence is also accumulating in support of the view that cellular communication can also influence early *in vitro* radiation responses, with possible effects on cellular recovery, genomic stability, and mutation rates. The present state of knowledge does not allow for extrapolation

of these findings to tumorigenesis *in vivo*, but some recent alpha-particle mutation data, if confirmed, may be of importance.

E. DNA REPAIR AND TUMORIGENESIS

398. For the purposes of relating mechanisms of radiation tumorigenesis to mechanisms that are believed to apply to spontaneously arising disease, it is important to consider in greater depth the evidence on the role of DNA repair and the uncertainties that attach to this association.

1. DNA repair as a determinant of oncogenic response

399. Data relating to the influence of DNA repair on mutagenic and other cellular radiation responses are discussed in Annex F, *"DNA repair and mutagenesis"*. Critical to the role of DNA repair in radiation tumorigenesis is the now unambiguous evidence that heritable human deficiency in genes controlling DNA repair and maintenance of genomic stability is frequently associated with an increased incidence of spontaneously arising neoplasms.

400. Thus, such DNA processing functions in normal somatic human cells must play a critical role in protecting against spontaneous neoplastic development. As discussed in the UNSCEAR 1993 Report [U3], these data also provide important support for the mutational origin of neoplasia via failures in repair of DNA damage.

401. With respect to tumours associated with human exposure to exogenous genotoxic agents, studies with two categories of genetic disorders, xeroderma pigmentosum (XP) [K8] and Li-Fraumeni syndrome (LFS) [H8], provide evidence that defects in DNA damage processing are also important to oncogenic development after ultraviolet and ionizing radiation, respectively.

402. In the case of xeroderma pigmentosum, there is unambiguous evidence that the inherited deficiency in repair of DNA photoproducts is associated with an excess of cancer in regions of skin receiving significant solar exposure [K8]. Unexposed skin of XP patients shows an unremarkable frequency of these neoplasms, indicating the critical importance of DNA photoproduct induction for skin carcinogenesis and the high level of protection afforded by high-fidelity DNA repair processes.

403. The cancer-prone genetic disorder Li-Fraumeni syndrome (LFS) is frequently, although not always, characterized by a deficiency in the *TP53* tumour-suppressor gene that normally plays a role in DNA damage sensing, cell-cycle control, and apoptosis (see Sections IV.B and IV.D). Although the data are less compelling than those for ultraviolet radiation exposure of XP patients, there is evidence that LFS and LFS-like patients exhibit, in childhood, an elevated risk of tumour induction after radiotherapy [H8, S19]. Thus, inherited human defects in DNA damage processing can be reflected in an increased risk of carcinogen-induced as well as spontaneously arising tumours.

404. In addition to these important human studies, experimental animal data relating to the role of DNA repair in radiation tumorigenesis are also beginning to emerge, largely through studies of mice that have been genetically manipulated to be deficient in specific genes involved in DNA repair and genomic stability [W2].

405. These animal data are discussed in Section IV.C. In brief, studies with *p53*-deficient mice give evidence of enhanced tumorigenic radiosensitivity associated with abrogation of a G_2/M cell-cycle checkpoint for chromosomal repair; radiation-induced *p53* gene loss via increased sensitivity to the induction of aneuploidy appeared to be the principal mechanism involved.

406. The *ATM* gene of human ataxia-telangiectasia is discussed in Annex F, *"DNA repair and mutagenesis"*, together with recent data relating to the radiosensitivity of mice manipulated to be deficient in this critical DNA damage response gene. In brief, *Atm*-deficient mice are highly radiosensitive, and while radiation tumorigenesis studies have yet to be reported, the animals, like their human counterparts, are prone to the spontaneous development of lymphoma and specific lymphoma-associated chromosomal rearrangement in haemopoietic cells [B1]. It may be anticipated that radiation tumorigenesis studies with these and other relevant genetically manipulated animals, e.g. those deficient in *BRCA1, BRCA2,* and *Rad51,* will be informative on the further relationships between DNA damage repair and tumorigenesis.

2. Implications and uncertainties

407. The Annex F, *"DNA repair and mutagenesis"*, provides evidence that certain forms of DNA double-strand lesions are the principal biologically relevant event induced by radiation in mammalian cells. Since current data imply that these lesions are usually repaired via a process of illegitimate rather than homologous recombination, there will be an inherent degree of error proneness in DNA repair after radiation exposure. Such misrepair events may be represented by gross chromosomal abnormality (deletion or rearrangement) or subchromosomal and intragenic events. Judging from molecular analyses of radiation-induced somatic cell mutants, these misrepair events take the form of DNA base-pair substitutions, frameshift mutations, or, more frequently, DNA deletions of varying size. Some data are suggestive of changes in mutational spectra with radiation dose rate and LET, but this issue remains controversial.

408. Also noted in Annex F, *"DNA repair and mutagenesis"*, is the growing evidence that DNA repair functions are important determinants of dose, dose rate, and radiation quality effects in mammalian cells. In brief, there is evidence that the extent and fidelity of repair

strongly influence the initial and final slopes of low-LET dose-effect relationships and the progressively steeper slopes of these relationships with increasing LET. It is concluded that RBE-LET effects are largely dependent on the repairability of initial DNA damage. To what extent are these *in vitro* data reflected in current knowledge of radiation tumorigenesis *in vivo*?

409. The data discussed in the UNSCEAR 1993 Report [U3] and in this Annex are broadly consistent with the single-cell mutational origin of most tumours. Loss-of-function mutations of critical gatekeeper genes appear to be early events in the genesis of many human solid tumours, and gain-of-function chromosomal events occur early in leukaemias and lymphomas (see Section B). There is evidence from animal studies, described earlier, that radiation-associated gene and chromosomal loss/deletion can act as an initiating event for tumorigenesis and that DNA damage processing is a crucial protective factor for *in vivo* radiation response. Given the obvious parallels between *in vitro* and *in vivo* data, it becomes possible to consider a general mechanistic framework within which to model dose-effect/RBE-LET relationships for radiation tumorigenesis and the protective functions that may operate. These modelling approaches will be discussed and developed later in this Annex.

410. There are, however, aspects of the data discussed in Annex F, *"DNA repair and mutagenesis*", that caution against seeking oversimplistic correlations between *in vitro* cellular response data and tumorigenesis *in vivo*. First is the issue of novel mechanisms of genetic change in mammalian cells. In addition to epigenetic changes such as imprinting and gene silencing noted in this Annex, it has also been speculated that radiation may induce unknown cellular pathways that promote untargeted mutation. In some studies the activity of these pathways has been shown to persist for many post-irradiation cell generations, leading to an apparent elevation of the spontaneous mutation rate. As discussed in Annex F, *"DNA repair and mutagenesis"*, such findings have been made with respect to lethal mutation, gene mutation, and unstable chromosomal damage.

411. Given the emphasis in this Annex on the early development of genomic instability in tumour development, it is possible to speculate that any persistent genomic instability induced by radiation in target somatic cells *in vivo* might make a significant, late-expressing contribution to tumorigenic risk [L24]. The cellular processes underlying this induced instability remain uncertain, however, and no single mechanism seems capable of explaining the various and sometimes inconsistent manifestations referred to in Annex F, *"DNA repair and mutagenesis"*. A collection of recent papers [M32] addresses various aspects of this developing field; of particular note is the finding of a possible genetic association in mouse strains between the post-irradiation expression of persistent chromosomal instability and susceptibility to radiation tumorigenesis [U25]. The authors were, however, cautious about the implications of these initial findings. In follow-up studies [O7], late-expressing chromosomal instability in the mammary-tumour-susceptible BALB/c mouse was shown to be genetically associated with changes in expression of repair-related DNA PK protein as well as with reduced DNA double-strand break repair. From these studies it is possible to indirectly implicate late-expressing genomic instability in radiation tumorigenesis in certain genetic settings, but whether a causal relationship applies is uncertain.

412. Overall, a general link between such induced instability and radiation tumour risk remains to be established. Indeed, the cytogenetic findings of *in vivo* studies relating to mechanisms of radiation-induced lymphomagenesis in *p53*-deficient mice and myeloid leukaemogenesis in CBA mice tend to argue against a significant contribution from induced and persistent genome-wide instability [B4, B20]. However, a specific and persistent clonal feature of chromosomal instability that has been closely associated with the development of human neoplasia is the so-called segmental jumping translocation. These events are not uncommon in spontaneous human lymphohaemopoietic tumours [U3] and were reported recently in myeloid neoplasms arising in irradiated survivors of the atomic bombings [N8]. It may be concluded that certain elements of radiation-associated persistent genomic instability probably do play a role in tumorigenic processes, but there is much uncertainty as to the overall importance of that role and therefore as to how it might be taken into account in the modelling of radiation risk.

413. A second source of uncertainty discussed in the UNSCEAR 1994 Report [U2] and in Annex F, *"DNA repair and mutagenesis"*, is the cellular phenomenon of adaptive response to DNA damage; the data describing such responses were reviewed recently [W6]. In brief, in certain experimental systems a small priming dose of radiation (or of some other genotoxic agents) can result in the development of partial resistance to a challenge by a second, higher dose. The radiobiological endpoint most frequently employed in cellular and animal systems has been cytogenetic damage, but there are also some data with respect to gene mutation and cell survival. In addition, some mechanistic studies have been undertaken of the possible role of activation/induction of novel proteins and cell-cycle perturbation. In principle, inducible DNA repair might underlie some manifestations of adaptive response, but as noted in Annex F, *"DNA repair and mutagenesis"*, evidence for the up-regulation of relevant repair genes is fragmentary. There is, however, growing evidence for subtle post-irradiation modification of repair-related protein complexes and the induction of stress-response genes. More detailed information on adaptive responses to cytogenetic and lethal cellular damage in mammalian cells are provided in Annex F, *"DNA repair and mutagenesis"*.

414. These studies lend credibility to the true existence of adaptive responses, but they also draw attention to the fact that the response is transient, not usually robust, and frequently lacking a clear mechanistic basis. For example, at the cellular level, adaptive responses have rather uncertain dose and dose-rate dependency and when expressed lead to only a modest decrease in sensitivity to

the subsequent radiation challenge. In addition, at the cytogenetic level this response is not consistently expressed, with cells from some humans and mouse strains failing, for unknown reasons, to show adaptive responses. Although some novel proteins have been detected in "adapted" cells, cell-cycle-related changes are not obvious, and the relationships between cytogenetic adaptive responses and known stress-related biochemical signalling pathways remain to be clarified [W6].

415. In the UNSCEAR 1994 Report [U2], the Committee reviewed animal studies of life-shortening and tumour induction that were relevant to the possible role of adaptive responses, but these studies did not report convincing evidence of such effects. Subsequently there have been reports of possible adaptive effects in relation to radiation lymphomagenesis [B21] and life-shortening in mice [Y4]. Of particular note is a recent report on a possible gamma-ray-induced adaptive response in respect of acute myeloid leukaemia (AML) induction in the mouse [M52]. This study reported that a chronic priming dose of 100 mGy did not influence the incidence of acute myeloid leukaemia following a chronic challenge dose of 1 Gy; the lifespan of mice without acute myeloid leukaemia was similarly unaffected. However, the animals receiving the priming dose showed modestly increased latency for induced acute myeloid leukaemia, implying that the later stages of the leukaemogenic process had been modified. The mechanistic basis of this unexpected result remains highly uncertain, but the authors speculate that the increased tumour latency might reflect the triggering of some form of persistent stress response.

416. Although the relevance of adaptive responses to human tumorigenesis should not be discounted, in the absence of a consistent body of *in vivo* tumorigenesis data and with current uncertainties on cellular mechanisms, it would be most difficult to include adaptive response parameters in mechanistic models of low-dose radiation tumorigenesis.

417. A third area of uncertainty surrounding DNA damage and repair concerns the relationships between spontaneous and radiation-induced damage and their implications for low-dose tumorigenic risk. Debate on these issues has been conducted for some years [A4, B22, C15, W7], and the main areas of contention may be outlined as follows.

418. In spite of its critical information-carrying role in the cell, genomic DNA has limited chemical stability. Via hydrolysis, oxidative attack, and chemical methylation processes, cellular DNA is constantly modified by its endogenous environment irrespective of the influence of exogenous agents such as electrophilic chemicals, ultraviolet radiation, and ionizing radiation [L25].

419. Endogenous damage to the mammalian genome may take the form of hydrolytic depurination and deamination of DNA bases, oxidative attack on DNA bases and the sugar-phosphate backbone, and non-enzymatic DNA methylation of certain bases. For largely technical reasons, estimates of the rate of accumulation and abundance of such endogenous DNA lesions vary considerably [L25].

420. Less uncertainty surrounds the general form that this DNA damage takes [L25]. By their very nature, hydrolytic, oxidative, and methylation events are random and unclustered, affecting chemical moieties on one or the other strands of the DNA duplex; examples are the formation of abasic sites due to hydrolytic depurination, 8-hydroxyguanine formation via hydroxyl radical attack, and uracil formation via deamination of 5-methylcytosine. DNA single-strand breaks as a consequence of base loss, oxidative attack, and as repair intermediates also arise spontaneously.

421. The evidence concerning the type, abundance, and repair of endogenously arising spontaneous DNA damage is summarized in Annex F, *"DNA repair and mutagenesis"*. The Annex emphasizes the technical uncertainties surrounding estimates of the abundance of such DNA damage.

422. The general conclusion that may be reached from these data is that while it is difficult to make precise quantitative comparisons, there are differences between the spectra of DNA damage types arising spontaneously and those induced by radiation; there are also differences in their repair characteristics (see also [C15, L25, W7]).

423. This view of endogenous damage and its consequences may be set against the following theoretical proposition: since the cell is able to repair a very high level of endogenous DNA damage without frequent mutagenic consequences, a further small increment of DNA damage from low doses of radiation will not impose significant risk; that risk only becomes significant at relatively high doses, when, at a given level of genomic damage, DNA repair capacity is exceeded.

424. The fundamental scientific uncertainty surrounding this proposition is that it assumes that the nature and reparability of spontaneous and radiation-induced DNA damage are essentially equivalent [C15, W7]. The data in Annex F, *"DNA repair and mutagenesis"*, provide evidence that although there are some similarities between the DNA lesion types arising spontaneously and those induced by radiation, DNA double-strand breaks almost certainly make a substantially greater relative contribution after radiation exposure. More important, however, is the evidence accumulating on the chemical nature of radiation-induced DNA double-strand breaks and other double-strand lesions.

425. Through a combination of cellular, biophysical, biochemical, and molecular approaches, it has become apparent that a high proportion of radiation-induced DNA double-strand breaks and related lesions are chemically complex and/or part of multiply damaged DNA sites. This feature stems from the requirement for local clustering of energy loss events from a given radiation track to effect coincident damage to both sugar-phosphate backbones of the DNA duplex [G5, G10]. This chemical complexity of

DNA double-strand breaks is apparent after low-LET radiation but will increase with LET.

426. As noted in Annex F, *"DNA repair and mutagenesis"*, the correct repair of such complex damage is difficult because of multiple and coincident damage to coding on both DNA strands. In most instances such repair is believed to proceed via illegitimate recombination, which is inherently error-prone, and it is this reparability factor that will principally distinguish spontaneous and radiation-induced DNA lesions. Accordingly, excess dicentric chromosomes have been recorded in human lymphocytes *in vitro* at low-LET doses of around 20 mGy, while the spontaneous rate of generation of these events is very low (~1 per 1,000 cell generations) [L8].

427. Stated simply, the relative abundance of complex and poorly repairable DNA lesions after radiation exposure is judged to be very much greater than that of lesions that arise spontaneously. Therefore, it is this feature rather than lesion abundance overall that should guide judgements on the role of DNA repair in low-dose response and radiation quality effects. Accordingly, the proposition stated in paragraph 423 runs counter to advances in fundamental knowledge and therefore has no obvious role in the modelling of tumorigenic risk.

3. Summary

428. A large body of information points to the crucial importance of DNA repair and other damage-response functions in tumorigenesis. Not only do these DNA damage-processing functions influence the appearance of initial events in the multi-stage process, but they also serve to reduce the probability that a benign neoplasm will spontaneously acquire the secondary mutations necessary for full malignant development. Thus, mutations of DNA damage-response genes in tumours play important roles in the spontaneous development of genomic instability. Various forms of radiation-induced persistent genomic instability have been recorded in experimental cellular systems. With the possible exception of instability at certain chromosomal translocation junctions, these phenomena are not well understood, and their association with *in vivo* tumorigenesis has yet to be established.

429. With respect to radiation damage to DNA, it is concluded that the repair of sometimes complex DNA double-strand lesions is inherently error-prone and is most likely to be an important determinant of dose, dose rate, and radiation quality effects for the induction of tumorigenic lesions. Uncertainties remain on the significance for tumorigenesis of adaptive responses to DNA damage; the mechanistic basis of such responses has yet to be clarified. In contrast, recent scientific advances provide clear evidence of the differences in complexity and reparability between spontaneously arising and radiation-induced DNA lesions. In the modelling of radiation tumorigenesis these data argue against basing judgements about low-dose response on uncritical comparisons between overall lesion abundance and repair capacity. Overall, the general concepts linking DNA damage repair and tumorigenesis that were summarized previously in the UNSCEAR 1993 Report [U3] remain valid. However, the data discussed here and in Annex F, *"DNA repair and mutagenesis"*, provide a far more robust scientific framework to support these concepts than was available to the Committee in 1993.

F. BIOLOGICAL MODELLING OF TUMORIGENIC RESPONSES

1. Implications of current data

430. As knowledge of the fundamental basis of multi-stage tumorigenesis continues to advance, it becomes possible to identify critical features of the process and the uncertainties that may attach to the development of biological models to describe risk at low doses.

431. The earliest phase of tumour development (initiation) appears, in the main, to involve loss- or gain-of-function mutation of single genes in single target stem-like cells in tissue. In the case of solid tumours, a set of tissue-/cell-type-specific gatekeeper genes in the tumour-suppressor category may be the principal loss-of-function gene targets. For lympho-haemopoietic tumours, both loss-of-function mutations and gain-of-function chromosomal translocations are likely to be important. In biological modelling, these two types of tumorigenic event may require different forms of treatment. Not only do mutational mechanisms differ but also gain-of-function mutations in leukaemia/lymphoma can have more profound cellular effects than loss of a single tumour-suppressor gene.

432. An important source of uncertainty is provided, however, by the non-mutational (epigenetic) events that can, for certain genes, substitute for gene mutation. The overall contribution of, for example, gene silencing and loss of imprinting to human tumorigenesis, although very difficult to quantify, seems likely to be significant.

433. Some uncertainties about the probability of accumulation of multiple genetic events in tumorigenesis have been reduced by the characterization of the spontaneous development of genomic instability at a relatively early point in neoplastic development. Some of the mutator genes that serve to drive tumorigenesis have been characterized, and from a modelling standpoint it seems most appropriate to view the development of the mutator phenotype as marking the transition between benign and malignant disease. Although critical evidence is lacking, the early appearance of a strongly expressing mutator phenotype with respect to spontaneously arising DNA damage may mean that low doses of exogenous carcinogens such as radiation will make a relatively small contribution to later phases of tumour development compared with those phases occurring before the spontaneous onset of genomic instability. This might serve as a mechanistic explanation for the observation that radiation usually acts only weakly on tumour promotion and progression [U3].

434. Evidence is growing, however, that in some circumstances, the expression of radiation-induced DNA/chromosomal damage in cells may be a persistent phenomenon. Secondary chromosomal change centred on primary exchange junctions, e.g. jumping translocations, is not unexpected and has been recorded in cellular systems and human/animal lymphohaemopoietic neoplasms [B44, G21, N8, U3]. The other cellular features of induced genomic instability discussed in this Annex and in Annex F, *"DNA repair and mutagenesis"* are more difficult to relate directly to tumorigenesis, so for this reason it may be premature to attempt to integrate them specifically into mechanistic models of tumour risk. The same general problem applies to bystander effects of radiation on cell inactivation and mutagenesis, although the alpha-particle data [N11] discussed earlier deserve some attention. That said, the Committee recognizes recent signs of progress in resolving the mechanistic uncertainties associated with the role of stress-related processes in cellular response to radiation and anticipates that much better informed judgements will be possible within a few years.

435. By contrast, many sources of data on tumorigenesis point toward the crucially important protective role played by high-fidelity DNA repair. In the light of information on DNA repair capacity in relation to the high flux of spontaneous DNA damage in mammalian cells, it has been suggested by some that low doses of radiation would be expected to contribute little to tumour risk. If true, this would have important implications for biological modelling. Uncertainties on this contentious issue have been reduced by the growing evidence that an important fraction of the DNA double-strand lesions induced by radiation is chemically complex, extremely difficult to repair correctly, and only very rarely occurs spontaneously. In the context of biological models of tumorigenesis, it is judged that overly simplistic analysis of data on DNA lesion abundance and repair can be most misleading. New data on the role of DNA repair in cellular dose and dose-rate effects and the associations between DNA lesion complexity and RBE-LET effects do, however, have implications for mechanistic models.

436. Other important protective factors in tumorigenesis include apoptotic cell death, cellular senescence, terminal differentiation of cells to a non-proliferating state, and the elimination of neoplastic cells by immunosurveillance mechanisms. Through their capacity to remove cells from neoplastic pathways, these processes collectively serve to provide a high level of protection against tumours arising spontaneously or induced by carcinogens. Critically, however, molecular genetic studies have provided compelling evidence of gene-specific neoplastic mutations that serve to block these processes. Thus, a given overtly malignant tumour will have succeeded via mutation or epigenetic change in evading or gaining tolerance to each of the protective challenges it has faced during development.

437. There are few quantitative data on which to base judgements on the relative magnitude or efficiency of the protective factors noted above. Indeed, it seems likely that this relative magnitude will vary from one tumour type to another, and the differences between virally associated and other tumour types with respect to immunological protection may be evidence of this. Thus, with limited knowledge, the modelling of protective factors in tumorigenesis can, at best, be only empirical.

438. In spite of this, a critically important question is whether the extent or effectiveness of such protective factors might be different for tumours arising spontaneously and those induced by radiation. As noted in the UNSCEAR 1993 Report [U3], exposure to high doses of radiation, where cell killing becomes important, would be expected to influence final tumour yield not only by initially reducing target cell numbers but also by subsequently mobilizing quiescent stem cells for tissue repopulation. High-dose suppression of immune functions might also be important for certain tumour types, principally those associated with oncogenic viruses.

439. At low doses and dose rates, where cell killing is not significant, there is, however, no specific reason to anticipate profound and long-term effects of radiation on the function of protective mechanisms. Transient changes in the activity of these systems resulting from stress-related effects on cellular biochemical signalling might be anticipated, but with current knowledge it is not possible to relate these to final tumour yields.

440. The possible exception to this are the adaptive responses to radiation noted in this Annex and discussed in depth in the UNSCEAR 1994 Report [U2]. These would include not only adaptive DNA damage responses but also possible stimulating effects on immune system and other potentially protective functions.

441. Stated simply, if low doses of radiation could be shown to enhance profoundly, over an extended period, the anti-tumour activities outlined in this Annex, then radiation-induced tumours would be expected to be subject to greater suppression than those arising spontaneously. Under these theoretical circumstances the shape of the dose-effect relationship for tumorigenesis would not be expected to be simple and might well depart from those for related radiobiological endpoints in single cells, i.e. the induction of chromosome aberrations, gene mutation, or cell transformation.

442. Although current knowledge does not exclude the possibility of this scenario, the data available on adaptive responses in cells or animals are judged to be insufficiently well developed for the purposes of biological modelling. Accordingly, the existence or otherwise of an adaptive response for radiation tumorigenesis remains a continuing source of debate.

2. Basic premises

443. Although much knowledge has been gained on the cytogenetic, molecular, and biochemical processes involved in the development of neoplasia, considerable uncertainty remains, particularly with respect to the quantitative

aspects of multi-stage tumour development. For this reason any attempts to include such data in the modelling of radiation tumorigenesis demands a set of simplifying judgements.

444. In full recognition of the uncertainties discussed in earlier Sections of this Annex, the following premises, based on the weight of current evidence, may be stated:

(a) The principal role of radiation in tumour development is to generate the DNA damage that can give rise to gene-specific mutation in critical target cells. Repair of that damage may be enhanced by cell-cycle checkpoints and, possibly, adaptive repair processes, but there is no specific expectation of wholly error-free repair, even at low doses and dose rates. Equally, the elimination of radiation-damaged cells by apoptotic processes is very unlikely to be complete;

(b) The vast majority of spontaneous and induced tumours have their origin in single specific mutations in single target cells in tissues. The probability of such a mutated cell progressing to overt malignancy is, however, very low because of the defences afforded by protective processes such as apoptosis, terminal differentiation, senescence, and cellular surveillance. Further mutation during pre-neoplastic clonal development can serve to bypass these defensive measures, and none are likely to be wholly protective or to be consistently enhanced by low doses of radiation;

(c) Although both loss-of-function (tumour-suppressor) and gain-of-function (proto-oncogene) gene mutations can contribute to multi-stage tumorigenesis, the DNA deletion mechanism characteristic of radiation will tend to make loss-of-function events the predominant process at all doses of radiation; and

(d) Radiation acts principally at the early stages of tumorigenesis by inducing specific mutations in normal stem-like cells. During protracted radiation exposures a contribution to the later stages of tumour development is possible, but during these later phases the acquisition of a mutator phenotype and/or one associated with epigenetic gene silencing/activation may be the primary driving force for neoplastic selection and progression.

445. Assuming these premises to be correct, it would seem that the dose-response parameters for radiation tumorigenesis at low doses are determined principally by factors that apply to the induction of the specific gene/chromosomal mutations in the target cells in question; the abundance and kinetics of these target cells will also be important determinants of the response. Stated simply, these radiation-induced mutations would be adding in a dose-dependent manner to the *in vivo* pool of tumour-initiating mutational events contributed by spontaneous processes and other genotoxic exposures. Thereafter, it seems reasonable to assume that all such events will be subject to the same variable sets of cellular and humoral factors that serve to suppress or enhance malignant development. On this basis, significant departure from a simple dose-response relationship would demand a dose-dependent change in the kinetics of one or more of these post-irradiation modifying processes. For example, if there were to be persistent post-irradiation elevation of error-free DNA repair, apoptosis, terminal differentiation, cellular senescence, or immunosurveillance, then the radiation cancer risk might be depressed. Conversely, if post-irradiation mutation rates are persistently high (as a form of induced genomic instability), tumour development might be enhanced.

446. Although it is possible to speculate on the roles that the above processes might play in determining tumorigenic responses, any such hypotheses currently lack critical experimental support and plausible mechanisms that might operate after low doses. It is not, however, difficult to envisage substantial modification of cellular/tissue behaviour during the long-term cellular repopulation required for tissue regeneration after high doses; these processes may well have consequences for local tumour development.

447. At low doses, transient changes in biochemical equilibria and cell kinetics should be expected, but these are probably part of the normal cellular damage response pathways associated with the cell-cycle checkpoint, DNA repair, and mutagenesis functions already discussed. There is, however, no reason to believe that induced transient changes are unique to radiation.

448. The overall judgement that, on mechanistic grounds, cancer risk at low doses will increase as a simple function of dose is, however, subject to a number of important caveats. Some of these have already been rehearsed in this Annex, but two deserve additional attention.

3. Error-free DNA repair at low doses

449. Recombinational repair involving fully homologous DNA sequences may be regarded as the sole source of potentially error-free repair, particularly with respect to DNA double-strand breaks/lesions that involve complexity of DNA damage at single sites, so-called multiply damaged sites. It has been argued that such homologous recombination is not the predominant mode of repair after ionizing radiation and that the majority of the relevant double-strand lesions are processed via error-prone pathways involving non-homologous recombination. The data that underpin this judgement have, however, been obtained largely through cellular and molecular studies conducted after relatively high radiation doses. For example, as discussed earlier, the formation of dicentric chromosome aberrations probably reflects such error-prone repair processes, and for these aberrations, the lowest dose at which an excess has been reproducibly obtained is around 20 mGy (low-LET). Human epidemiological studies, also outlined in this Annex, reveal evidence of excess cancer risk at somewhat higher doses. Below these doses there must be complete dependence on an understanding of mechanistic processes and, critically, on the contention that error-prone DNA repair processes remain in place in cells down to single-track intersections of DNA.

450. Although there is no specific reason to depart from this position, it remains possible that at low doses, below, say, 10 mGy (low-LET), where radiogenic DNA lesions are few, error-free homologous recombination predominates. In this hypothetical situation, error-prone repair would be a secondary response that applied only when the abundance of DNA lesions increased above some critical level. Under these conditions the form of the dose-response relationship for mutational/tumorigenic risk would be expected to have a threshold-like component at very low doses.

451. Formal experimental approaches to this problem are beyond the resolution of current quantitative techniques in cellular radiobiology. One particular set of observations argues, however, against the proposition of wholly error-free repair at very low abundance of radiogenic DNA lesions.

452. As noted in this Annex and in Annex F, *"DNA repair and mutagenesis"*, spontaneously arising DNA double-strand breaks have a relatively low abundance in mammalian cells. In spite of this, dicentric chromosome aberrations, a manifestation of DNA break misrepair, occur at a reproducibly measurable spontaneous rate of about 1 per 1,000 cell generations [L8]. These observations argue that such DNA misrepair processes are not solely a product of a high incidence of DNA lesions and therefore that error-free repair at low doses is unlikely. Although somewhat less certain, a proportion of the DNA deletions/rearrangements that characterize some spontaneously arising gene mutations in mammalian cells may also arise as a consequence of DNA break misrepair mechanisms (see Annex F, *"DNA repair and mutagenesis"*).

453. Finally, although in a repair context homologous recombination is potentially error-free, it may carry its own risk in that it can serve to duplicate genes from the undamaged homologous chromosome of a given target cell. These recombinational processes are well-recognized mechanisms for the unmasking of variant heterozygous genes that can contribute to tumorigenic development, i.e. homologous recombination as well as DNA deletion results in the loss of heterozygosity in DNA that characterizes many human and animal tumours [V2]. Thus, it may be that there is no risk-free way in which complex DNA double-strand lesions can be processed in the cells of genetically heterozygous organisms such as humans.

4. Epigenetic events in tumorigenesis

454. Stable epigenetic effects on gene activity such as gene silencing via heterochromatization and gene activation via loss of imprinting have been described in this Annex as being involved in tumour development. Whereas there is a wealth of information on dose-response relationships for the induction of gene/chromosomal mutations, it remains most uncertain whether radiation damage contributes directly to the establishment of these stable epigenetic events and whether there is any form of dose response. In general, the implications for low-dose cancer risk remain a matter of speculation, but a number of issues may be raised.

455. First and most simply, in some instances the translocation of intact genetic material from one genomic location to another can lead to changes in gene activity, so-called position effects (see [P12]). The dose response for such effects should follow that of chromosomal exchange, so there should be no major uncertainty. Cellular targets for loss of imprinting and/or changes in the status of DNA methylation and/or heterochromatin *in situ* remain obscure, however.

456. Second, some *in vivo/in vitro* studies with rodents noted in the UNSCEAR 1993 Report [U3] and in this Annex imply that the rate of induction by radiation of early tumour-associated events in clonogens of thyroid and breast tissue is too high to be explained by conventional mutational mechanisms. These observations, together with those made in certain cellular transformation systems, have led to suggestions that induced epigenetic processes may play an important role in radiation tumorigenesis [C19].

457. The cellular and molecular basis of these high-frequency events remains unresolved. Nevertheless, the finding of induced frequencies for tumour-initiated clonogens of around 10^{-2} cannot possibly be explained by a gene-specific deletion mechanism such as that which applies to *HPRT* in cultured cells, where induced frequencies after low-LET irradiation rarely exceed a value of 10^{-4}. As noted in Annex F, *"DNA repair and mutagenesis"*, induced mutation frequency is, however, influenced strongly by genetic context, with tolerance of DNA loss an important factor; recent observations concerning tumorigenic mechanisms in mice, outlined below, suggest that extreme forms of such tolerance may not be unusual and may be misleading with respect to gene targets.

458. In studies of radiation tumorigenesis in *p53*-deficient (+/−) mice, Kemp et al. [K2] noted the very high frequency at which tumour-initiating events appeared to be induced and suggested that radiation-induced persistent genomic instability with respect to wild type *p53* might be responsible. Follow-up studies [B4] suggest, however, that this high frequency of initial events can be explained by a mechanism involving whole chromosome gain and loss (aneuploidy), where the target size for *p53* gene loss from critical cells may be orders of magnitude greater than the gene itself. In the same way, loss of wild-type *Apc* during the early development of intestinal neoplasia in $Apc^{+/-}$ mice frequently involves loss of the whole of the encoding chromosome [L20, S18] and is also likely to be a much more frequent event than single gene deletion. Thus, alone, frequency of tumour initiation may not be a reliable indicator of epigenetic involvement in radiation tumorigenesis. In this respect the extrapolation of mechanistic data from rodent experimental systems to human tumorigenesis should be undertaken with caution.

459. Nevertheless, these findings indicate that in seeking to model multi-stage radiation tumorigenesis, it is not always necessary to be constrained by conventional values of induced mutation frequency that apply to single genes in cells irradiated *in vitro*. Very large chromosomal deletions

and specific forms of aneuploidy can be tolerated and selected during *in vivo* tumour development, and the rate of many forms of gene/chromosomal mutation is often enhanced following the spontaneous development of mutator phenotypes. In addition, the suppression of apoptosis during tumorigenesis may allow the proliferation of genomically aberrant cells that would otherwise have been eliminated. In effect, the loss of apoptotic processes can further enhance the rate at which viable mutant cells appear within evolving neoplastic clones.

G. SUMMARY

460. Current evidence suggests that the biological modelling of radiation tumorigenesis might best proceed on the initial assumption that at low doses radiation acts primarily as a mutational initiator of neoplasia. The situation regarding protracted low-dose irradiation is biologically more complex, and mechanistic studies have yet to comment specifically upon the extent to which radiation may influence the later stages of tumorigenesis. The possible existence of error-free DNA repair in target cells that might generate a low-dose threshold for tumour induction is recognized but judged on fundamental grounds to be unlikely. Other cellular and humoral factors would need to be modulated in a dose-dependent fashion to specifically change the initial slope of the dose response for tumour induction. At present, conclusive evidence for these radiation-specific modulations operating at low doses is lacking, but such effects would not be unexpected after high-dose irradiation. An additional uncertainty is the balance between mutational and epigenetic contributions to induced neoplasia, particularly the role and possible dose response for epigenetic effects. Epigenetic effects following radiation could, in principle, impact all stages of tumour development. Transient biochemical stress responses and bystander effects are most likely to influence the mutagenic and apoptotic aspects of tumour initiation, while induced and persistent genomic instability may be envisaged to impact pre-neoplastic clonal evolution and malignant development. Although fundamental knowledge is increasing rapidly, the extent to which such processes specifically determine low-dose tumorigenic response remains largely a matter of speculation.

461. Overall, evidence on the fundamental aspects of radiation action and its relationship to tumour induction provide no firm scientific reasons to believe that at low doses the form of cellular dose response is related in a complex fashion to increasing dose. Employing the principle of parsimony, it is therefore suggested that low-dose cellular mutagenic risk and, by implication, that for tumorigenesis rises from the zero-dose baseline as a simple function of dose. The linear form is the simplest of these responses and is not inconsistent with the majority of the quantitative data discussed in this Annex. Irrespective of future scientific developments, however, it may well be impossible to provide formal scientific proof of linearity or any other form of low-dose radiation response for tumorigenesis *in vivo*.

462. In addition, for a complex multifactorial response such as *in vivo* tumorigenesis, the expression of initial *in vivo* cellular events may in some circumstances be subject to high dose modification. Accordingly, caution needs to be exercised in interpreting dose-response data for *in vivo* tumorigenesis that encompass wide dose ranges, say, 0–5 Gy low-LET.

463. The observation of apparently simple forms of *in vivo* dose response over such a dose range can, in principle, disguise competing dose-dependent elements in the tumorigenic process. For example, at high doses, the suppressive effects of initial inactivation of target cells may compete with subsequent tumour promotion in damaged normal tissue. For the purposes of modelling the biological elements of radiation tumorigenesis, it is not possible at present to quantify such competing effects, but as knowledge accumulates these problems will demand increasing attention.

V. BIOLOGICALLY BASED MODELLING OF RADIATION CARCINOGENESIS

464. The principal aims of this Chapter are (a) to review general aspects of the computational models that seek to interpret epidemiological data on cancer risk, (b) to describe the empirical and mechanistic models that have been developed, and (c) to illustrate and compare the predictive features of empirical and mechanistic models with emphasis on risk at low doses. Computational models of cancer risk can also play a role in describing the possible interactions between radiation and other agents. This complex issue is explored in Annex H, *"Combined effects of radiation and other agents"*.

465. In Chapter IV the review of fundamental data on radiation tumorigenesis allowed proposals to be made on how evolving knowledge might guide the development of mechanistic models of radiation risk. At this early stage of understanding, much of the guidance remains to be implemented, but three main principles can be considered: (a) radiation will tend to act at the earliest stage of tumorigenesis (initiation), (b) in general, no low-dose threshold should be expected, and (c) time-constant relative risk is suggested on the basis that radiation-induced and spontaneously arising tumorigenic events will be subject to the same host and environmental modifications although this is recognized as being somewhat simplistic.

466. The biological uncertainties noted in earlier Chapters suggest, however, that dose-dependent differences between tumour types with respect to their induction and the mechanisms involved should be expected. Accordingly, general comment will be provided on the predictive value of

biologically based models with respect to the projection of risk with time and dose response for radiation tumorigenesis. At the outset it is however important to stress that although a number of valuable mathematical and statistical tools have been developed, the outcome of cancer risk modelling is often dependent on the initial biological assumptions made. Even using the same data sets, different groups of workers can arrive at different optimal mathematical/statistical solutions depending on these assumptions. This is clearly a significant source of uncertainty.

A. GENERAL ASPECTS OF THE PROBLEM

467. One of the principal uncertainties that surround the calculation of cancer risks from epidemiological data results from the fact that few radiation-exposed cohorts have been followed up to extinction. For example, 50 years after the atomic bombings of Hiroshima and Nagasaki, about half of the survivors were still alive [P2]. In attempting to calculate lifetime population cancer risks it is therefore important to predict how risks might vary as a function of time after radiation exposure, in particular for that group for whom the uncertainties in projection of risk to the end of life are most uncertain, namely those who were exposed in childhood.

468. One way to model the variation in risk is to use empirical models incorporating adjustments for a number of variables (e.g. age at exposure, time since exposure, sex) and indeed this approach was used by the BEIR V Committee [C1] in its analyses of data on the Japanese atomic bomb survivors and various other irradiated groups. Recent analyses of solid cancers for these groups have found that the radiation-induced excess risk can be described fairly well by a relative risk model [I2]. The time-constant relative risk model assumes that if a dose of radiation is administered to a population, then, after some latent period, there is an increase in the cancer rate, the excess rate being proportional to the underlying cancer rate in an unirradiated population. For leukaemia, this model provides an unsatisfactory fit, consequently a number of other models have been used for this group of malignancies, including one in which the excess cancer rate resulting from exposure is assumed to be constant i.e. the time-constant additive risk model [U4].

469. It is well known that for all cancer subtypes (including leukaemia) the excess relative risk (ERR) diminishes with increasing age at exposure [U2]. For those irradiated in childhood there is evidence of a reduction in the excess relative risk of solid cancer 25 or more years after exposure [L6, L33, P2, T4]. For solid cancers in adulthood the excess relative risk is more nearly constant, or perhaps even increasing over time [L32, L33], although there are some indications to the contrary [W9]. Clearly then, even in the case of solid cancers various factors have to be employed to modify the excess relative risk.

470. Associated with the issue of projection of cancer risk over time is that of projection of cancer risk between two populations with differing underlying susceptibilities to cancer. Analogous to the relative risk time projection model one can employ a multiplicative transfer of risks, in which the ratio of the radiation-induced excess cancer rates to the underlying cancer rates in the two populations might be assumed to be identical. Similarly, akin to the additive risk time projection model one can use an additive transfer of risks, in which the radiation-induced excess cancer rates in the two populations might be assumed to be identical. The data that are available suggests that there is no simple solution to the problem [U2]. For example, there are weak indications that the relative risks of stomach cancer following radiation exposure may be more comparable than the absolute excess risks in populations with different background stomach cancer rates [U2]. The breast cancer relative risks observed in the most recent analysis of the Japanese atomic bomb survivor incidence data [T4] are rather higher than those seen in various other data sets, particularly for older ages at exposure [B6, M2, S20]. The observation that sex differences in solid tumour excess relative risk are generally offset by differences in sex-specific background cancer rates [U2] might suggest that absolute excess risks are more alike than excess relative risks. Taken together, these considerations suggest that in various circumstances relative or absolute transfers of risk between populations may be advocated, or indeed, the use of some sort of hybrid approach such as that which has been employed by Muirhead and Darby [M12] and Little et al. [L56].

471. The exposed populations that are often used for deriving cancer risks e.g. the Japanese atomic bomb survivors, were exposed to ionizing radiation at high doses and high dose rates. However, it is the possible risks arising from low dose and low dose-rate exposure to ionizing radiation which are central to the setting of standards for radio-logical protection. The ICRP [I2] recommended application of a dose and dose-rate effectiveness factor of 2 to scale cancer risks from high dose and high dose-rate exposure to low dose and low dose-rate exposure on the basis of animal data, the shape of the cancer dose response in the bomb survivor data and other epidemiological data. Although the linear-quadratic dose-response model (with upward curvature) found for leukaemia is perhaps the most often employed departure from linearity in analyses of cancer in radiation-exposed groups [P1, P2], other shapes are possible for the dose-response curve [U3]. While for most tumour types in the Japanese data linear-quadratic curvature adequately describes the shape of the dose-response curve, for non-melanoma skin cancer (NMSC) there is evidence for departures from linear-quadratic curvature. The non-melanoma skin cancer dose response in the Japanese cohort is consistent with a dose threshold of 1 Sv [L7, L30] or with an induction term proportional to the fourth power of dose, with, in each case, an exponential cell sterilization term to reduce non-melanoma skin cancer risk at high doses (>3 Sv).

472. Arguably, models which take account of the biological processes leading to the development of cancer can provide insight into these related issues of projection of cancer risk over time, transfer of risk across population and extrapolation of risks from high doses and dose-rates to low doses and dose-

rates. For example, Little and Charles [L32] have demonstrated that a variety of mechanistic models of carcinogenesis predict an excess relative risk which reduces with increasing time after exposure for those exposed in childhood, while for those exposed in adulthood the excess relative risk might be approximately constant over time. Mechanistic considerations also imply that the interactions between radiation and the various other factors that modulate the process of carcinogenesis may be complex [L2], so that in general one would not expect either relative or absolute risks to be invariant across populations. Some of the general features of interaction between radiation and other factors are described in Annex H, *"Combined effects of radiation and other agents"*.

B. EMPIRICAL AND MECHANISTIC MODELS

1. Armitage-Doll multi-stage model

473. Mechanistic models of carcinogenesis were originally developed to explain phenomena other than the effects of ionizing radiation. One of the more commonly observed patterns in the age-incidence curves for epithelial cancers is that the cancer incidence rate varies approximately as Ct^{β} for age t and some constants C and β. At least for most epithelial cancers in adulthood, the exponent β of age seems to lie between 4 and 6 [D5]. The so-called multi-stage model of

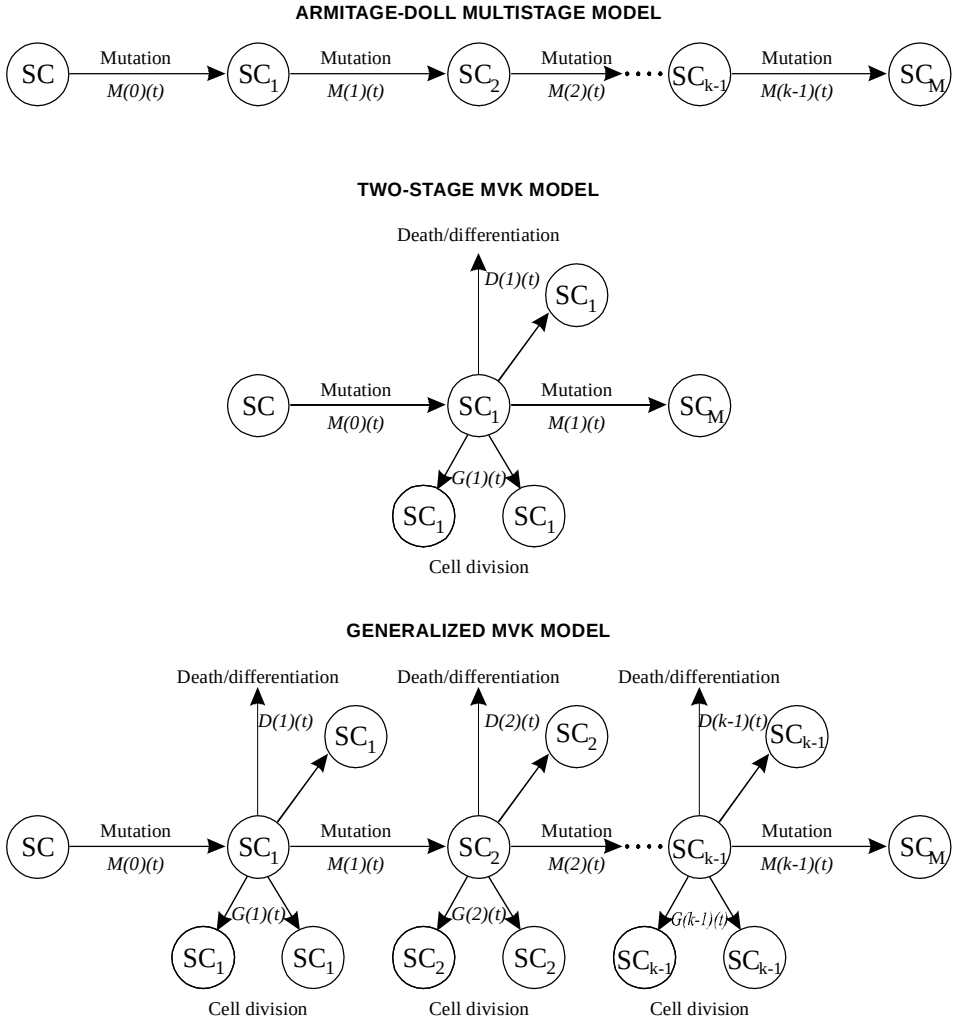

Figure XXIII. Empirical/mechanistic models of multi-stage carcinogenesis.

SC: Initial stem cell
SC_i: Stem cell of stage i
SC_M: Malignant stem cell
M(i)(t): Mutation rate at stage i and age t
G(i)(t): Stem cell rate at stage i and age t
D(i)(t): Death/differentiation rate at stage i and age t

carcinogenesis of Armitage and Doll [A1] was developed in part as a way of accounting for this approximately log-log variation of cancer incidence with age. The model supposes that at age t an individual has a population of X(t) completely normal (stem) cells and that these cells acquire one mutation at a rate M(0)(t). The cells with one mutation acquire a second mutation at a rate M(1)(t), and so on until at the (k − 1)th stage the cells with (k − 1) mutations proceed at a rate M(k − 1)(t) to become fully malignant. The model is illustrated schematically in Figure XXIII. It can be shown that when X(t) and M(i)(t) are constant, a model with k stages predicts a cancer incidence rate that is approximately given by the expression Ct^{k-1}, with C = M(0) M(1)...M(k − 1)/(1 × 2 ... (k − 1)) [A1, M27].

474. In developing their model, Armitage and Doll [A1] were driven largely by epidemiological findings and, in particular, by the age distribution of epithelial cancers. In the intervening 30 years, substantial biological evidence has been gathered that cancer is a multi-step process involving the accumulation of a number of genetic and epigenetic changes in a clonal population of cells. This evidence was reviewed in the UNSCEAR 1993 Report [U3], and subsequent data and concepts are outlined in Chapter IV of this Annex. However, there are certain problems with the model proposed by Armitage and Doll [A1] associated with the fact that to account for the observed age-incidence curve Ct^{β} with β between 4 and 6, between five and seven stages are needed. For colon cancer there is evidence that six stages might be required [F1, U3]. However, for other cancers there is, at present, insufficient evidence to conclude that there are as many rate-limiting stages as this. BEIR V [C1] surveyed evidence for all cancers and found that two or three stages might be justifiable, but not a much larger number. To this extent, the large number of stages predicted by the Armitage-Doll model appears to be verging on the biologically unlikely. Related to the large number of stages required by the Armitage-Doll multi-stage model are the high mutation rates predicted by the model. Moolgavkar and Luebeck [M28] fitted the Armitage-Doll multi-stage model to data sets describing the incidence of colon cancer in a general population and in patients with familial adenomatous polyposis. Moolgavkar and Luebeck [M28] found that Armitage-Doll models with five or six stages gave good fits to these data sets, but that both of these models implied mutation rates that were too high by at least two orders of magnitude. The discrepancy between the predicted and experimentally measured mutation rates might be eliminated, or at least significantly reduced, if account is taken of the fact that the experimental mutation rates are locus-specific. A "mutation" in the sense in which it is defined in this model might result from the "failure" of any one of a number of independent loci, so that the "mutation" rate would be the sum of the failure rates at each individual locus.

475. Notwithstanding these problems, much use has been made of the Armitage-Doll multi-stage model as a framework for understanding the time course of carcinogenesis, particularly for the interaction of different carcinogens [P10]. Thomas [T3] has fitted the Armitage-Doll model with one and two radiation-affected stages to the solid cancer data in the Japanese Life Span Study 11 cohort of bomb survivors. Thomas [T3] found that a model with a total of five stages, of which either stages one and three or stages two and four were radiation-affected, fitted significantly better than models with a single radiation-affected stage. Little et al. [L5, L35] also fitted the Armitage-Doll model with up to two radiation-affected stages to the Japanese Life Span Study 11 data set and also to data on various medically exposed groups, using a slightly different technique to that of Thomas [T3]. Little et al. [L5, L35] found that the optimal solid cancer model for the Japanese data had three stages, the first of which was radiation affected, while for the Japanese leukaemia data the best fitting model had three stages, the first and second of which were radiation affected. A version of the Armitage-Doll has also been fitted to the Life Span Study solid tumour incidence data by Pierce and Mendelsohn [P22], who found that a model with five or six stages gave the best fit to this data.

476. Both the paper of Thomas [T3] and those of Little et al. [L5, L35] assumed the ith and the jth stages or mutation rates [M(i −1), M(j − 1)] (j > i) in a model with k stages to be (linearly) affected by radiation and the transfer coefficients (other than M(i −1) and M(j −1)) to be constant, as is the stem-cell population X(t). In these circumstances, it can be shown [L5] that if an instantaneously administered dose of radiation d is given at age a, then at age t (>a) the cancer rate is approximately as follows:

$$\mu\, t^{k-1} + \alpha\, d\, a^{i-1}\, t^{k-i-1} + \beta\, d\, a^{i-1}\, t^{k-j-1} + \gamma\, d^2\, a^{i-1}\, t^{k-j-1} \qquad (5)$$

for some positive constants μ, α and β, and where γ is given by

$$\gamma = \frac{\alpha\,\beta\,\Gamma(k-i)\,\Gamma(j)}{2\,\mu\,\Gamma(k)} \quad \text{if } j = i+1 \qquad (6)$$

$$\textit{and} \qquad = 0 \quad \text{if } j > i+1$$

and $\Gamma(i)$ is the gamma function [A6].

477. The first term (μt^{k-1}) in expression (5) corresponds to the cancer rate that would be observed in the absence of radiation, while the second term ($\alpha d a^{i-1} t^{k-i-1}$) and the third term ($\beta d a^{i-1} t^{k-j-1}$) represent the separate effects of radiation on the ith and jth stages, respectively. The fourth term ($\gamma d^2 a^{i-1} t^{k-j-1}$), which is quadratic in dose, d, represents the consequences of interaction between the effects of radiation on the ith and the jth stages and is only non-zero when the two radiation-affected stages are adjacent (j = i + 1). Thus if the two affected stages are adjacent, a quadratic (dose plus dose-squared) relationship will occur, whereas the relationship will be approximately linear if the two affected stages have at least one intervening stage. Another way of considering the joint effects of radiation on two stages is that for a brief exposure, unless the two radiation-affected stages are adjacent, there will be insignificant interaction between the cells affected by radiation in the earlier and later of the two radiation-affected cell compartments. This is simply because very few cells will move between the two compartments in the course of the radiation exposure. If the ith and the jth stages are radiation-affected, the result of a brief dose of radiation will be to cause some of the cells that have already accumulated (i -1) mutations to acquire an extra mutation and move from the (i - 1)th to the ith compartment. Similarly, it will cause some of the cells that have already acquired (j - 1) mutations to acquire an extra mutation and so move from the (j - 1)th to the jth compartment. It should be noted that the model does not require that the same cells be hit by the radiation at the ith and jth stages, and in practice for low total doses, or whenever the two radiation-affected stages are separated by an additional unaffected stage or stages, an insignificant

proportion of the same cells will be hit (and mutated) by the radiation at both the ith and the jth stages. The result is that unless the radiation-affected stages are adjacent, for a brief exposure the total effect on cancer rate is approximately the sum of the effects, assuming radiation acts on each of the radiation-affected stages alone. One interesting implication of models with two or more radiation-affected stages is that as a result of interaction between the effects of radiation at the various stages, protraction of dose, in general, results in an increase in cancer rate, i.e. an inverse dose-rate effect [L5]. However, it can be shown that in practice the resulting increase in cancer risk is likely to be small [L5].

478. The variant of the Armitage-Doll model fitted by Pierce and Mendelsohn [P22] is unusual in that it assumes that radiation equally affects all k mutation rates in the model except the last. (In the last stage, radiation is not assumed to have any effect.) This assumption distinguishes their use of this model from the approaches of Little et al. [L5] or Thomas [T3], both of which assumed that radiation affected at most two of the mutation rates (and neither of which constrained the effects of radiation to be equal in these stages). There are some technical problems with the paper of Pierce and Mendelsohn [P22] arising from the authors' failure to take account of interactions between the effects of radiation on the $(k - 2)$ pairs of adjacent stages. These interactions contribute significantly by adding a quadratic term in the dose response and cannot be ignored, even to a first-order approximation. The fact that in general there is little evidence for upward curvature in the solid cancer dose response in the Life Span Study [P1, L7, L44] argues that if proper account had been taken of these interaction terms, the model of Pierce and Mendelsohn [P22] would not fit the data well. Moreover, one implication of the model of Pierce and Mendelsohn [P22] is that the excess relative risk will be proportional to $1/a$, i.e. the inverse of attained age. However, this is known to provide a poor description of the excess relative risk of solid cancer, even within the Life Span Study cohort [L56, L57].

479. The optimal leukaemia model found by Little et al. [L5, L35], having adjacent radiation-affected stages, predicts a linear-quadratic dose response, in accordance with the significant upward curvature which has been observed in the Japanese data set [P1, P2, P3]. This leukaemia model, and also that for solid cancer, predicts the pronounced reduction of excess relative risk with increasing age at exposure which has been seen in the Japanese atomic bomb survivors and other data sets [U2]. The optimal Armitage-Doll leukaemia model predicts a reduction of excess relative risk with increasing time after exposure for leukaemia. At least for those exposed in childhood, the optimal Armitage-Doll solid cancer model also predicts a reduction in excess relative risk with time for solid cancers. These observations are consistent with the observed pattern of risk in the Japanese and other data sets [L33, U2]. Nevertheless, there are indications that the Armitage-Doll model may not provide an adequate fit to the Japanese data [L6]. For this reason, and because of the other problems with the Armitage-Doll model discussed above, a slightly different class of models need to be considered.

2. Two-mutation models

480. In order to reduce the biologically implausible number of stages required by their first model, Armitage and Doll [A7] developed a further model of carcinogenesis, which postulated a two-stage probabilistic process whereby a cell following an initial transformation into a pre-neoplastic state (initiation) was subject to a period of accelerated (exponential) growth. At some point in this exponential growth a cell from this expanding population might undergo a second transformation (promotion) leading directly to the development of a neoplasm. Like their previous model, it satisfactorily explained the incidence of cancer in adults, but was less successful in describing the pattern of certain childhood cancers.

481. The two-mutation model developed by Knudson [K16] to explain the incidence of retinoblastoma in children took account of the process of growth and differentiation in normal tissues. Subsequently, the stochastic two-mutation model of Moolgavkar and Venzon [M7] generalized Knudson's model, by taking account of cell mortality at all stages as well as allowing for differential growth of intermediate cells. The two-stage model developed by Tucker [T7] is very similar to the model of Moolgavkar and Venzon but does not take account of the differential growth of intermediate cells. The two-mutation model of Moolgavkar, Venzon, and Knudson (MVK) supposes that at age t there are $X(t)$ susceptible stem cells, each subject to mutation to an intermediate type of cell at a rate $M(0)(t)$. The intermediate cells divide at a rate $G(1)(t)$; at a rate $D(1)(t)$ they die or differentiate; at a rate $M(1)(t)$ they are transformed into malignant cells. The model is illustrated schematically in Figure XXIII. In contrast to the case of the (first) Armitage-Doll model, there is a considerable body of experimental biological data supporting this initiation-promotion type of model (see, e.g. [M5, T6]). The model has been developed to allow for time-varying parameters at the first stage of mutation [M30]. An additional generalization of this model (to account for time-varying parameters at the second stage of mutation) was presented by Little and Charles [L32], who also demonstrated that the excess relative risk predicted by the model when the first mutation rate was subject to instantaneous perturbation decayed at least exponentially for a sufficiently long time after the perturbation. Moolgavkar et al. [M29] and Luebeck et al. [L12] and Heidenreich et al. [H2] used the two-mutation model to describe the incidence of lung cancer in rats exposed to radon, and in particular to describe the inverse dose-rate effect that has been observed in these data. Other groups have also modelled lung tumour risk in rats exposed to radon [H35, L2], and in this experimental system the modelling suggests effects of radiation on the later stages of tumorigenesis. Moolgavkar et al. [M13] and Luebeck et al. [L16] also applied the model to describe the interaction of smoking and radiation as causes of lung cancer in the Colorado Plateau uranium miner cohort. More recently the two-mutation model has been utilized to model lung, stomach, and colon cancer in the atomic bomb survivor incidence data [K17].

482. Moolgavkar and Luebeck [M28] have used models with two or three mutations to describe the incidence of colon cancer in a general population and in patients with familial adenomatous polyposis. They found that both models gave good fits to both data sets, but that the model with two mutations implied biologically implausibly low mutation rates. The three-mutation model, which predicted mutation rates more in line with biological data, was therefore somewhat preferable. The problem of implausibly low mutation rates implied by the two-mutation model is not specific to the case of colon cancer, and is discussed at greater length by Den Otter et al. [D6] and Derkinderen et al. [D7], who argue that for most cancer sites a model with more than two stages is required.

3. Generalized Moolgavkar-Venzon-Knudson (MVK) multi-stage models

483. A number of generalizations of the Armitage-Doll and two- and three-mutation models have been developed [L31, T6]. In particular, two closely related models have been developed, whose properties have been described by Little [L31]. The first model is a generalization of the two-mutation model of Moolgavkar, Venzon, and Knudson and so will be termed the *generalized MVK model*. The second model generalizes the multi-stage model of Armitage and Doll and will be termed the *generalized multi-stage model*.

For the generalized MVK model it may be supposed that at age t there are X(t) susceptible stem cells, each subject to mutation to a type of cell carrying an irreversible mutation at a rate of M(0)(t). The cells with one mutation divide at a rate G(1)(t); at a rate D(1)(t) they die or differentiate. Each cell with one mutation can also divide into an equivalent daughter cell and another cell with a second irreversible mutation at a rate M(1)(t). For the cells with two mutations there are also assumed to be competing processes of cell growth, differentiation, and mutation taking place at rates G(2)(t), D(2)(t), and M(2)(t), respectively, and so on until at the (k – 1)th stage the cells that have accumulated (k – 1) mutations proceed at a rate M(k – 1)(t) to acquire another mutation and become malignant. The model is illustrated schematically in Figure XXIII. The two-mutation model of Moolgavkar, Venzon, and Knudson corresponds to the case k = 2. The generalized multi-stage model differs from the generalized MVK model only in that the process whereby a cell is assumed to split into an identical daughter cell and a cell carrying an additional mutation is replaced by the process in which only the cell with an additional mutation results, i.e. an identical daughter cell is not produced. The classical Armitage-Doll multi-stage model corresponds to the case in which the intermediate cell proliferation rates G(I)(t) and the cell differentiation rates D(i)(t) are all zero.

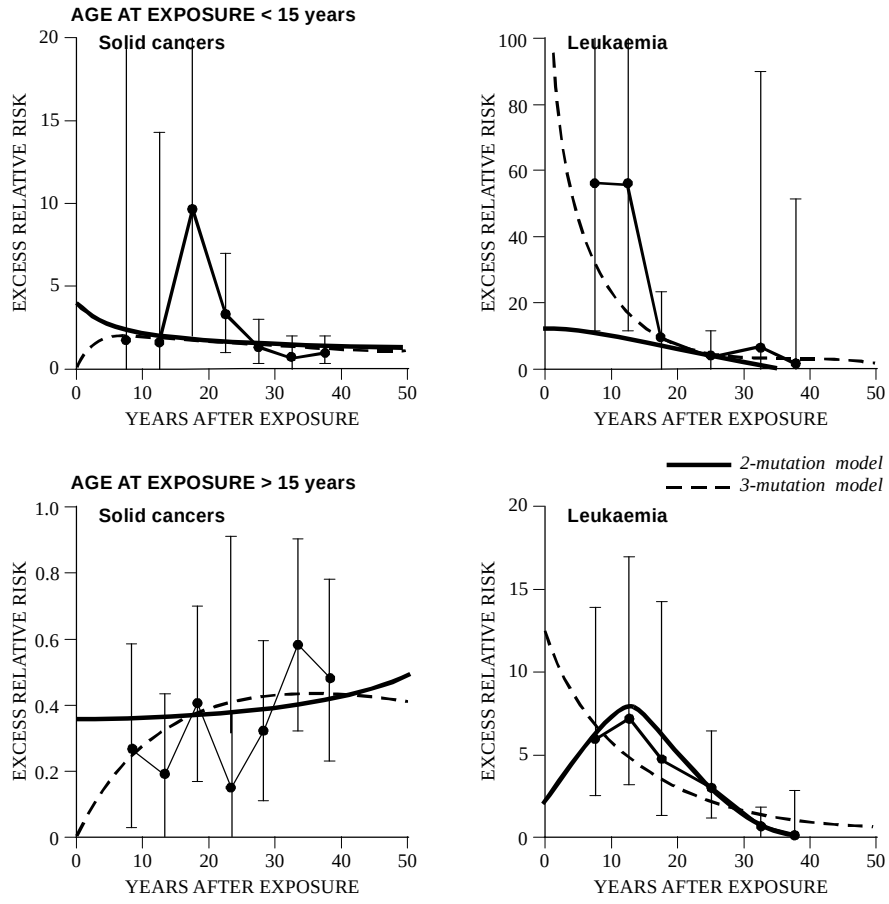

Figure XXIV. Comparison of generalized MVK models fitted to the observed excess relative risk Sv^{-1} (CI: 90%) in survivors of the atomic bombings in Japan [L36].

484. It can be shown [L31] that the excess relative risk for either model following a perturbation of the parameters will tend to zero as the attained age tends to infinity. One can also demonstrate that perturbation of the parameters M(k – 2), M(k – 1), G(k – 1), and D(k – 1) will result in an almost instantaneous change in the cancer rate [L31].

485. Generalized MVK models have been fitted to the atomic bomb survivor mortality data [L36]. For both leukaemia and solid cancers, the only models with a single radiation-affected parameter that give even slightly satisfactory fits are those in which radiation is assumed to affect M(0) [L36]. For both leukaemia and solid cancer, generalized two- and three-mutation MVK models fit equally well. For leukaemia, the three-mutation model provides a satisfactory fit only when M(0) and M(1) are assumed affected by radiation. For solid cancer and leukaemia there are indications of lack of fit to the youngest age-at-exposure group for the three-mutation model; there is also some lack of fit of the optimal solid cancer three-mutation model to this age-at-exposure group (Figure XXIV).

486. For solid cancer, only M(0) is (linearly) affected by radiation for two- or three-mutation generalized MVK models. In contrast to the solid cancer models, both leukaemia models assume a linear-quadratic dose dependence of the M(i). The non-linearity found in the leukaemia M(i) dose response reflects known curvature in the leukaemia dose response in the atomic bomb survivor data [C1, P1]. There is some evidence, e.g. for chromosome aberrations, that the mutation induction curve is linear-quadratic at least for low-LET radiation, although linearity is generally observed for high-LET radiation [L34].

487. Despite the indications of lack of fit discussed above, the variation of excess relative risk with time since exposure and age at exposure predicted by the optimal two- and three-mutation models for solid cancer (Figure XXIV) is in qualitative agreement with the variation seen in the Japanese bomb survivors and in other irradiated groups [U2]. In particular the optimal models demonstrate the progressive reduction in excess relative risk with increasing age at exposure seen in many data sets [U2], together with the marked reduction in excess relative risk with increasing time since exposure observed in various groups exposed in childhood [L42, P2].

488. Figure XXIV reinforces the theoretical predictions of an earlier paper by Little [L31] and shows that immediately after perturbing M(0) in the two-mutation model, the excess relative risk for solid cancers and leukaemia quickly increases. However, there are no data in the first five years of follow-up in the survivor cohort [P2], so that it is difficult to test the predictions [L31] in respect of the variation in risk shortly after exposure using that data set.

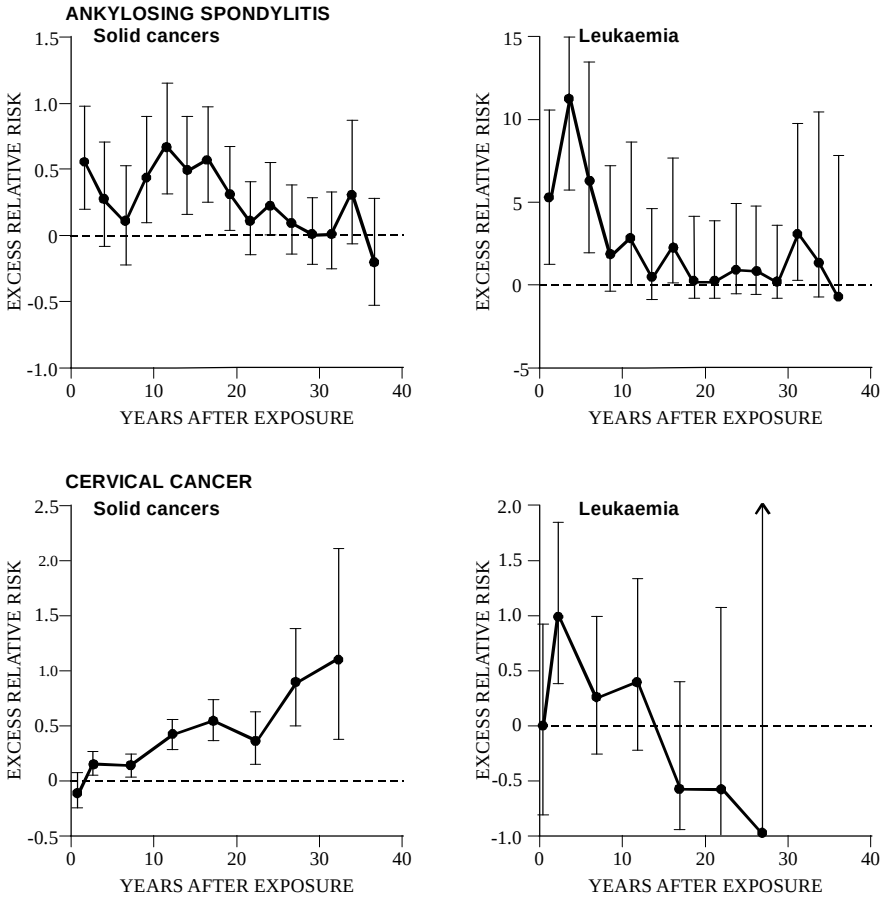

Figure XXV. Excess relative risk (CI: 90%) of solid cancers and leukaemia in Ankylosing Spondylitis Study in United Kingdom and the International Study of Cervical Cancer [B5, D8].

489. There is a suggestive increase in the excess relative risk of cancers other than leukaemia and colon cancer in the UK ankylosing spondylitis patients <5 years after first treatment (the first two datapoints in the top-left panel of Figure XXV), but the authors caution against interpreting this as the effect of the x-irradiation [D8]. There are no strong indications of an elevation in risk in the first five years after radiotherapy for cancers other than leukaemia and of the reproductive organs in a study of women followed up for second cancer after radiotherapy for cervical cancer [B5]. This corresponds to the first two datapoints in the bottom panel of Figure XXV. (Lung cancers are also excluded from the International Radiation Study of Cervical Cancer (IRSCC) data shown in the lower left panel of Figure XXV because of indications of above-average smoking rates in this cohort [B5].) In general there are no strong indications of an elevation in solid cancer risk soon after irradiation in other exposed groups [U2]. To this extent there are indications of inconsistency for solid cancers between the predictions of the two-mutation model and the observed variation in risks shortly after exposure.

490. In their analysis of the Colorado uranium miners data, Moolgavkar et al. [M13] partially overcame the problem posed by this instantaneous rise in the hazard after perturbation of the two-mutation model parameters by assuming a fixed period (3.5 years) between the appearance of the first malignant cell and the clinical detection of malignancy. However, the use of such a fixed latency period only translates a few years into the future the sudden step-change in the hazard. To achieve the observed gradual increase in excess relative risk shortly after exposure, a stochastic process must be used to model the transition from the first malignant cell to detectable cancer; such a process is provided by the final stage(s) in the three- or four-mutation generalized MVK models used in the analysis of Little [L36]. In particular, an exponentially growing population of malignant cells could be modelled by a penultimate stage with $G(k-1) > 0$ and $D(k-1) = 0$, the probability of detection of the clone being determined by $M(k-1)$. In their analysis of lung, stomach, and colon cancer in the atomic bomb survivor incidence data, Kai et al. [K17] did not assume any such period of latency, perhaps because of the long time after the bombings (12.4 years) before solid cancer incidence follow-up began in the Life Span Study.

491. The evidence with respect to the variation in excess relative risk shortly after exposure for leukaemias is rather different from that for solid cancers. In the United Kingdom ankylosing spondylitis patients [D8] there is significant excess risk even in the period <2.5 years after first treatment (first datapoint in top-right panel of Figure XXV). The IRSCC data [B5] shows a significant excess risk for acute non-lymphocytic leukaemia in the period 1–4 years after first treatment (the second datapoint in the lower-right panel of Figure XXV), and this pattern is observed in many other groups [U2]. More detailed analysis of UK leukaemia incidence data indicate that the age-incidence curves for all subtypes of lymphocytic leukaemia can be adequately modelled by two- and three-mutation generalized MVK models [L26, L36]. However, the two-mutation models for acute lymphocytic leukaemia (ALL) imply a very small number of stem cells ($<10^4$ cells) if the model is not to yield implausibly low mutation rates [L26].

492. Little [L55] fitted various generalized MVK models to the three main radiogenic leukaemia subtypes, namely acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML) and acute lymphocytic leukaemia (ALL) in two incidence data sets, one relating to a subset of the population of the United Kingdom recently assembled by the Leukaemia Research Fund (LRF) [C21] and the second the Japanese atomic bomb survivors [P3]. The results of this model fitting are illustrated by Figures XXVI and XXVII. Figure XXVI shows that the optimal two-mutation models adequately describe the background incidence of all three leukaemia subtypes in the United Kingdom Leukaemia Research Fund data [L55]. The optimal two-mutation model for AML assumes a step change in the numbers of susceptible stem cells and a simultaneous change in the intermediate cell proliferation parameters, G(1)(t) and D(1)(t). The optimal two-mutation model for CML assumes a step change in the numbers of susceptible stem cells and a simultaneous change in the number of susceptible stem cells and a simultaneous change in the intermediate cell growth parameter, G(1)(t), although the cell death or differentiation rate, D(1)(t), is constant. The optimal two-mutation model for ALL assumes a susceptible stem cell population of the form $X = X_0 \exp [X_1 t + 1_{[1>T]} X_+]$ and a step change in the intermediate cell proliferation parameters, G(1)(t) and D(1)(t). As can be seen from Figure XXVI, three-mutation models provide a rather worse fit for all leukaemia subtypes, particularly for ALL [L55]. For ALL, two-mutation models which assumed ionizing radiation acts to elevate mutation rates for life fitted the Japanese atomic bomb survivor incidence data rather worse than models which assumed ionizing radiation acts to elevate the first mutation rate instantaneously [L55] (see Figure XXVII, lower panel). For CML, two-mutation models which assumed ionizing radiation acts to elevate mutation rates for life fitted the Japanese atomic bomb survivor incidence data rather better than models which assumed ionizing radiation acts to elevate the first mutation rate instantaneously [L55] (see Figure XXVII, center panel). For AML (Figure XXVII, upper panel), both sorts of two-mutation models fitted equivalently well [L55].

4. Multiple pathway models

493. Little et al. [L6] fitted a generalization of the Armitage-Doll model to the Japanese atomic bomb survivor and IRSCC leukaemia data which allowed for two cell populations at birth, one consisting of normal stem cells carrying no mutations, the second a population of cells each of which has been subject to a single mutation. The leukaemia risk predicted by such a model is equivalent to that resulting from a model with two pathways between the normal stem cell

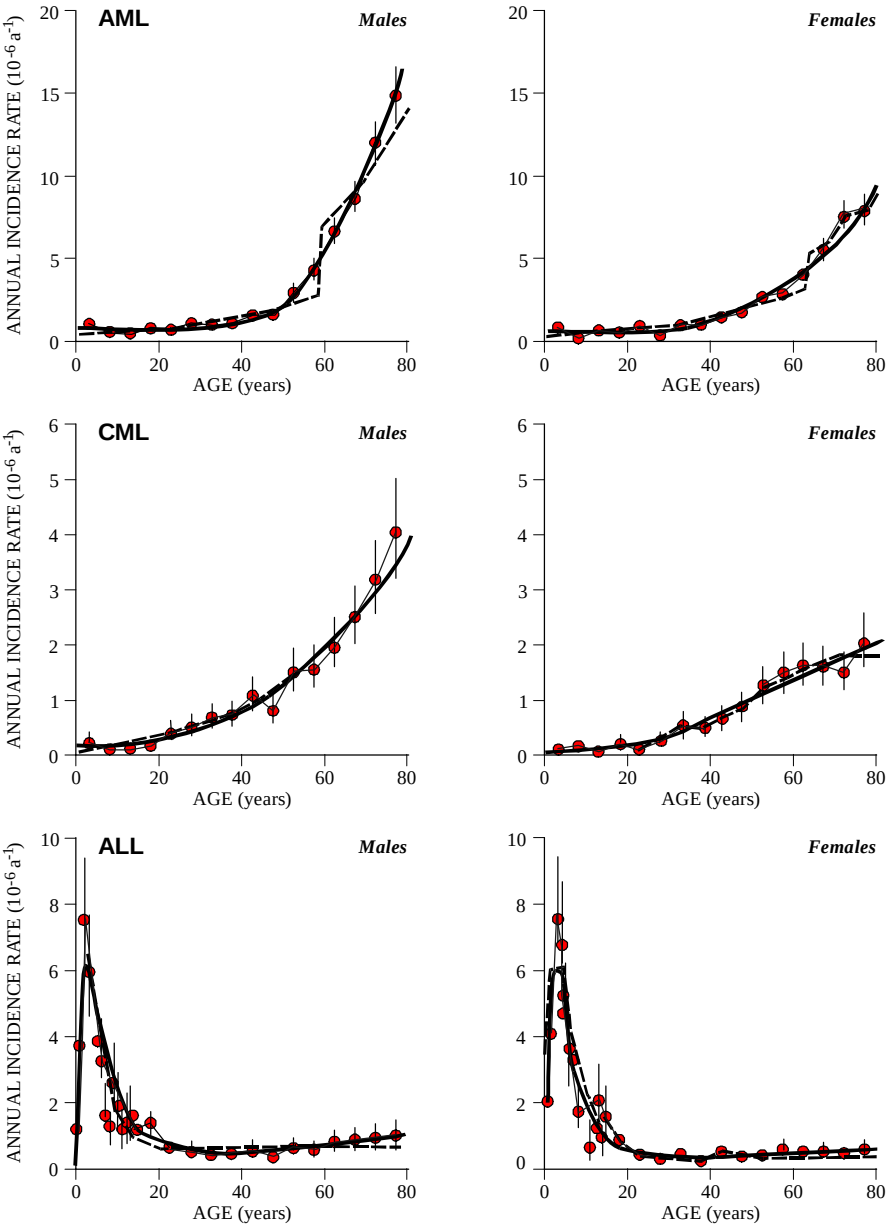

Figure XXVI. Fit of optimal two-stage and three-stage generalized MVK models to Leukeamia Research Fund data for acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML), and acute lymphocytic leukaemia (ALL) [L55].

Observed risks are shown with 95% CI.

Observed — *Two-stage model* - - - *Three-stage model*

compartment and the final compartment of malignant cells, the second pathway having one fewer stage than the first. This model fitted the Japanese and IRSCC leukaemia data sets significantly better, albeit with biologically implausible parameters, than a model which assumed just a single pathway [L6]. The findings of Kadhim et al. [K4], namely that the exposure of mammalian haemopoietic stem cells to alpha particles could generally elevate mutation rates to very much higher than normal levels, imply (if they are at all relevant to tumorigenesis) that there might be multiple pathways in the progression from normal stem cells to malignant cells (discussed in Chapter IV). The mutation rates and indeed the number of rate-limiting stages might be substantially different in these two or more pathways. A number of such models are described by Tan [T6], who also discusses at some length the biological and epidemiological evidence for such models.

C. DOSE-RESPONSE RELATIONSHIPS

494. The shape of the cancer dose response is largely driven by assumptions made about the shape of the dose-response curve for the initiating lesion or lesions. In particular, if a lesion induced by a single acutely delivered dose D of ionizing radiation at age α has a dose response given by the function F(D,a) and this is assumed to act on a single stage (not necessarily the first) in the multi-stage process of carcinogenesis and assuming also that the dose is low enough to avoid saturation effects, then it can be shown [L5, L31] that

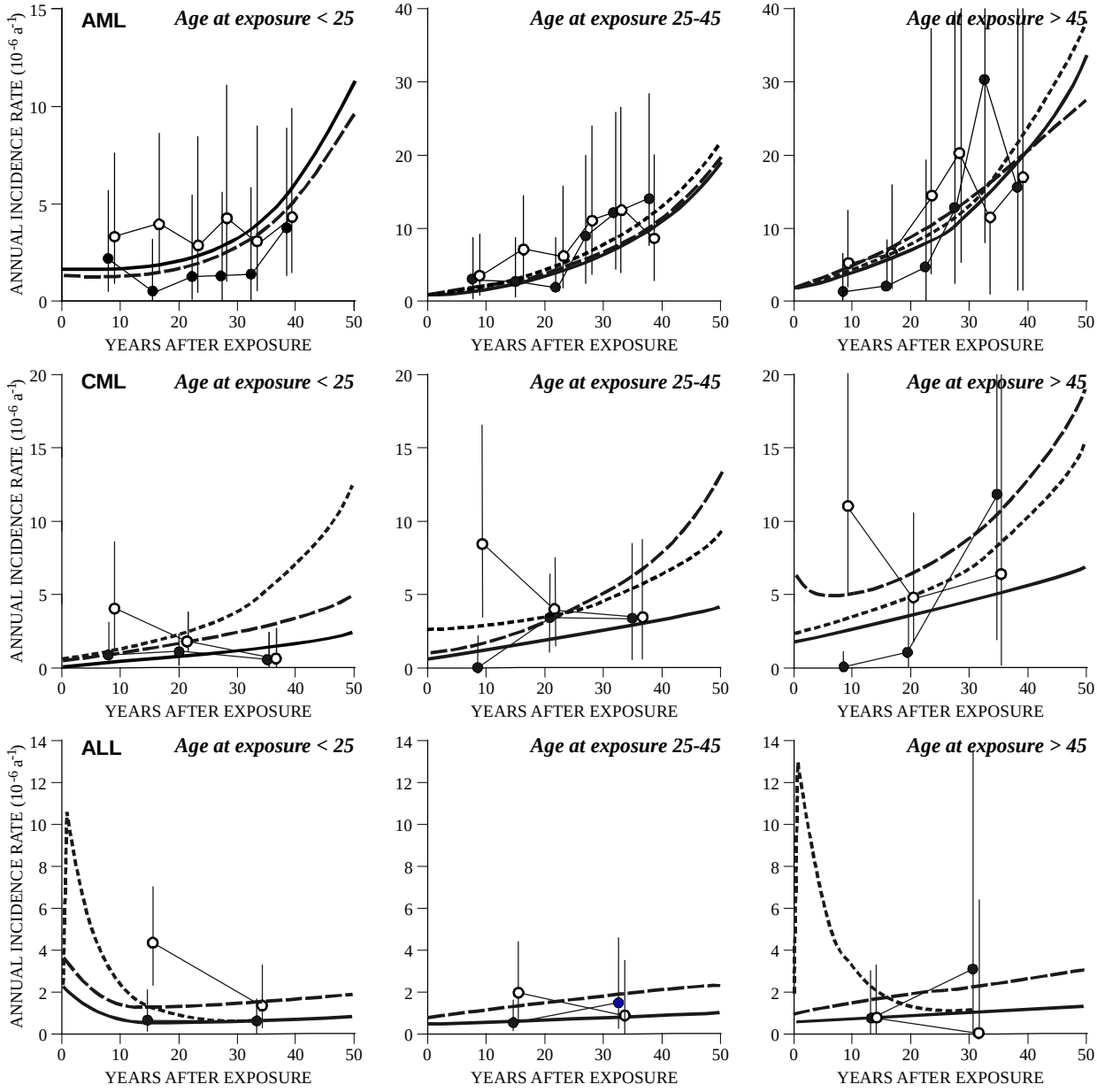

Figure XXVII. Fit of optimal two-stage MVK models to Japanese data for acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML), and acute lymphocytic leukaemia (ALL) [L55].

Observed risks are shown with 95% CI.

Japanese unexposed *Japanese exposed*

MVK unexposed

MVK exposed, M(0) elevated instantaneously

MVK exposed, M(i) elevated lifetime

the dose-response curve for carcinogenesis i years after exposure is given by w_0 (t,a) + F(D,a) − w_1(t,a) for some functions w_0(t,a) and w_1(t,a). In other words, the dose response for cancer has the same shape as the dose response for initial lesion production. In particular, if the initial lesion production is a linear-quadratic function of dose D, $F(D) = \sigma_0 + \sigma_1 - D + \sigma_2 - D^2$, then the cancer dose response will also be linear-quadratic, with the same ratio of quadratic to linear coefficients.

495. It is a crucial assumption underlying this invariance in "shape" of the dose-response curves for the production of initial lesions and cancer that the radiation-induced lesions act only at a single "stage" in the carcinogenic process. If, for example, there is a quadratic term in the dose response resulting from interactions between the (linear) effects of radiation on different "stages" (e.g. adjacent "stages" in the classical multistage model of carcinogenesis) then the ratio of the quadratic to the linear coefficients for the cancer dose response would change with time after exposure [L5]. It has been hypothesized that the quadratic term in the dose-response curve for chromosome aberrations results from interactions between the effects of two radiation tracks through the cell nucleus [E10, M42].

496. Comparison of the shape of dose-response relationships for tumour induction with that of *in vitro* cellular endpoints such as chromosome aberration induction is not straightforward. Chapter IV provides evidence of the cellular complexity of multistage tumour development, and some distortion of dose-response parameters for initial events in single cells seems likely. Accordingly, such dose-response comparisons need to be made with some caution.

497. Nevertheless, the assumption made here is that tumorigenic dose response is determined largely by the dose response for the production of initial lesions. The shape of the dose response for the various candidate lesions that might be associated with cancer has been discussed in earlier Chapters of this Annex. There is some information on this question that can be obtained from the epidemiological data, although this is generally obtained from moderately high dose studies (with total dose up to 5 Gy). There is very little reliable epidemiological data relating to total doses less than 20 mGy. In most analyses of epidemiological data, linear dose-response models are used, and give satisfactory fit. In particular this is the case for most solid tumours in the Japanese atomic bomb survivors [P2, T4] and in many other radiation-exposed groups [U2]. The linear-quadratic dose response (with upward curvature) that is found for leukaemia is perhaps the most often employed departure from linearity. However, in analyses of the shape of the cancer dose-response curve in radiation-exposed groups [P1, S1], there are various other possible shapes to the dose-response curve [U3]. For the class of deterministic effects defined by the ICRP [I2], it is assumed that there is a threshold dose, below which there is no effect [E9]. Such a form of dose response has also been employed in analyses of brain damage among those exposed *in utero* to the atomic bombings in Hiroshima and Nagasaki [O9, O10]. Arguments have been put forward that sufficiently small doses of radiation induce either no increase in cancer risk (i.e. a dose threshold), or a reduction in cancer risk (i.e. hormesis) [L45, K19, K22, P9], although these interpretations have been challenged [C15, U3].

498. Recently there have appeared a number of assessments of possible threshold-type departures from linear-quadratic curvature in the cancer dose-response curve in the Japanese atomic bomb survivor tumour incidence and mortality data. These data are noted earlier in the Annex and are discussed in detail below.

499. Analysis by Little and Muirhead [L7, L43] and by Hoel and Li [H26] of the Japanese atomic bomb survivor incidence data demonstrated a significant improvement in fit to the leukaemia incidence data when a threshold is incorporated in a linear-quadratic relative risk model, albeit at borderline levels of statistical significance (two-sided p=0.04). Little and Muirhead [L7, L43] examined the three radiogenic leukaemia subtypes (AML, ALL, CML), as well as the principal solid cancer sites in the incidence data, and found that, apart from leukaemia, only for non-melanoma skin cancer was there evidence of a threshold (at about 1 Sv); this last finding was reinforced by a more detailed examination of this cancer type [L30]. The evidence for there being a significant excess risk of non-melanoma skin cancer at relatively low doses (<1 Gy) in other (Caucasian) populations [S31] may indicate the limited relevance of these findings in the Japanese data to Western populations with respect to this cancer type. Paralleling the analysis of Little and Muirhead [L7, L43]. Hoel and Li [H26] also examined the fit of linear-threshold models to a number of solid cancer sites in both the Japanese incidence and mortality data and found that for none of the cancer sites was there evidence that incorporation of a threshold significantly improved the fit.

500. It is well recognized that errors in the estimates of dose can substantially alter the shape of the dose-response relationship and hence the evidence both for a dose response and also for any possible curvature in that dose response. The problem of random dosimetric errors for the Radiation Effects Research Foundation (RERF) data has been previously investigated by Jablon [J5], Gilbert [G2], Pierce et al. [P7] and Pierce and Vaeth [P1]. Such random errors in doses were taken into account in the analysis of the tumour incidence and mortality data by Little and Muirhead [L7, L32, L44], but not by Hoel and Li [H26]. The issue of dosimetric errors in epidemiological data is considered in Annex I, *"Epidemiological evaluation of radiation-induced cancer"*.

501. There are certain technical problems associated with use of threshold models. In general, the asymptotic (χ^2) distribution of the deviance difference statistic used for significance tests is not guaranteed, because of a lack of sufficient smoothness in the likelihood as a function of the dose threshold parameter [S30]. This problem is circumvented by the likelihood-averaging techniques used to take account of dosimetric errors in the analysis by Little and Muirhead [L7, L43, L44]; this problem is not addressed in the analyses of Hoel and Li [H26].

502. Other subtle problems affect the interpretation of the results of both Hoel and Li [H26] and Little and Muirhead [L7, L43, L44]. These problems are connected with the use of the grouped form of the data, and in particular the grouped dose categories, in the publicly available forms of both the Japanese incidence and mortality data sets. A likelihood-averaging technique used to take account of dosimetric errors [L7, L43, L44] is one possible way around this problem. Little and Muirhead [L7, L43, L44], following the methodology of Pierce et al. [P7], evaluated the average, for a given "nominal" dose, d, of the relative risk, RR(i,D), evaluated at the "true" dose D: $\mathrm{Avg}[RR(i,D)|d]$. The data set used was in grouped form, the strata being defined in each case by the variables city, sex, age at exposure, time since exposure, and dose. (In the mortality data set [P2], there is additional stratification by attained age.) For each such stratum, i, the average "nominal" dose was available for the persons in that stratum $\mathrm{Avg}_i[d]$. Ideally one should calculate for each strata $\mathrm{Avg}_i[\mathrm{Avg}[RR(i,D)|d]]$, i.e. the average of $\mathrm{Avg}[RR(i,D)|.]$ over all individuals in the stratum i. It was not possible to calculate this quantity using the grouped data that were publicly available, so that in the analyses of Little and Muirhead [L7, L43, L44] the quantity $\mathrm{Avg}[RR(i,D)|\mathrm{Avg}_i[d]]$

Table 16
Leukaemia cases and deaths in various dose groups in the Japanese atomic bomb survivor incidence and mortality data

Bone marrow dose group [a]	*Number of cases*	
	Incidence [b] *[P3]*	*Deaths* [c] *[P2]*
0.0-0.1	128	125
0.1-0.2	8	11
0.2-0.5	27	24
0.5-1.0	24	22
1.0-1.5	19	16
1.5-2.0	8	9
2.0-3.0	17	18
3.0-4.0	2	7
≥4.0	4	4
Total	237	236

a In the incidence data a neutron relative biological effectiveness (RBE) of 1 is used to calculate the neutron component of bone marrow dose (in Gy), for the mortality data a neutron RBE of 10 is used to calculate dose (in Sv).
b All leukaemia cases over the years 1950-1987.
c All leukaemia deaths over the years 1950-1987.

was evaluated, i.e. the value of Avg[RR(i,D)|.] evaluated at the average "nominal' dose ($Avg_i[d]$) within stratum i. Even for linear dose-response models there can be differences between Avg[RR(i,D)|Avg_i[d]] and Avg_i[Avg[RR(i,D)|d]]. As shown by Little and Muirhead [L43], at least when 35% dosimetric errors were assumed, this approximation did not introduce appreciable errors for the optimal linear-quadratic-threshold model for leukaemia; errors were at most 5% [L43].

503. Although the errors introduced by this approximation were small, nevertheless they may be sufficiently great to question the analyses of Little and Muirhead [L7, L43] and Hoel and Li [H26]. As discussed previously, one of the main findings of the analysis of leukaemia incidence among the Japanese atomic bomb survivors by Little and Muirhead [L7] was that incorporation of a threshold in the linear-quadratic model yielded an improvement in fit at borderline levels of statistical significance (best estimate of threshold for a linear-quadratic-threshold model was 0.12 Sv, 95% CI: 0.01–0.28; two-sided p=0.04). In contrast, the fits of a linear-quadratic-threshold model to the mortality data by the same authors demonstrated that the threshold was not significantly different from zero (best estimate of threshold for a linear-quadratic-threshold model was 0.09 Sv, 95% CI: <0.00–0.29; two-sided p=0.16) [L44]. Comparison of the leukaemia incidence and mortality data in Figure XXVIII and Table 16 demonstrates their similarity. Little and Muirhead [L44] concluded that the most probable reason for the difference between the reported findings in the incidence and mortality data sets was the finer subdivision of dose groups in the mortality data set. (There are 14 dose groups in the mortality data sets in a publicly available form, compared with 10 dose groups in the incidence data sets.)

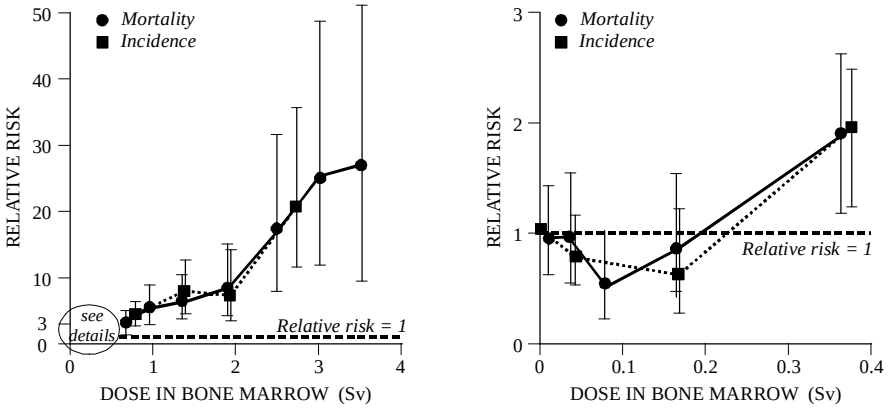

Figure XXVIII. Relative risk of leukaemia in survivors of the atomic bombings [L44].
The diagram on the right shows the low-dose region in detail.

504. Substantial low-dose curvilinearity in dose response has been observed for skin cancer in some human populations [L30] (although not in all [S31, R15]) and in CBA/CaH mice [P8], which is consistent either with a threshold or with a power of dose substantially greater than 2. Low-dose curvature, consistent with a quadratic-exponential dose response, has also been observed for myeloid leukaemia in CBA mice [M4, D11]. For a specific endpoint a threshold dose response,

or something approximating to it (e.g. a dose response proportional to some high power (>2) of dose), might be expected if radiation must hit a large number of targets relevant to that endpoint, for example in order to inactivate a critical number of cells in a particular tissue [E9]. In particular, there are grounds for believing that this might be the case for induction of cataract, sterility, *in utero* severe mental retardation and various other deterministic effects [E9]. There is substantial evidence that oncogenesis arises from damage to a single cell, and in particular from damage to the genetic material in a cell, as reviewed by UNSCEAR [U3], in Chapter IV of the present Annex and discussed by Little and Muirhead [L7]. Given the evidence that single tracks of all types of ionizing radiation can induce a variety of damage, including DNA double-strand breaks [C15, G10, G12], a dose threshold for cancer induction is judged to be unlikely but cannot be excluded formally. These mechanistic issues have been discussed in depth in Chapter IV. The finding of a significant excess leukaemia risk in various occupationally exposed groups [C20], in which total doses generally are administered in an episodic manner, and also among those exposed to small doses (<0.02 Sv) of x-irradiation *in utero* [K23], provide further evidence that argues against a low dose threshold in the leukaemia dose response.

505. In conclusion, although there is evidence at borderline levels of statistical significance for threshold departures from linear-quadratic curvature for leukaemia incidence in the Japanese atomic bomb survivor data, the grouped nature of the Japanese data make inferences on a possible dose threshold problematic. Based on the most current analysis of the mortality data [L44], there is no evidence for a threshold departure from linear-quadratic curvature for leukaemia in the Japanese atomic bomb survivor data, nor is there for any other cancer type, with the possible exception of non-melanoma skin cancer. In arriving at these conclusions the Committee recognizes the uncertainties that attach to current modelling approaches to cancer risk and the shape of the dose-response relationship.

D. SUMMARY

506. The classical multi-stage model of Armitage and Doll and the two-mutation model of Moolgavkar, Venzon, and Knudson, and various generalizations of them also, are capable of describing, at least qualitatively, many of the observed patterns of excess cancer risk following ionizing radiation exposure. However, there are certain inconsistencies with the biological and epidemiological data for both the multi-stage and two-mutation models. In particular, there are indications that the two-mutation model is not totally suitable for describing the pattern of excess risk for solid cancers that is often seen after exposure to ionizing radiation, although leukaemia may be better fitted by this type of model. Generalized MVK models which require three or more mutations are easier to reconcile with biological and epidemiological data relating to solid cancers. Firm statements on the relative validity of different biologically based models of radiation tumorigenesis must await further developments. In general, however, it appears that those models retaining biologically realistic parameters while providing satisfactory fits to the data tend to require radiation action at the early stage of tumorigenesis. This feature is consistent with the conclusions reached in Chapter IV following review of mechanistic data. At the same time it is recognized that the optimal solutions in such modelling can often depend on initial assumptions made on the role of radiation-induced damage in complex multi-stage tumorigenic processes. Some influence of radiation on the later stages of tumorigenesis should be anticipated, particularly perhaps with respect to protracted exposures.

507. Although there is evidence at borderline levels of statistical significance for threshold departures from linear-quadratic curvature for leukaemia incidence data in the Japanese atomic bombings, the grouped nature of the Japanese data make inferences on a possible dose threshold problematic. Based on the most current analysis of the mortality data, there is no evidence for a threshold departure from linear-quadratic curvature for leukaemia in the Japanese atomic bomb survivor data, nor is there for any other cancer type, with the possible exception of non-melanoma skin cancer. Thus, as is the case for data from animal, cellular and molecular studies, evidence from the modelling of epidemiological data tends to favour the view that, in general, cancer risk at low doses rises as a simple function of dose. It is recognized, however, that, at present, the descriptions of dose-effect relationships that have been published are principally qualitative in nature and the choice of models for the quantitative estimation of risk remains to be satisfactorily resolved. Substantial uncertainties attach to the true form of these dose-response relationships and the extent to which they are determined by biological assumptions. The Committee is supportive of further work aimed at the further development and validation of these biologically-based models; it is believed that they will have an important role in the future work of the Committee.

SUMMARY AND CONCLUSIONS

508. A number of considerations are important in determining the risks of exposures to radiation at low doses and low dose rates. These include (a) analysis of epidemiological and experimental studies to determine the lowest doses at which, for statistical and methodological reasons, radiation effects are directly observable; (b) examination of the shape of the dose-response relationships in the low-dose region using available epidemiological and experimental data; and (c) assessment of the possibilities for extrapolation to lower levels of dose based on an understanding of the mechanisms involved in radiation response. The aim of this Annex has been to provide an overview of the data available on the relationship between radiation exposure and the induction of cancer and hereditary disease, with emphasis on the limits of detection of effects at low doses of low-LET radiation and the associated uncertainties. This information, coupled with the understanding to date of mechanisms of damage to cells and tissues, provides a basis for reasoned judgements to be made about the likely form of the dose response at exposures below those at which direct information is available.

509. ***DNA damage.*** It is generally recognized that damage to DNA in the nucleus is the main initiating event by which radiation causes long-term damage to organs and tissues of the body. Double-strand breaks in DNA are generally regarded as the most likely candidate for causing the critical damage. Single radiation tracks have the potential to cause double-strand breaks and in the absence of 100% efficient repair could result in long-term damage, even at the lowest doses, although with a low probability. Damage to other cellular components (epigenetic changes) may influence the functioning of the cell and progression to the malignant state.

510. ***Direct observations.*** Studies of cellular systems, animal experiments and human epidemiological investigations provide direct and relatively consistent evidence of linear or linear-quadratic dose-response relationships at high to intermediate levels of dose and dose rate. However, all such studies are hampered by statistical limitations in providing clear indications of effects at acute doses much less than about 100 mGy (low-LET). Epidemiological studies at low doses are also subject to uncertainties due to methodological issues related to bias and confounding that can limit interpretation of the data. Some exceptions are the induction of cancer following irradiation *in utero* for which an increase in risk has been observed at doses of about 10–20 mGy, experimental data on mouse hair mutations at 10 mGy, and unstable chromosomal aberrations at 20 mGy. In the case of high-LET radiation, the experimental data on cellular damage generally indicate a linear dose-response relationship.

511. For the induction of unstable chromosome aberrations and mutations, a small priming dose of low-LET radiation can sometimes reduce the effect caused by a subsequent higher dose. This adaptive response seems to be a consequence of stimulating the expression/production of genes/proteins in cells involved in DNA damage response and takes a few hours to become effective. Such adaptive responses appear to be transient and have also been observed for cell transformation.

512. Animal studies are valuable for determining the shapes of dose-response relationships and examining how the biological and physical conditions of exposure may influence radiation responses. For many tumour types, the dose response following exposure to both low- or high-LET radiation can be reasonably well represented by a linear or linear-quadratic function. In many cases, however, alternative fits to the data are also possible. Other model fits include the possibility of a threshold dose below which tumours do not occur, as well as more complex functions in which the time for the tumour to appear is much later at low dose rates, which can also suggest the presence of a threshold for response. Animal studies do not, and probably cannot, provide direct information at acute doses much less than about 100 mGy. Values for the lowest doses to give a significant increase in tumour yield following chronic irradiation are generally higher than those for acute irradiation.

513. For radiation-induced hereditary disease, the most comprehensive information comes from measurements of specific locus mutations in mouse spermatogonia. The dose-response relationship for low-dose exposures from low-LET radiation is well fitted by a linear response. The lowest dose tested in these studies was 380 mGy (low-LET). The incidence of mutations in male mice falls by a factor of about three for a reduction in dose rate from 800–900 mGy min^{-1} to 0.007 mGy min^{-1}. This suggests that a substantial fraction of the damage to DNA that results in the induction of heritable mutations is not amenable to effective repair.

514. Epidemiological studies provide a substantial amount of direct quantitative data on the risks of cancer in humans following radiation exposure. The main source of information is the Japanese Life Span Study (LSS), which gives information on the effects of whole-body irradiation following exposure at different ages. The follow-up study indicates a significant ($p=0.05$) increase in the risk of radiation-induced fatal solid cancers in the 0–50 mSv dose range.

515. The dose-response relationship for mortality from leukaemia has been fitted by a linear-quadratic function, while for all solid cancers taken together, a linear dose response provides a best fit for the data for doses up to about 3 Sv. A linear dose response can also be fitted to the data for a number of individual tumour types. There are a number of cancers that have not been significantly increased, including those of the rectum, bone, prostate and testes. Further follow-up and better information on the doses received will be needed before the shape of the dose response for both morbidity and mortality can be determined with confidence at doses below about 100 mSv.

516. Dose-response data from a number of other epidemiological studies can also be fitted with a linear or

linear-quadratic dose response at doses up to a few gray, but alternative relationships have also been obtained. Thus, in radium dial painters exposed to the alpha emitters $^{226/228}$Ra, the best fit to the data on bone tumour induction can be obtained with a model indicating a "practical threshold". Data are also available on an increased risk of bone sarcomas in patients given ^{224}Ra. It has been proposed that some of these tumours would be expected to arise only in tissue with deterministic radiation damage and only above a threshold dose. Similar conclusions have been drawn for the bone tumours arising in the radium dial painters. For exposure to radon and its decay products a constant-relative-risk model without any modifying factors, such as attained age and exposure rate, appear to give a good fit to the data at low doses.

517. Data on patients irradiated for medical reasons are generally consistent with a linear dose-response relationship at doses below a few gray. Results suggest a statistically significant increase in the risk of thyroid cancer at external radiation doses above about 100 mGy received in childhood.

518. A number of studies provide information on the risk of childhood cancer following obstetric radiography at low doses. A statistically significant, 40% increase in the relative risk of leukaemia and other childhood cancers (up to 15 years of age) has been seen following doses in the 10–20 mGy (low-LET) range. The principal reason for being able to determine this increase in risk, which in absolute terms is modest, is the low background incidence of cancer in childhood.

519. Data on the effects of low-dose, chronic exposure in radiation workers are generally consistent with results obtained from the high-dose-rate studies on leukaemia induction, although having wide statistical uncertainties. A longer period of follow-up and pooling of data from different studies will, however, be necessary if information on the slope of the dose-response relationship is to be obtained.

520. Some data are available on the risks of cancer in areas of high natural background. Comparative studies of groups exposed to different levels of natural background radiation do not have the statistical power to detect predicted effects on cancer incidence. Generally, there are substantial difficulties in interpreting the data because of uncertainties in the doses actually received, geographical variation in the accuracy of cancer diagnoses, and confounding by environmental factors.

521. ***Mechanistic considerations.*** Proto-oncogenes and tumour-suppressor genes control a complex array of biochemical pathways involved in cellular signalling and interaction, growth, mitogenesis, apoptosis, genomic stability, and differentiation. Mutation of these genes can, in an often pleiotropic fashion, compromise these controls and contribute to the multi-stage development of neoplasia.

522. On the basis of accumulating knowledge it is argued that early gain-of-function proto-oncogene activation by chromosomal translocation is often associated with the development of human lympho-haemopoietic neoplasia, although gene loss is not infrequent. For many solid tumours there is a requirement for loss of function mutation of tissue-specific tumour-suppressor genes that act as cellular gatekeepers. It has also been proposed that the subsequent onset of spontaneous genomic instability via further clonal mutation is a critical event in neoplastic conversion from a benign to a malignant phenotype. Loss of apoptotic control is also believed to be an important feature throughout neoplastic development.

523. Much information on multi-stage tumorigenesis still remains to be learned. Although the concept of sequential and interacting gene mutations as the driving force for neoplasia is more firmly established, there is insufficient understanding of the complex physiological interplay between these events and the consequences for cellular behaviour and tissue homeostasis.

524. Uncertainty also surrounds the degree to which non-mutational (epigenetic) changes to the genomes of neoplastic cells contribute to tumorigenesis. Increases in the methylation status of critical tumour-suppressor genes is known to be an alternative to mutational inactivation in a range of neoplasms, and loss of methylation imprints may also serve to increase the activity of some growth-promoting genes. DNA methylation is also believed to be involved in genomic imprinting processes. Loss of such imprinting may be important in a number of tumour types. New evidence also implicates histone acetylation in genomic heterochromatization and gene silencing; this process is suggested to be a potentially important contributor to epigenetic change. Epigenetic processes (bystander effects and induced genomic instability) have been shown to influence certain aspects of cellular response *in vitro*. The relevance of these poorly understood processes to *in vivo* tumour induction at low doses of radiation remains to be established.

525. Studies have clarified the role of specific gene mutations in tumours that serve to destabilize the genome, thereby allowing for the accelerated spontaneous development of clonal heterogeneity and tumour progression. Although critical evidence is lacking, it is possible to envisage that after this transition point is reached, tumour development may be relatively independent of exogenously induced DNA damage. Cellular selection during neoplastic development is judged to be of crucial importance at all stages of tumorigenesis. Overall it is judged that most tumours have their origin in gene/chromosomal mutations affecting single target stem-like cells in tissues.

526. Direct evidence on the nature of radiation-associated initiating events in human tumours is sparse, and rapid progress in this area should not be anticipated. By contrast, good progress is being made in resolving early events in radiation-associated tumours in mouse models. These molecular observations strengthen the view expressed in the UNSCEAR 1993 Report [U3] that radiation-induced tumorigenesis will tend to proceed via gene-specific losses; a contribution from early arising epigenetic events should not, however, be discounted.

527. Neoplastic development is subject to a large number of cellular constraints, which provide a high level of protection against neoplastic growth and development. Principal of these are control of cellular proliferation/genomic stability, the induction of apoptosis, and terminal differentiation to a non-proliferative cellular state. For at least certain tumour types there is evidence that immunosurveillance mechanisms can recognize and restrict the growth of neoplastic cells. In spite of these constraints, resistance to or tolerance of all these countermeasures can be developed via gene-specific mutation. On the basis of current molecular genetic knowledge, different modes of *in vivo* constraint are unlikely to apply to spontaneously arising and radiation-induced tumours.

528. Much information points to the crucial importance of DNA repair and other damage-response functions in tumorigenesis. DNA damage response functions influence the appearance of initial events in the multi-stage process, and reduce the probability that a benign neoplasm will spontaneously acquire the secondary mutations necessary for full malignant development. Thus, mutations of DNA damage-response genes in tumours play an important role in the spontaneous development of genomic instability.

529. The repair of sometimes complex DNA double-strand lesions is largely error-prone, and is an important determinant of dose, dose rate, and radiation quality effects in cells. Uncertainties continue to surround the significance to tumorigenesis of adaptive responses to DNA damage; the mechanistic basis of such responses has yet to be well characterized although associations with the induction of biochemical stress responses seems likely. Recent scientific advances highlight the differences in complexity and reparability between spontaneously arising and radiation-induced DNA lesions. These data argue against basing judgements concerning low-dose response on comparisons of overall lesion abundance rather than their nature.

530. ***Biological uncertainties and dose-response models.*** Evidence suggesting the predominance of error-prone repair of radiation damage to cellular DNA has grown, implying that mutational/ tumorigenic risk should be expected at low doses. Important uncertainties remain, however, on whether error-free DNA repair might apply at very low doses, although there are some arguments against it.

531. There are also uncertainties about whether radiation-induced non-mutational (epigenetic) events, such as induced genomic instability, contribute significantly to tumour risk. The dose-response characteristics of such events are obscure, and there is no way to judge the ensuing risk at low doses, if indeed it exists. The involvement of such processes cannot be inferred solely on the basis of the frequency of phenotypic effects after radiation.

532. Since tumorigenic processes are highly complex, attention is drawn to the problems of judging the shape of the low-dose response on data sets that are over-reliant on high-dose estimates of effect. Apparently simple dose-response relationships may disguise competing processes that have different dose dependencies.

533. In spite of these uncertainties the weight of evidence from fundamental studies favours the mutagenic action of radiation acting primarily at a very early stage of tumorigenesis (initiation), with risk rising as a function of dose. Thus the risk of developing malignant tumours should follow the dose response for initiating lesions unless there are dose-dependent effects on the later phases of tumorigenesis.

534. ***Computational modelling of tumorigenesis.*** The different characteristics of empirical and biologically based models of radiation tumorigenesis have been considered by the Committee. The classical multi-stage model of Armitage and Doll and the two-mutation model of Moolgavkar, Venzon, and Knudson, and various generalizations of both, are capable of describing, at least qualitatively, many of the observed patterns of excess cancer risk following ionizing radiation exposure. However, different solutions have been obtained by different investigators and there are certain inconsistencies with the biological and epidemiological data for both the multi-stage and two-mutation models. Generalized MVK models that require three or more mutations are easier to reconcile with biological and epidemiological data relating to solid cancers.

535. Although there is evidence at borderline levels of statistical significance for threshold departures from linear-quadratic curvature for leukaemia incidence in the Japanese atomic bomb survivor data, the grouped nature of the Japanese data make inferences on a possible dose threshold problematic. The most current analysis of the mortality data provides no evidence for a threshold departure from linear-quadratic curvature for leukaemia in the Japanese atomic bomb survivor data, nor is there evidence of this for any other cancer type, with the possible exception of non-melanoma skin cancer.

536. ***Conclusions.*** DNA is the principal target for the initiation of radiation-induced cancer and for radiation-induced hereditary disease. Experimental studies of the effects of ionizing radiation on cellular systems, including the induction of chromosome aberrations, cell transformation and somatic mutations are of value for providing information on damage to DNA. The data obtained have been generally consistent with a linear or linear-quadratic dose response at exposures below those at which cell killing becomes significant (a few gray). In general, significant radiation effects can be detected at doses of about 100 mGy (low-LET) and above, although there are some experimental systems for which effects at lower doses have been observed. In the case of high-LET radiation, the experimental data generally indicate a linear dose-response relationship in the absence of cell killing.

537. For most tumour types in experimental animals and in man a significant increase in risk is only detectable at doses above about 100 mGy. An exception is for human exposures *in utero* when a significant increase in tumour induction in children has been found for doses in the 10–20 mGy range (low-LET). No such excess was observed in the studies of Japanese atomic bomb survivors irradiated *in utero*.

538. In both experimental animals and in humans the dose-response data for tumour induction can be frequently fitted by a linear or linear-quadratic dose response at doses below a few gray. There is evidence though that for some cancer types this form of response does not apply and there may be a practical threshold for a response. Other forms of dose response can also be fitted for the induction of some tumour types.

539. With respect to direct observations of radiation effects, which all carry statistical and/or methodological uncertainty, there are no circumstances where it is scientifically valid to equate the absence of an observable biological effect with the absence of risk.

540. Although mechanistic uncertainty remains, studies on DNA repair and the cellular/molecular processes of radiation tumorigenesis provide no good reason to assume that there will be a low-dose threshold for the induction of tumours in general. However, curvilinearity of the dose response in the low-dose region, perhaps associated with biochemical stress responses and/or changing DNA repair characteristics, cannot be excluded as a general feature. The mechanistic modelling of radiation tumorigenesis is at a relatively early stage of development, but the data available tend to argue against a dose threshold for most tumour types.

541. Until the above uncertainties on low-dose response are resolved, the Committee believes that an increase in the risk of tumour induction proportionate to the radiation dose is consistent with developing knowledge and that it remains, accordingly, the most scientifically defensible approximation of low-dose response. However, a strictly linear dose response should not be expected in all circumstances.

542. The dose response for the induction of heritable disease carries fewer low-dose biological uncertainties than that of multi-stage tumorigenesis, but the same uncertainties surrounding DNA damage response remain; an increase in the risk of germ-cell mutation that is proportionate to radiation dose is judged to be a scientifically reasonable approximation for the induction of heritable effects at low doses.

543. The Committee recognizes that ongoing and future studies in epidemiology and animal sciences, while remaining of great importance for quantitative risk assessment, will not resolve the uncertainties surrounding the effects in humans of low-dose radiation. Accordingly, there will be an increasing need for weight-of-evidence judgements based on largely qualitative data from cellular/molecular studies of the biological mechanisms that underlie health effects; the provision of such judgements demands strong support from biologically validated computational models of risk. With ever-improving experimental technology, fundamental knowledge will continue to grow. On this basis, the Committee emphasizes the need for further work on the mechanisms of DNA damage response/cellular stress and studies of the consequences of these responses for neoplastic development. Current uncertainties on the role of epigenetic factors, such as bystander effects and induced genomic instability, are expected to be reduced, but it may remain difficult to estimate their overall contribution to risk. However, the development of mechanistic models of radiation risk demands more than a simple improvement in the understanding of cellular/molecular processes. Issues such as target-cell identity/multiplicity; the kinetics of pre-neoplastic clonal development; and rates of cell mutation, clonal proliferation, differentiation, and apoptosis, as well as the pattern of energy deposition in critical cellular targets, all need to be better understood in order to define biological parameters for use in the biological modelling of tumorigenesis. These are difficult areas of research, and it is not easy to anticipate the rate of progress. In spite of such experimental difficulties, the Committee believes that advances in computational modelling of the physical and biological aspects of radiation tumorigenesis will provide an essential tool for estimating radiation risk at low doses and low dose rates.

References

A1 Armitage, P. and R. Doll. The age distribution of cancer and a multi-stage theory of carcinogenesis. Br. J. Cancer 8: 1-12 (1954).

A2 Arnheim, N. and D. Shibata. DNA mismatch repair in mammals: role in disease and meiosis. Curr. Opin. Genet. Dev. 3: 364-370 (1997).

A3 Alexander, P. Do cancers arise from a single transformed cell or is monoclonality of tumours a late event in carcinogenesis? Br. J. Cancer 51: 453-457 (1985).

A4 Abelson, P.H. Risk assessments of low-level exposures. Science 265: 1507 (1994); *also* Correspondence. Science 266: 114-113 (1994).

A5 Albert, R.E., F.J. Burns and P. Bennett. Radiation-induced hair follicle damage and tumour formation in mouse and rat skin. J. Natl. Cancer Inst. 49: 1131-1137 (1972).

A6 Abramowitz, M. and I.A. Stegun. Handbook of Mathematical Functions. National Bureau of Standards, Washington, D.C., 1964.

A7 Armitage, P. and R. Doll. A two-stage theory of carcinogenesis in relation to the age distribution of human cancer. Br. J. Cancer 9: 161-169 (1957).

A8 Albert, M.L., J. Darnell, A. Bender et al. Tumour-specific killer cells in paraneoplastic cerebellar degeneration. Nature Med. 4: 1321-1325 (1998).

A9 Académie des sciences. Problèmes liés aux effets des faibles doses de radiations ionisantes. Rapport No. 34, Tec Doc, Paris (1995).

A10 Albertini, R.J., L.S. Clark, J.A. Nicklas et al. Radiation quality affects the efficiency of induction and the molecular spectrum of *HPRT* mutations in human T cells. Radiat. Res. 148: S76-S86 (1997).

A11 Amundson, S.A., F. Xia, K.B. Wolfson et al. Different cytotoxic and mutagenic responses induced by X-rays in two human lymphoblastoid cell lines derived from a single donor. Mutat. Res. 286: 233-241 (1993).

A12 Amundson, S.A., D.J. Chen and R.T. Okinaka. Alpha particle mutagenesis of human lymphoblastoid cell lines. Int. J. Radiat. Biol. 70(2): 219-226 (1996).

A13 Adams, L.M., S.P. Ethier and R.L. Ullrich. Enhanced *in vitro* proliferation and *in vivo* tumorigenic potential for mammary epithelium from BALB/c mice exposed *in vivo* to γ-radiation and/or 7,12-dimethylbenz(a)anthracene. Cancer Res. 47: 4425-4431 (1987).

A14 Ames, B.N. Endogenous DNA damage as related to cancer and ageing. Mutat. Res. 214: 41-46 (1989).

A15 Albert, R.E., F.J. Burns and R.D. Heimbach. The effect of penetration depth of electron radiation on skin tumour formation in the rat. Radiat. Res. 30: 515-524 (1967).

A16 Aghamohammadi, S.Z, D.T. Goodhead and J.R.K. Savage. Induction of sister chromatid exchanges (SCE) in G lymphocytes by plutonium-238 α-particles. Int. J. Radiat. Biol. 53: 909-915 (1988).

A17 Ashmore, J.P., D. Krewski, J.M. Zielinski et al. First analysis of mortality and occupational radiation exposure based on the National Dose Registry of Canada. Am. J. Epidemiol. 148: 564-574 (1998).

A18 Amundson, S.A., K.T. Do and A.J. Fornace. Induction of stress genes by low doses of gamma rays. Radiat. Res. 152: 225-231 (1999).

A19 Azzam, E., S. de Toledo, T. Gooding et al. Inter-cellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles. Radiat. Res. 159: 497-504 (1998).

A20 Azzam, E.I., G.P. Raaphorst and R.E.J. Mitchel. Radiation-induced adaptive response for protection against micronucleus formation and neoplastic transformation in C3H 10T1/2 mouse embryo cells. Radiat. Res. 138: S28-S31 (1994).

A21 Azzam, E.I., S.M. de Toledo, G.P. Raaphorst et al. Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells. Radiat. Res. 146: 369-373 (1996).

B1 Barlow, C., S. Hirotsume, R. Paylor et al. *Atm*-deficient mice: a paradigm of ataxia-telangiectasia. Cell 86: 159-171 (1996).

B2 Barendsen, G.W. Do fast neutrons at low dose rate enhance cell transformation *in vitro*? A basic problem of micro-dosimetry and interpretation. Int. J. Radiat. Biol. 47: 731-734 (1985).

B3 Barendsen, G.W. Physical factors influencing the frequency of radiation induced transformation of mammalian cells. p. 315-324 in: Cell Transformation and Radiation Induced Cancer (K.H. Chadwick et al. eds.). Adam Hilger, Bristol, 1989.

B4 Bouffler, S.D., C.J. Kemp, A. Balmain et al. Spontaneous and ionizing radiation-induced chromosomal abnormalities in *p53*-deficient mice. Cancer Res. 55: 3883-3889 (1995).

B5 Boice, J.D., N.E. Day, A. Andersen et al. Second cancers following radiation treatment for cervical cancer. An international collaboration among cancer registries. J. Natl Cancer Inst. 74: 955-975 (1985).

B6 Boice, J.D., D.L. Preston, F.G. Davis et al. Frequent chest x-ray fluoroscopy and breast cancer incidence among tuberculosis patients in Massachusetts. Radiat. Res. 125: 214-222 (1991).

B7 Boice, J.D., M. Blettner, R.A. Kleinerman et al. Radiation dose and leukemia risk in patients treated for cancer of the cervix. J. Natl. Cancer Inst. 79: 1295- 1311 (1987).

B8 Bithell, J.F. and A.M. Stewart. Prenatal irradiation and childhood malignancy: a review of British data from the Oxford Survey. Br. J. Cancer 35: 271-287 (1975).

B9 Bishop, J.M. Molecular themes in oncogenesis. Cell 64: 235-248 (1991).

B10 Boland, C.R., J. Sato, H.D. Appelman et al. Microallelotyping defines the sequence and tempo of allelic losses at tumour suppressor gene loci during colorectal cancer progression. Nature Med. 1: 902-909 (1995).

B11 Balmain, A. Exploring the bowels of DNA methylation. Curr. Biol. 5: 1013-1016 (1995).

B12 Barlow, D.P. Gametic imprinting in man. Science 270: 1610-1613 (1995).

B13 Borrow, J., V.P. Stanton, J.M. Andersen et al. The translocation t(18;16) (p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. Nature Genet. 14: 33-41 (1996).

B14 Bongarzone, I., M.G. Butti, L. Fugazzola et al. Comparison of the breakpoint regions of *ELE1* and *RET* genes involved in the generation of *RET/PTC3* oncogene in sporadic and in radiation-associated papillary thyroid carcinomas. Genomics 42: 252-259 (1997).

B15 Breckon, G., D. Papworth and R. Cox. Murine radiation myeloid leukaemogenesis: a possible role for radiation sensitive sites on chromosome 2. Genes, Chromosome, Cancer 3: 367-375 (1991).

B16 Bouffler, S.D., E.I.M. Meijne, D.J. Morris et al. Chromosome 2 hypersensitivity and clonal development in murine radiation acute myeloid leukaemia. Int. J. Radiat. Biol. 72: 181-189 (1997).

B17 Bryan, T.M., A. Englezou, L. Dalla-Pozza et al. Evidence for an alternate mechanism for maintaining telomere length in human tumours and tumour-derived cell lines. Nature Med. 3: 1271-1274 (1997).

B18 Beverley, P. Cell mediated immune responses to cancer. p. 311-329 in: Introduction to the Cellular and Molecular Biology of Cancer (L.M. Franks and N.M. Teich, eds.). Oxford University Press, Oxford, 1997.

B19 Branch, P., D.C. Bicknell, A. Rown et al. Immune surveillance in colorectal carcinoma. Nature Genet. 9: 231-232 (1995).

B20 Bouffler, S.D., E.I.M. Meijne, R. Huiskamp et al. Chromosomal abnormalities in neutron-induced acute myeloid leukaemias in CBA/H mice. Radiat. Res. 146: 349-352 (1996).

B21 Bhattarcharjee, D. Role of radio-adaptation on radiation-induced thymic lymphoma in mice. Mutat. Res. 358: 231-235 (1996).

B22 Billen, D. Spontaneous DNA damage and its significance for the 'negligible' dose controversy in radiation protection. Radiat. Res. 124: 242-245 (1990).

B23 Burns, F.J. and R.E. Albert. Radiation carcinogenesis in rat skin. p. 199-214 in: Radiation Carcinogenesis (A.C. Upton, R.E. Albert, F.J. Burns et al., eds.). Elsevier, New York, 1986.

B24 Berke, G. The function and mechanism of action of cytolytic lymphocytes. p. 965-1014 in: Fundamental Immunology, 3rd edition (W.E. Paul, ed.). Raven Press, New York, 1993.

B25 Benedict, W.F., A. Banerjee, A. Gardner et al. Induction of morphological transformation in C3H 10T½ cells clone 8 cells and chromosomal damage in hamster A(Tl) Cl-3 cells by cancer chemotherapeutic agents. Cancer Res. 37: 2202-2208 (1977).

B26 Brenner, D.J. and E.J. Hall. The inverse dose-rate effect for oncogenic transformation by neutrons and charged particles: a plausible interpretation consistent with published data. Int. J. Radiat. Biol. 58: 745-758 (1990).

B27 Blank, K.R., M.S. Rudoltz, D.G. Kao et al. Review: the molecular regulation of apoptosis and implications for radiation oncology. Int. J. Radiat. Biol. 71: 455-466 (1997).

B28 Bithell, J.F. Epidemiological studies of children irradiation *in utero*. p. 77-87 in: Low Dose Radiation: Biological Bases of Risk Assessment (K.F. Baverstock and J.W. Stather, eds.). Taylor and Francis, London, 1989.

B29 Balcer-Kubiczek, E.K. and G.H. Harrison. Survival and oncogenic transformation of C3H10T½ cells after extended x irradiation. Radiat. Res. 104: 214-223 (1985).

B30 Balcer-Kubiczek, E.K. and G.H. Harrison. Effect of x-ray dose protraction and a tumour promoter on transformation induction *in vitro*. Int. J. Radiat. Biol. 54: 81-89 (1988).

B31 Bond, V.P., L.E. Feinendegen and J. Booz. What is a "low dose" of radiation? Int. J. Radiat. Biol. 53(1): 1-12 (1988).

B32 Booz, J. and L.E. Feinendegen. A microdosimetric understanding of low-dose radiation effects. Int. J. Radiat. Biol. 53(1): 13-21 (1988).

B33 Borsa, J., M.D. Sargent, M. Einspenner et al. Effects of oxygen and misonidazole on cell transformation and cell killing in C3H10T½ cells by x-rays *in vitro*. Radiat. Res. 100: 96-103 (1984).

B34 Broerse, J.J. Influence of physical factors on radiation carcinogenesis in experimental animals. p. 181-194 in: Low Dose Radiation: Biological Bases of Risk Assessment (K.F. Baverstock and J.W. Stather, eds.). Taylor and Francis, London, 1989.

B35 Bodnar, A.G., M. Onellette, M. Frolkis et al. Extension of life-span by introduction of telomerase into normal human cells. Science 279: 349-352 (1998).

B36 Boecker, B.B. Development and use of biokinetic models for incorporated radionuclides. Radiat. Prot. Dosim. 79: 223-228 (1998).

B37 Batsakis, J.G. Tumours of the Head and Neck. Williams and Wilkins, Baltimore, Maryland, 1996.

B38 Boice, J.D. Carcinogenesis - a synopsis of human experience with external exposure in medicine. Health Phys. 55: 621-630 (1988).

B39 Boice, J.D. and R.J.M. Fry. Radiation carcinogenesis in the gut. p. 291-306 in: Radiation and Gut (C.S. Potten and J.H. Hendry, eds.). Elsevier, Oxford, 1995.

B40 Blocher, D. DNA double-strand break repair determines the RBE of α-particles. Int. J. Radiat. Biol. 54: 761-771 (1988).

B41 Boecker, B.B., F.F. Hahn, B.A. Muggenburg et al. The relative effectiveness of inhaled alpha and beta-emitting radionuclides in producing lung cancer. p. 1059-1062 in: Proceedings IRPA7: 7th International Congress of the International Radiation Protection Association, Sydney, April 1988. Volume 2. Pergamon Press, London, 1988.

B42 Brenner, D.J. The microdosimetry of radon daughters and its significance. Radiat. Prot. Dosim. 31: 399-403 (1990).

B43 Bertrand, P., D. Rouillard, A. Boulet et al. Increase of spontaneous intrachromosomal recombination in mammalian cells expressing a mutant p53 protein. Oncogene 14: 1117-1122 (1997).

B44 Bouffler, S.D., G. Breckon and R. Cox. Chromosomal mechanisms in murine radiation acute myeloid leukaemogenesis. Carcinogenesis 17: 101-105 (1996).

B45 Boice, J.D. Jr. and R.W. Miller. Childhood and adult cancer after intrauterine exposure to ionizing radiation. Teratology 59: 227-233 (1999).

C1 Committee on the Biological Effects of Ionizing Radiations (BEIR V). Health Effects of Exposure to Low Levels of Ionizing Radiation. United States National Academy of Sciences, National Research Council. National Academy Press, Washington, 1990.

C2 Cardis, E., J. Estève and B.K. Armstrong. Meeting recommends international study of nuclear industry workers. Health Phys. 63: 405-406 (1992).

C3 Carpenter, L., C. Higgins, A. Douglas et al. Combined analysis of mortality in three United Kingdom nuclear industry workforces. Radiat. Res. 138: 224-238 (1994).

C4 Canman, C.E. and M.B. Kastan. Three paths to stress relief. Nature 384: 213-214 (1996).

C5 Clark, D.J., E.I.M. Meijne, S.D. Bouffler et al. Microsatellite analysis of recurrent chromosome 2 deletions in acute myeloid leukaemia induced by radiation in F1 hybrid mice. Genes, Chromosome Cancer 16: 238-246 (1996).

C6 Coggle, J.E. Lung tumour induction in mice by x-rays and neutrons. Int. J. Radiat. Biol. 53: 585-598 (1988).

C7 Covelli, V., M. Coppola, V. DiMajo et al. Tumour induction and life shortening in $BC3F_1$ mice at low doses of fast neutrons and x-rays. Radiat. Res. 113: 362-374 (1988).

C8 Covelli, V., V. DiMajo, M. Coppola et al. The dose-response relationships for myeloid leukaemia and malignant lymphoma in $Bc3F_1$ mice. Radiat. Res. 119: 553-561 (1989).

C9 Cox, R. Mechanisms of radiation oncogenesis. Int. J. Radiat. Biol. 65: 57-64 (1994).

C10 Cross, S.H. and A.P. Bird. CpG islands and genes. Curr. Opin. Genet. Dev. 5: 309-314 (1995).

C11 Chao, L-Y., V. Huff, G. Tomlinson et al. Genetic mosaicism in normal tissues of Wilms' tumour patients. Nature Genet. 3: 127-131 (1993).

C12 Cosset, J-M. Secondary cancers after radiotherapy. Radioprotection 32: C1241-C1248 (1997).

C13 Chauveinc, L., M. Ricoul, L. Sabatier et al. Radiation-induced malignant tumours: a specific cytogenetic profile? Radioprotection 32: C1249-C1250 (1997).

C14 Counter, C.M., A.A. Avillon, C.R. Lefeuvre et al. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomere activity. EMBO J. 11: 1921-1929 (1992).

C15 Cox, R., C.R. Muirhead, J.W. Stather et al. Risk of radiation-induced cancer at low doses and low dose rates for radiation protection purposes. Doc. NRPB 6(1): (1995).

C16 Chinnaiyan, A. and V. Dixit. The cell-death machine. Curr. Biol. 6: 555-562 (1996).

C17 Committee on the Biological Effects of Ionizing Radiations (BEIR I). The Effects on Populations of Exposure to Low Levels of Ionizing Radiation. National Academy of Sciences, National Research Council. National Academy Press, Washington, 1972.

C18 Cameron, E.A., K.E. Bachman, S. Myohanen et al. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nature Genet. 21: 103-107 (1999).

C19 Clifton, K.H. Comments on the evidence in support of the epigenetic nature of radiogenic initiation. Mutat. Res. 350: 77-80 (1996).

C20 Cardis, E., E.S. Gilbert, L. Carpenter et al. Effects of low doses and low dose rates of external ionizing radiation: cancer mortality among nuclear industry workers in three countries. Radiat. Res. 142: 117-132 (1995).

C21 Cartwright, R.A., R.J.Q. McNally, D.J. Rowland et al. The Descriptive Epidemiology of Leukaemia and Related Conditions in Parts of the United Kingdom 1984-1993. Leukaemia Research Fund, London, 1997.

C22 Cross, F.T. A review of experimental animal radon health effects data. p. 476-481 in: Radiation Research: A Twentieth-Century Perspective, Vol. II (J.D. Chapman et al., eds.). Academic Press, San Diego, 1992.

C23 Chadwick, K.H., R. Cox, H.P. Leenhouts et al. Molecular mechanisms in radiation mutagenesis and carcinogenesis. EUR 13294 (1994).

C24 Cera, F., R. Cherubini, M. Dalla Vecchia et al. Cell inactivation, mutation and DNA damage induced by light ions: Dependence on radiation quality. p. 191-194 in: Microdosimetry: An Interdisciplinary Approach (D.T. Goodhead, P. O'Neill and H.G. Menzel, eds.). Royal Society of Chemistry, Cambridge, 1997.

C25 Cucinotta, F.A., J.W. Wilson, M.R. Shavers et al. Effects of track structure and cell inactivation on the calculation of heavy ion mutation rates in mammalian cells. Int. J. Radiat. Biol. 69: 593-600 (1995).

C26 Committee on the Biological Effects of Ionizing Radiations (BEIR VI). The Health Effects of Exposure to Indoor Radon. National Academy of Sciences, National Research Council. National Academy Press, Washington, 1999.

C27 Cox, R. and W.K. Masson. Mutation and activation of cultured mammalian cells exposed to beams of accelerated heavy ions. III. Human diploid fibroblasts. Int. J. Radiat. Biol. 36: 149-160 (1979).

C28 Chauveinc, L., M. Ricoul, L. Sabatier et al. Dosimetric and cytogenetic studies of multiple radiation-induced meningiomas for a single patient. Radiother. Oncol. 43: 285-288 (1997).

C29 Chadwick, K.H., G. Moschini and M.M. Varma (eds.). Biophysical Modelling of Radiation Effects. Adam Hilger, Bristol, Philadelphia, New York, 1992.

D1 Darby, S.C., J.H. Olsen, R. Doll et al. Trends in childhood leukaemia in the Nordic countries in relation to fallout from atmospheric nuclear weapons testing. Br. Med. J. 304: 1005-1009 (1992).

D2 Delongchamp, R.R., K. Mabuchi, Y. Yasuhiko et al. Cancer mortality among atomic bomb survivors exposed *in utero* or as young children, October 1950-May 1992. Radiat. Res. 147: 385-395 (1997).

D3 Doll, R. and R. Wakeford. Risk of childhood cancer from fetal irradiation. Br. J. Radiol. 79: 130-139 (1997).

D4 de Bustros, A., D.D. Nelkin, A. Silverman et al. The short arm of chromosome 11 is a "hot spot" for hypermethylation in human neoplasia. Proc. Natl. Acad. Sci. U.S.A. 85: 5693-5697 (1988).

D5 Doll, R. The age distribution of cancer: implications for models of carcinogenesis. J. R. Stat. Soc., Ser. A 132: 133-166 (1971).

D6 Den Otter, W., J.W. Koten, B.J.H. van der Vegt et al. Oncogenesis by mutations in anti-oncogenes: a view. Anticancer Res. 10: 475-488 (1990).

D7 Derkinderen, D.J., O.J. Boxma, J.W. Koten et al. Stochastic theory of oncogenesis. Anticancer Res. 10: 497-504 (1990).

D8 Darby, S.C., R. Doll, S.K. Gill et al. Long term mortality after a single treatment course with X-rays in patients treated for ankylosing spondylitis. Br. J. Cancer 55: 179-190 (1987).

D9 Dove, W.F., R.T. Cormier, K.A. Gould et al. The intestinal epithelium and its neoplasms: genetic, cellular and tissue interactions. Philos. Trans. R. Soc. Lond. 353: 915-923 (1998).

D10 Darby, S.C. The contribution of natural ionizing radiation to cancer mortality in the United States. p. 183-190 in: The Origins of Human Cancer (J. Brugge et al., eds.). Cold Spring Harbour Laboratory Press, New York, 1991.

D11 Di Majo, V., M. Coppola, S. Rebessi et al. Dose-response relationship of radiation-induced harderian gland tumors and myeloid leukemia of the CBA/Cne mouse. J. Natl. Cancer Inst. 76: 955-966 (1986).

D12 Darby, S.C., E. Nakashima and H. Kato. A parallel analysis of cancer mortality among atomic bomb survivors and patients ankylosing with spondylitis given x-ray therapy. J. Natl. Cancer Inst. 75: 1-21 (1985).

D13 Deshpande, A., E.H. Goodwin, S.M. Bailey et al. Alpha particle induced sister chromatid exchange in normal human lung fibroblasts. Evidence for an extracellular target. Radiat. Res. 145: 260-267 (1996).

D14 de Vathaire, F., C. Hardiman, A. Shamsaldin et al. Thyroid carcinoma following irradiation for a first cancer during childhood. Arch. Intern. Med. 159: 2713-2719 (2000).

E1 Evans, R.D. The effect of skeletally deposited alpha-ray emitters in man. Br. J. Radiol. 39: 881-895 (1966).

E2 Edwards, A. Communication to the UNSCEAR Secretariat (1998).

E3 Elledge, R. and W.H. Lee. Life and death by p53. BioEssays 17: 923-930 (1995).

E4 Elgin, S.C.R. and S.P. Jackson (eds). Chromosomes and expression mechanisms. Curr. Opin. Genet. Dev. 7: (1997).

E5 Ellender, M., S.M. Larder, J.D. Harrison et al. Radiation-induced intestinal neoplasia in a genetically-predisposed mouse (*min*). Radioprotection 32: C1-287 (1997).

E6 Elliot, T. Tapping into tumours. Nature Genet. 13: 139-140 (1996).

E7 Evans, H.H., M. Nielsen, J. Mencl et al. The effect of dose rate on x-radiation-induced mutant frequency and the nature of DNA lesions in mouse lymphoma L5178Y cells. Radiat. Res. 122: 316-325 (1990).

E8 Edwards, A.A. The use of chromosomal aberrations in human lymphocytes for biological dosimetry. Radiat. Res. 148: S39-S44 (1997).

E9 Edwards, A.A. and D.C. Lloyd. Risk from deterministic effects of ionising radiation. Doc. NRPB 7(3): 1-31 (1996).

E10 Edwards, A.A., V.V. Moiseenko and H. Nikjoo. On the mechanism of formation of chromosomal aberrations by ionising radiation. Radiat. Environ. Biophys. 35: 25-30 (1996).

E11 Evans, R.D., A.T. Keane, R.J. Kolenkow et al. Radiogenic tumours in the radium and mesothorium cases studied at M.I.T. p. 157-194 in: Delayed Effects of Bone-Seeking Radionuclides (C.W. Mays et al., eds.). University of Utah Press, Salt Lake City, 1969.

E12 Edwards, A.A., R.J. Purrott, J.S. Prosser et al. The induction of chromosome aberrations in human lymphocytes by alpha-radiation. Int. J. Radiat. Biol. 38: 83-91 (1980).

E13 Edwards, A.A., D.C. Lloyd and J.S. Prosser. The induction of chromosome aberrations in human lymphocytes by accelerated charged particles. Radiat. Prot. Dosim. 13: 205-209 (1985).

E14 Edwards, A.A., D.C. Lloyd and J.S. Prosser. The induction of chromosome aberrations in human lymphocytes by 24 keV neutrons. Radiat. Prot. Dosim. 31: 265-268 (1990).

E15 Edwards, A.A., D.C. Lloyd and J.S. Prosser. 38 chromosome aberrations in human lymphocytes - a radiobiological review. p. 423-432 in: Low Dose Radiation: Biological Bases for Risk Assessment (K.F. Baverstock and J.W. Stather, eds.). Taylor and Francis, London, 1989.

E16 Edwards, A.A. Communication to the UNSCEAR Secretariat based on calculations by H. Nikjoo, Medical Research Council (MRC), Radiobiology Unit, Chilton, United Kingdom (2000).

F1 Fearon, E.R. and B. Vogelstein. A genetic model for colorectal tumourigenesis. Cell 61: 759-767 (1990).

F2 Feinberg, A.P. Genomic imprinting and gene activation in cancer. Nature Genet. 4: 110-113 (1993).

F3 Fishel, R. and R.D. Kolodner. The identification of mismatch repair genes and their role in the development of cancer. Curr. Opin. Genet. Dev. 5: 382-395 (1995).

F4 Folkman, J. Angiogenesis in cancer, vascular rheumatoid and other disease. Nature Med. 1: 27-31 (1995).

F5 Foulds, L. Neoplastic Development, Volume 2. Academic Press, New York, 1975.

F6 Ford, A.M., S.A. Ridge, M.E. Cabrera et al. *In utero* rearrangements in the trithorax-related oncogene in infant leukaemias. Nature 363: 358-360 (1993).

F7 Fialkow, P.J. Clonal origin of human tumours. Annu. Rev. Med. 30: 135-143 (1979).

F8 Fearon, E.R., K.R. Cho, J.M. Nigro et al. Identification of a chromosome 18q gene that is altered in colorectal cancers. Science 247: 49-56 (1991).

F9 Ferrara, N. Natural killer cells, adhesion and tumour angiogenesis. Nature Med. 2: 971-972 (1996).

F10 Freedman, V.H. and S. Shin. Cellular tumorigenicity in male mice: correlation with cell growth in semi solid medium. Cell 3: 355-359 (1974).

F11 Finkel, M.P. and B.O. Biskis. Toxicity of plutonium in mice. Health Phys. 8: 565-579 (1962).

F12 Finkel, M.P., B.O. Biskis and P.B. Jinkins. Toxicity of ^{226}Ra in mice in: Radiation Induced Cancer (A. Ericson, ed.). IAEA, Vienna, 1971.

F13 Finkel, A.J., C.E. Miller and R.J. Hasterlik. Radium-induced malignant tumours in man. p. 195-225 in: Delayed Effects of Bone-Seeking Radionuclides (C.W. Mays et al., eds.). University of Utah Press, Salt Lake City, 1969.

F14 Furuno-Fukushi, I. and H. Matsudaira. Mutation induction by different dose rates of γ rays in radiation-sensitive mutants of mouse leukaemia cells. Radiat. Res. 120: 370-374 (1989).

F15 Fry, R.J.M. Time-dose relationship and high-LET radiation. Int. J. Radiat. Biol. 58: 866-870 (1990).

G1 Groupe de travail de l'Académie des Sciences. Problèmes liés aux effets des faibles doses de radiations ionisantes. Rapport No. 34. Académie des Sciences, Paris (1995).

G2 Gilbert, E.S. Some effects of random dose measurement errors on analyses of atomic bomb survivor data. Radiat. Res. 98: 591-605 (1984).

G3 Gilbert, E.S., D.L. Cragle and L.D. Wiggs. Updated analyses of combined mortality data on workers at the Hanford site, Oak Ridge National Laboratory and Rocky Flats weapons plant. Radiat. Res. 136: 408-421 (1993).

G4 Gribben, M.A., J.L. Weeks and G.R. Howe. Cancer mortality (1956-85) among male employees of Atomic Energy of Canada Limited with respect to occupational exposure to external low-linear-energy-transfer ionising radiation. Radiat. Res. 133: 375-380 (1993).

G5 Goodhead, D.T., P. O'Neill and H.G. Menzel (eds.). Microdosimetry: An Interdisciplinary Approach. Royal Society of Chemistry, Cambridge, 1997.

G6 Goodhead, D.T. Biophysical models of radiation-action - introductory review. p. 306-311 in: Proceedings of the 8th International Congress of Radiation Research (Abstract), Edinburgh, 1987, Volume 2 (E.M. Fielden et al. eds.). Taylor and Francis, London, 1987.

G7 Goodhead, D.T. Physical basis for biological effect. p. 37-53 in: Nuclear and Atomic Data for Radiotherapy and Related Radiobiology. STI/PUB/741. IAEA, Vienna, 1987.

G8 Greenblatt, M.S., W.P. Bennett, M. Hollstein et al. Mutations in the p53 tumour suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res. 54: 4855-4878 (1994).

G9 Greider, C.W. Telomere length regulation. Annu. Rev. Biochem. 65: 337-365 (1996).

G10 Goodhead, D.T. Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. Int. J. Radiat. Biol. 65: 7-17 (1994).

G11 Griffiths, D.F.R., P. Sacco and D. Williams. The clonal origin of experimental large bowel tumours. Br. J. Cancer 59: 385-387 (1989).

G12 Goodhead, D.T. and H. Nikjoo. Track structure analysis of ultrasoft x-rays compared to high- and low-LET radiations. Int. J. Radiat. Biol. 55(4): 513-529 (1989).

G13 Granath, F., F. Darroudi, A. Auvinen et al. Retrospective dose estimates in Estonian Chernobyl clean-up workers by means of FISH. Mutat. Res. 369: 7-12 (1996).

G14 Grahn, D. and G.A. Sacher. Fractionation and protraction factors and the late effects of radiation in small mammals. p. 2.1-2.27 in: Proceedings of a Symposium on Dose Rate in Mammalian Radiation Biology (D.G. Brown et al., eds.). CONF-680410 (1968).

G15 Gössner, W., R.R. Wick and H. Spiess. Histopathological review of ^{224}Ra induced bone sarcomas. p. 255-260 in: Health Effects of Internally Deposited Radionuclides: Emphasis on Radium and Thorium (G. van Kaick, A. Karaoglou and A.M. Kellerer, eds.). World Scientific, London, 1995.

G16 Griffith, W.C., B.B. Boecker, N.A. Gillett et al. Comparison of risk factors for bone cancer induced by inhaled $^{90}SrCl_2$ and $^{238}PuO_2$. in: Proceedings EULEP/DoE Joint Bone Radiobiology Workshop, Toronto, July 1991. USDOE Report UCD-472-136 (1991).

G17 Gössner, W. Pathology of radium-induced bone tumours: new aspects of histopathology and histogenesis. Radiat. Res. 152: S12-S15 (1999).

G18 Gray, R.G., J. Lafuma and S.E. Paris. Lung tumours and radon inhalation in over 2000 rats: approximate linearity across a wide range of doses and potentiation by tobacco smoke. p. 592-607 in: Lifespan Radiation Effects Studies in Animals: What Can They Tell Us? (R.C. Thompson and J.A. Mahaffey, eds.). CONF-830951 (1986).

G19 Guarente, L. Diverse and dynamic functions of the Sir silencing complex. Nature Genet. 23: 281-285 (1999).

G20 Grosovsky, A.J. Radiation-induced mutations in unirradiated DNA. Proc. Natl. Acad. Sci. (USA) 96: 5346-5347 (1999).

G21 Grosovsky, A.J., K.K. Parks and S.L. Nelson. Clonal analysis of delayed karyotypic abnormalities and gene mutations in radiation-induced genetic instability. Mol. Cell Biol. 16: 6252-6262 (1996).

G22 Goodhead, D.T. Spatial and temporal distribution of energy. Health Phys. 55(2): 231-240 (1988).

G23 Goodhead, D.T. Microscopic features of dose from radionuclides, particularly emitters of α-particles and Auger electrons. Int. J. Radiat. Biol. 60(3): 550-553 (1991).

H1 Han, A. and M.M. Elkind. Transformation of mouse C3H10T½ cells by single and fractionated doses of x-rays and fission-spectrum neutrons. Cancer Res. 39: 123-130 (1979).

H2 Heidenreich, W.F., P. Jacob, H.G. Paretzke et al. Two-step model for the risk of fatal and incidental lung tumors in rats exposed to radon. Radiat. Res. 151: 209-217 (1999).

H3 Holliday, R. Endless quest. BioEssays 18: 3-5 (1996).

H4 Hart, I.R. and I. Saini. Biology of tumour metastasis. Lancet 339: 1453-1457 (1992).

H5 Holliday, R. Understanding Ageing. Cambridge University Press, Cambridge, 1995.

H6 Hartwell, L.H. and M.B. Kastan. Cell cycle control and cancer. Science 266: 1821-1828 (1994).

H7 Hartwell, L.H., T. Weinert, L. Kadyk et al. Cell cycle checkpoints, genomic integrity, and cancer. Cold Spring Harbor Symp. Quant. Biol. 59: 259-263 (1994).

H8 Heyn, R., V. Haeberlen, W.A. Newton et al. Second malignant neoplasms in children treated for rhabdomyosarcoma. J. Clin. Oncol. 11: 262-270 (1993).

H9 Hall, A. Ras-related proteins. Curr. Opin. Cell Biol. 5: 63-69 (1993).

H10 Hinds, P.W. and R.A. Weiberg. Tumour suppressor genes. Curr. Opin. Genet. Dev. 4: 135-141 (1994).

H11 Haber, D. and E.D. Harlow. Tumour-suppressor genes: evolving definitions in the genomic age. Nature Genet. 16: 320-322 (1997).

H12 Hartwell, L. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. Cell 71: 543-546 (1992).

H13 Haffner, R. and M. Oren. Biochemical properties and biological effects of p53. Curr. Opin. Genet. Dev. 5: 84-90 (1995).

H14 Hartwell, L., T. Weinert, L. Kadyk et al. Cell cycle checkpoints, genomic integrity and cancer. Cold Spring Harbor Symp. Quant. Biol. 59: 259-263 (1994).

H15 Hayata, I., M. Seki, K. Yoshida et al. Chromosomal aberrations observed in 52 mouse myeloid leukaemias. Cancer Res. 43: 367-373 (1983).

H16 Harley, C.B. Telomere loss: mitotic clock or genetic time bomb? Mutat. Res. 256: 271-282 (1991).

H17 Hemminki, A., P. Peltomaki and J.P. Mecklin. Loss of the wild type MLH1 gene is a feature of hereditary non-polyposis colorectal cancer. Nature Genet. 8: 405-410 (1994).

H18 Hall, E.J. Finding a smoother pebble: a workshop summary. p. 401-402 in: Cell Transformation and Radiation Induced Cancer (K.H. Chadwick et al., eds.). Adam Hilger, Bristol, 1989.

H19 Hei, T.K., K. Komatsu, E.J. Hall et al. Oncogenic transformation by charged particles of defined LET. Carcinogenesis 9: 747-750 (1988).

H20 Hall, E.J., R.C. Miller and D.J. Brenner. Neoplastic transformation and the inverse dose rate effect for neutrons. Radiat. Res. 128: S75-S80 (1991).

H21 Hasterlik, R.J. The delayed toxicity of radium deposited in the skeleton of human beings. p. 149-155 in: Proceedings of the International Conference on the Peaceful Uses of Atomic Energy, Volume 11, Geneva, 1956.

H22 Hockenbery, D.M. Bcl-2, a novel regulator of cell death. BioEssays 17: 631-638 (1995).

H23 Huang, L.C., K.C. Clarkin and G.M. Wahl. Sensitivity and selectivity of the DNA damage sensor responsible for activating p53-dependent G1 arrest. Proc. Natl. Acad. Sci. U.S.A. 93: 4827-4832 (1996).

H24 Hall, E.J. and R.C. Miller. The how and why of *in vitro* oncogenic transformation. Radiat. Res. 87: 208-223 (1981).

H25 Hill, C.K. and L. Zhu. Energy and dose-rate dependence of neoplastic transformation and mutations induced in mammalian cells by fast neutrons. Radiat. Res. 128: S53-S59 (1991).

H26 Hoel, D.G. and P. Li. Threshold models in radiation carcinogenesis. Health Phys. 75: 241-250 (1998).

H27 Hill, C.K., A. Han and M.M. Elkind. Promotion, dose-rate, and repair processes in radiation-induced neoplastic transformation. Radiat. Res. 109: 347-351 (1987).

H28 Heimbach, R.D., F.J. Burns and R.E. Albert. An evaluation by alpha-particle Bragg peak radiation of the critical depth in rat skin for tumour induction. Radiat. Res. 39: 332-344 (1969).

H29 Hei, T.K., E.J. Hall and C.A. Waldren. Neutron risk assessment based on low dose mutation data. p. 481-490 in: Low Dose Radiation: Biological Bases for Risk Assessment (K.F. Baverstock and J.W. Stather, eds.). Taylor and Francis, London, 1989.

H30 Hahn, F.F., W.C. Griffith and B.B. Boecker. Comparison of the effects of inhaled $^{238}PuO_2$ and β-emitting radionuclides on the incidence of lung carcinomas in

laboratory animals. p. 916-919 in: Proceedings IRPA8: 8th International Congress of the International Radiation Protection Association, Montreal, 1991. Volume 1. Pergamon Press, London, 1991.

H31 Howe, G.R. and J. McLaughlin. Breast cancer mortality between 1950 and 1987 after exposure to fractionated moderate-dose-rate ionizing radiation in the Canadian fluoroscopy cohort study and a comparison with breast cancer mortality in the atomic bomb survivors study. Radiat. Res. 145: 694-707 (1996).

H32 Haines, J., R. Dunford, J. Moody et al. Loss of heterozygosity in spontaneous and x-ray-induced intestinal tumors arising in F1 hybrid *Min* mice: evidence for sequential loss of Apc^+ and *Dpc4* in tumour development. Genes Chrom. Cancer 28(4): 387-394 (2000).

H33 Hayata, I., C. Wang, W. Zhang et al. Chromosome translocation in residents of the high background radiation area in Southern China. J. Radiat. Res. 41: (2000, in press).

H34 Hayata, I. Insignificant risk at low dose (rate) radiation predicted by cytogenetic studies. p. 268 (T-17-3) in: Proceedings of the 10th International Congress of the International Radiation Protection Association, Hiroshima, Japan, 14-19 May 2000.

H35 Harms, M.D., H.P. Leenhouts and P.A.M. Uijt deHaag. A two mutation model of carcinogenesis: application to lung tumours using rat experimental data. RIVM Report No. 610065.006 (1998).

H36 Hyun, S-J., M-Y. Yoon, T-I. Kum et al. Enhancement of mitogen-stimulated proliferation of low dose radiation-adapted mouse splenocytes. Anticancer Res. 17: 225-230 (1997).

I1 International Agency for Research on Cancer Study Group on Cancer Risk among Nuclear Industry Workers. Direct estimates of cancer mortality due to low doses of ionising radiation: An international study. Lancet 344: 1039-1043 (1994).

I2 International Commission on Radiological Protection. 1990 Recommendations of the International Commission on Radiological Protection. Annals of the ICRP 21(1-3). ICRP Publication 60. Pergamon Press, Oxford, 1991.

I3 Issa, J-P., Y.L. Ottaviano, P. Celano et al. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nature Genet. 7: 536-540 (1994).

I4 Issa, J-P. and S.B. Baylin. Epigenetics and human disease. Nature Med. 2: 281-282 (1996).

I5 International Commission on Radiological Protection. Biological Effects of Inhaled Radionuclides. ICRP Publication 31. Annals of the ICRP 4(1-2). Pergamon Press, Oxford, 1980.

I6 International Commission on Radiological Protection. Recommendations of the International Commission on Radiological Protection. ICRP Publication 1. Pergamon Press, Oxford, 1959.

I7 Iwasaki, T., M. Minowa, S. Hashimoto et al. Non-cancer mortality and life expectancy in different natural background radiation levels of Japan. p. 107-112 in: Low Dose Irradiation and Biological Defense Mechanisms (T. Sugahara et al., eds.). Elsevier Science Publishers, Amsterdam, 1992.

I8 International Commission on Radiological Protection. Recommendations of the International Commission on Radiological Protection. ICRP Publication 26. Pergamon Press, Oxford, 1977; reprinted (with additions) in 1987.

I9 International Commission on Radiological Protection. Limits on Intakes of Radionuclides by Workers. ICRP Publication 30, Part 1. Annals of the ICRP 2(3/4). Pergamon Press, Oxford, 1979

I10 International Commission on Radiological Protection. Age-dependent Doses to Members of the Public from Intake of Radionuclides. Part 2: Ingestion Dose Coefficients. ICRP Publication 67. Annals of the ICRP 23(3/4). Pergamon Press, Oxford, 1993.

I11 International Commission on Radiological Protection. Human Respiratory Tract Model for Radiological Protection. ICRP Publication 66. Annals of the ICRP 24(1/3). Pergamon Press, Oxford, 1994.

I12 International Commission on Radiological Protection. Lung Cancer Risk from Indoor Exposures to Radon Daughters. ICRP Publication 50. Annals of the ICRP 17(1). Pergamon Press, Oxford, 1987.

I13 International Commission on Radiological Protection. RBE for Deterministic Effects. ICRP Publication 58. Annals of the ICRP 20(4). Pergamon Press, Oxford, 1989.

I14 Iwamoto, K.S., S. Fujii, A. Kurata et al. p53 mutations in tumour and non-tumour tissues of Thorotrast recipients: a model for cellular selection during radiation carcinogenesis in the liver. Carcinogenesis 20: 1283-1291 (1999).

I15 Ishii, K. and M. Watanabe. Participation of gap-junctional cell communication on the adaptive response in human cells induced by low doses of X-rays. Int. J. Radiat. Biol. 69: 291-299 (1996).

J1 Jablon, S. and H. Kato. Childhood cancer in relation to prenatal exposure to atomic bomb radiation. Lancet i: 1000-1003 (1970).

J2 Jones, P.A., W.M.I. Rideout, J-C. Shen et al. Methylation, mutation and cancer. Bioessays 14: 33-36 (1992).

J3 Jones, P.A., M.J. Wolkewicz, W.M. Ridout et al. *De novo* methylation of the MyoD1 CpG island during the establishment of immortal cell lines. Proc. Natl. Acad. Sci. U.S.A. 87: 6117-6121 (1990).

J4 Jiang, X-R., G. Jiminez, E. Chang et al. Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. Nature Genet. 21: 111-114 (1999).

J5 Jablon, S. Atomic bomb radiation dose estimation at ABCC. ABCC Technical Report 23-71 (1971).

J6 Jenner, T.J., C.M. DeLara, P. O'Neill et al. Induction and rejoining of double strand breaks in V79-4 mammalian cells following γ- and α-irradiation. Int. J. Radiat. Biol. 64: 265-273 (1993).

J7 Johnson, N.F., A.F. Hobbs and D.G. Thomassen. Epithelial progenitor cells in the rat respiratory tract. p. 88-98 in: Biology, Toxicology and Carcinogenesis of Respiratory Epithelium (D.G. Thomassen and P. Nettesheim, eds.). Hemisphere Publishing Co., Washington D.C., 1990.

J8 Joiner, M.C., P. Lamblin and B. Marples. Adaptive response and induced resistance. C.R. Acad. Sci. Ser. 3 (322): 167-175 (1999).

J9 Jiang, T., I. Hayata, C. Wang et al. Dose-effect relationship of dicentric and ring chromosomes in lymphocytes of individuals living in high background radiation area in China. J. Radiat. Res. 41: (2000, in press).

K1 Karp, J.E. and S. Broder. Molecular foundations of cancer: new targets for intervention. Nature Med. 1: 309-320 (1995).

K2 Kemp, C.J., T. Wheldon and A. Balmain. *p53*-deficient mice are extremely susceptible to radiation-induced tumorigenesis. Nature Genet. 8: 66-69 (1994).

K3 Kendall, G.M., C.R. Muirhead, B.H. MacGibbon et al. Mortality and occupational exposure to radiation: first analysis of the National Registry for radiation workers. Br. Med. J. 304: 220-225 (1992).

K4 Kadhim, M.A., D.A. Macdonald, D.T. Goodhead et al. Transmission of chromosomal instability after plutonium α-particle irradiation. Nature 355: 738-740 (1992).

K5 Kinzler, K.W. and B. Vogelstein. Life and death in a malignant tumour. Nature 379: 19-20 (1996).

K6 Korsmeyer, S.J. Regulators of cell death. Trends Genet. 11: 101-105 (1995).

K7 Kroemer, G. The proto-oncogene Bcl-2 and its role in regulating apoptosis. Nature Med. 3: 614-620 (1997).

K8 Kraemer, K.H., D.D. Levy, C.N. Parris et al. Xeroderma pigmentosum and related disorders: examining the linkage between defective DNA repair and cancer. J. Invest. Dermatol. 103: 96-101 (1994).

K9 Kučerová, M., A.J.B. Anderson, K.E. Buckton et al. X-ray-induced chromosome aberrations in human peripheral blood leucocytes: the response to low levels of exposure *in vitro*. Int. J. Radiat. Biol. 21: 389-396 (1972).

K10 Knox, E.G., A.M. Stewart, G.W. Kneale et al. Prenatal irradiation and childhood cancer. J. Soc. Radiol. Prot. 7: 177-189 (1987).

K11 Kastan, M.B. On the TRAIL from p53 to apoptosis. Nature Genet. 17: 130-131 (1997).

K12 Kinzler, K.W. and B. Vogelstein. Lessons from hereditary colorectal cancer. Cell 87: 159-170 (1996).

K13 Klugbauer, S., E. Lengerfelder, E.P. Demidchik et al. High prevalence of *ret* rearrangements in thyroid tumours of children from Belarus after the Chernobyl reactor accident. Oncogene 11: 2459-2467 (1995).

K14 Kipling, D. The Telomere. Oxford University Press, Oxford, 1995.

K15 Keane, A.T., J. Rundo and M.A. Essling. Postmenopausal loss of Ra acquired in adolescence or young adulthood: quantitative relationship to radiation-induced skeletal damage and dosimetric implications. Health Phys. 54: 517-527 (1988).

K16 Knudson, A.G. Mutation and cancer: statistical study of retinoblastoma. Proc. Natl. Acad. Sci. U.S.A. 68: 820-823 (1971).

K17 Kai, M., E.G. Luebeck and S.H. Moolgavkar. Analysis of the incidence of solid cancer among atomic bomb survivors using a two-stage model of carcinogenesis. Radiat. Res. 148: 348-358 (1997).

K18 Kouzarides, T. Histone acetylases and deacetylases in cell proliferation. Curr. Opin. Genet. Dev. 9: 40-48 (1999).

K19 Kondo, S. Health Effects of Low-Level Radiation. Kinki University Press, Osaka, Japan and Medical Physics Publishing, Madison, WI USA, 1993.

K20 König, F. and J. Kiefer. Lack of dose-rate effect for mutation induction by γ-rays in human TK_6 cells. Int. J. Radiat. Biol. 54(6): 891-897 (1988).

K21 Kelsey, K.T., A. Memisoglu, D. Frenkel et al. Human lymphocytes exposed to low doses of X-rays are less susceptible to radiation-induced mutagenesis. Mutat. Res. 263: 197-201 (1991).

K22 Kathren, R.L. Pathway to a paradigm: the linear non-threshold dose-response model in historical context: the American Academy of Health Physics 1995 Radiology Centennial Hartman oration. Health Phys. 70: 621-635 (1996).

K23 Kneale, G.W. and A.M. Stewart. Age variation in the cancer risks from foetal irradiation. Br. J. Cancer 35: 501-510 (1977).

K24 Katz, R., R. Zachariah, F.A. Cucinotta et al. Survey of radiosensitivity parameters. Radiat. Res. 14: 356-365 (1994).

K25 Kellerer, A.M. and E. Nekolla. Neutron versus γ-ray risk estimates. Inferences from the cancer incidence and mortality data in Hiroshima. Radiat. Environ. Biophys. 36: 73-83 (1997).

K26 Koshurnikova, N.A., G.D. Bysogolov, M.G. Bolotnikova et al. Mortality among personnel who worked at the Mayak complex in the first years of its operation. Health Phys. 71: 90-93 (1996).

K27 Kellerer, A.M. and D. Barclay. Age dependencies in the modelling of radiation carcinogenesis. Radiat. Prot. Dosim. 41: 273-281 (1992).

K28 Kiefer, J. Quantitative Mathematical Models in Radiation Biology. page 208. Springer Verlag, Berlin, Heidelberg, New York, 1987.

K29 Kostyuchenko, V.A. and L.Yu. Krestinina. Long-term irradiation effects in the population evacuated from the East-Urals radioactive trace area. Sci. Total Environ. 142: 119-125 (1994).

K30 Kusunoki, Y., S. Kyoizumi, Y. Hirai et al. Flow of cytometry measurements of subsets of T, B and NK cells in peripheral blood lymphocytes of atomic bomb survivors. Radiat. Res. 150: 227-236 (1998).

L1 Lanfrancone, L., G. Pelicci and P.E. Pelicci. Cancer genetics. Curr. Opin. Genet. Dev. 4: 109-119 (1994).

L2 Leenhouts, H.P. and K.H. Chadwick. A two-mutation model of radiation carcinogenesis: application to lung tumours in rodents and implications for risk evaluation. J. Radiol. Prot. 14: 115-130 (1994).

L3 Lengauer, C., K.W. Kinzler and B. Vogelstein. Genetic instability in colorectal cancers. Nature 386: 623-626 (1997).

L4 Little, J.B. The relevance of cell transformation to carcinogenesis *in vivo*. p. 396-413 in: Low Dose Radiation: Biological Bases of Risk Assessment (K.F. Baverstock and J.W. Stather, eds.). Taylor and Francis, London, 1989.

L5 Little, M.P., M.M. Hawkins, M.W. Charles et al. Fitting the Armitage-Doll model to radiation-exposed cohorts and implications for population cancer risks. Radiat. Res. 132: 207-221 (1992).

L6 Little, M.P., C.R. Muirhead, J.D. Boice et al. Using multistage models to describe radiation-induced leukaemia. J. Radiol. Prot. 15: 315-334 (1995).

L7 Little, M.P. and C.R. Muirhead. Evidence for curvilinearity in the cancer incidence dose-response in the Japanese atomic bomb survivors. Int. J. Radiat. Biol. 70: 83-94 (1996).

L8 Lloyd, D.C., A.A. Edwards, A. Leonard et al. Chromosomal aberrations in human lymphocytes induced *in vitro* by very low doses of x-rays. Int. J. Radiat. Biol. 61: 335-343 (1992).

L9 Lloyd, D.C., A.A. Edwards and J.S. Prosser. Chromosome aberrations induced in human lymphocytes by *in vitro* acute x and gamma radiation. Radiat. Prot. Dosim. 15: 83-88 (1986).

L10 Loeb, L. Mutator phenotype may be required for multi-stage carcinogenesis. Cancer Res. 51: 3075-3079 (1991).

L11 Little, M.P. Comments on the article "Studies of the mortality of atomic bomb survivors. Report 12, Part I. Cancer: 1950-1990" by D.A. Pierce et al. (Radiat. Res. 146: 1-27 (1996)). Radiat. Res. 148: 399-401 (1997).

L12 Luebeck, E.G., S.B. Curtis, F.T. Cross et al. Two stage model of radiation-induced malignant lung tumours in rats: effects of cell killing. Radiat. Res. 145: 163-173 (1996).

L13 Luchnik, N.V. and A.V. Sevan'kaev. Radiation-induced chromosomal aberrations in human lymphocytes. I. Dependence on the dose of gamma-rays and an anomaly at low doses. Mutat. Res. 36: 363-378 (1976).

L14 Levine, A.J. and J.R. Broach (eds). Oncogenes and cell proliferation. Curr. Opin. Genet. Dev. 5(1): (1995).

L15 Levine, A.J. The tumour suppressor genes. Annu. Rev. Biochem. 62: 623-651 (1993).

L16 Luebeck, E.G., W.F. Heidenreich, W.D. Hazelton et al. Biologically based analysis of the data for the Colorado uranium miners cohort: age, dose and dose-rate effects. Radiat. Res. 152: 339-351 (1999).

L17 Loeb, L.A. Microsatellite instability: marker of a mutator phenotype in cancer. Cancer Res. 54: 5059-5063 (1994).

L18 Levy, D.B., K.J. Smith, Y. Beazer-Barclay et al. Inactivation of both *Apc* alleles in human and mouse tumours. Cancer Res. 54: 5953-5958 (1994).

L19 Luongo, C., A.R. Moser, S. Gledhill et al. Loss of Apc^+ in intestinal adenomas from Min mice. Cancer Res. 54: 5947-5952 (1994).

L20 Luongo, C. and W.F. Dove. Somatic genetic events linked to the *Apc* locus in intestinal adenomas of the Min mouse. Genes, Chromosome Cancer 17: 194-198 (1996).

L21 Lundblat, V. and W.E. Wright. Telomeres and telomerase: a simple picture becomes complex. Cell 87: 369-375 (1996).

L22 Little, M.P., C.R. Muirhead and C.A. Stiller. Modelling lymphocytic leukaemia incidence in England and Wales using generalisations of the two-mutation model of carcinogenesis of Moolgavkar, Venzon and Knudson. Stat. Med. 15: 1003-1022 (1996).

L23 Leach, D.R., M.F. Krummel and J.P. Allison. Enhancement of antitumour immunity by CTLA-4 blockade. Science 271: 1734-1736 (1996).

L24 Little, J.B. Induced genetic instability as a critical step in radiation carcinogenesis. p. 597-601 in: Radiation Research 1895-1995, Vol. 2 (U. Hagen, D. Harder, H. Jung et al., eds.). H. Sturtz AG, Wurzburg, 1995.

L25 Lindahl, T. Instability and decay of the primary structure of DNA. Nature 362: 709-715 (1993).

L26 Little, M.P., C.R. Muirhead and C.A. Stiller. Modelling acute lymphocytic leukaemia using generalizations of the MVK two-mutation model of carcinogenesis: implied mutation rates and the likely role of ionising radiation. p. 244-247 in: Microdosimetry. An Interdisciplinary Approach (D.T. Goodhead, P. O'Neill and H.G. Menzel, eds.). Royal Society of Chemistry, Cambridge, 1997.

L27 Lloyd, R.D., G.N. Taylor, W. Angus et al. Bone cancer occurrence among beagles given ^{239}Pu as young adults. Health Phys. 64: 45-51 (1993).

L28 Lundgren, D.L., F.F. Hahn and W.W. Carlton. Dose response from inhaled monodisperse aerosols of $^{244}Cm_2O_3$ in the lung, liver and skeleton of F344 rats and comparison with $^{239}PuO_2$. Radiat. Res. 147: 598-612 (1997).

L29 Lewis, E.B. Leukaemia and ionizing radiation. Science 125: 965-972 (1957).

L30 Little, M.P. and M.W. Charles. The risk of non-melanoma skin cancer incidence in the Japanese atomic bomb survivors. Int. J. Radiat. Biol. 71(5): 589-602 (1997).

L31 Little, M.P. Are two mutations sufficient to cause cancer? Some generalizations of the two-mutation model of carcinogenesis of Moolgavkar, Venzon and Knudson and of the multistage model of Armitage and Doll. Biometrics 51: 1278-1291 (1995).

L32 Little, M.P. and M.W. Charles. Time variations in radiation-induced relative risk and implications for population cancer risks. J. Radiol. Prot. 11: 91-110 (1991).

L33 Little, M.P. Risks of radiation-induced cancer at high doses and dose rates. J. Radiol. Prot. 13: 3-25 (1993).

L34 Lloyd, D.C. and A.A. Edwards. Chromosome aberrations in human lymphocytes: effect of radiation quality, dose and dose rate. p. 23-49 in: Radiation-induced Chromosome Damage in Man (T. Ishihara and M.S. Sasaki, eds.). Alan Liss, New York, 1983.

L35 Little, M.P., M.M. Hawkins, M.W. Charles et al. Letter to the Editor. Corrections to the paper "Fitting the Armitage-Doll model to radiation-exposed cohorts and implications for population cancer risks". Radiat. Res. 137: 124-128 (1994).

L36 Little, M.P. Generalisations of the two-mutation and classical multi-stage models of carcinogenesis fitted to the Japanese atomic bomb survivor data. J. Radiol. Prot. 16: 7-24 (1996).

L37 Li, F., G. Ambrosini, E.Y. Chu et al. Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 396: 580-582 (1998).

L38 Lloyd, D.C. New developments in chromosomal analysis for biological dosimetry. Radiat. Prot. Dosim. 77 (1/2): 33-36 (1998).

L39 Lundgren, D.L., F.F. Hahn, W.C. Griffith et al. Pulmonary carcinogenicity of relatively low doses of beta-particle radiation from inhaled $^{144}CeO_2$ in rats[1,2]. Radiat. Res. 146: 525-535 (1996).

L40 Lyon, M.F., R.J.S. Phillips and H.J. Bailey. Mutagenic effect of repeated small radiation doses to mouse spermatogonia. I. Specific locus mutation rates. Mutat. Res. 15: 185-190 (1972).

L41 Lutz, W.K., T. Fekete and S. Vamvakas. Position and base pair specific comparison of p53 mutation spectra in human tumours: elucidation of relationships between organs for cancer etiology. Environ. Health Perspect. 106: 207-211 (1998).

L42 Little, M.P., M.M. Hawkins, R.E. Shore et al. Time variations in the risk of cancer following irradiation in childhood. Radiat. Res. 126: 304-316 (1991).

L43 Little, M.P. and C.R. Muirhead. Curvilinearity in the dose-response curve for cancer in Japanese atomic bomb survivors. Environ. Health Perspect. 105 (Suppl. 6): 1505-1509 (1997).

L44 Little, M.P. and C.R. Muirhead. Curvature in the cancer mortality dose response in Japanese atomic bomb survivors: absence of evidence of threshold. Int. J. Radiat. Biol. 74: 471-480 (1998).

L45 Luckey, T.D. Radiation Hormesis. CRC Press, Boca Raton, 1991.

L46 Little, M., M.W. Charles, J.W. Hopewell et al. Assessment of skin doses. Doc. NRPB 8(3) (1997).

L47 Lord, B.I. The architecture of bone marrow cell populations. Int. J. Cell Cloning 8: 317-331 (1990).

L48 Lubin, J.H., J.D. Boice Jr., C. Edling et al. Radon and lung cancer risk: a joint analysis of 11 underground miners studies. NIH Publication No. 94-3644 (1994).

L49 Little, M.P. Comments on the article "Threshold models in radiation carcinogenesis" by D.G. Hoel and P. Li (Health Phys. 75: 241-250 (1998)). Health Phys. 76: 432-434 (1999).

L50 Lucas, J.N., W. Deng, D. Moore et al. Background ionizing radiation plays a minor role in the production of chromosome translocations in a control population. Int. J. Radiat. Biol. 75: 819-827 (1999).

L51 Lubin, J.H., J.D. Boice Jr., C. Edling et al. Lung cancer risk in radon-exposed miners and estimation of risk from indoor exposure. J. Natl. Cancer Inst. 87: 817-827 (1995).

L52 Little, M.P. and C.R. Muirhead. Derivation of low dose extrapolation factors from analysis of curvature in the cancer incidence dose response in the Japanese atomic bomb survivors. Int. J. Radiat. Biol. 76: 939-953 (2000).

L53 Lubin, J.H. and J.D. Boice. Lung cancer risk from residential radon: meta analysis of eight epidemiologic studies. J. Natl. Cancer Inst. 89: 49-57 (1997).

L54 Lloyd, D.C., R.J. Purrott, G.W. Dolphin et al. Chromosome aberrations induced in human lymphocytes by neutron irradiation. Int. J. Radiat. Biol. 29: 169-182 (1976).

L55 Little, M.P. Modelling leukaemia risk in the Japanese atomic bomb survivors and in a UK population using multistage generalizations of the two-mutation model of carcinogenesis of Moolgavkar, Venzon and Knudson. Br. J. Cancer (2000, in press).

L56 Little, M.P., C.R. Muirhead and M.W. Charles. Describing time and age variations in the risk of radiation-induced solid tumour incidence in the Japanese atomic bomb survivors using generalized relative and absolute risk models. Stat. Med. 18:17-33 (1999).

L57 Little, M.P., F. de Vathaire, M.W. Charles et al. Variations with time and age in the relative risks of solid cancer incidence after radiation exposure. J. Radiol. Prot. 17: 159-177 (1997).

L58 Liu, S.Z., W.H. Liu and J.B. Sun. Radiation hormesis: its expression in the immune system. Health Phys. 52: 579-583 (1987).

M1 Merlo, A., J.E. Herman, D.J. Lee et al. 5'CpG island methylation is associated with transcriptional silencing of the tumour suppressor (*p16/CDKN2/MTS*) in human cancers. Nature Med. 1: 686-692 (1995).

M2 Miller, A.B., G.R. Howe, G.J. Sherman et al. Mortality from breast cancer after irradiation during fluoroscopic examinations in patients being treated for tuberculosis. N. Engl. J. Med. 321: 1285-1289 (1989).

M3 Mole, R.H. and I.R. Major. Myeloid leukemia frequency after protracted exposure to ionising radiation: experimental confirmation of the flat dose-response found in ankylosing spondylitis after a single treatment course with x-ray. Leuk. Res. 7: 295-300 (1983).

M4 Mole, R.H., D.G. Papworth and M.J. Corp. The dose response for x-ray induction of myeloid leukaemia in male CBA/H mice. Br. J. Cancer 47: 285-291 (1983).

M5 Moolgavkar, S.H. and A.G. Knudson. Mutation and cancer: a model for human carcinogenesis. J. Natl. Cancer Inst. 66: 1037-1052 (1981).

M6 Mole, R.H. Fetal dosimetry by UNSCEAR and risk coefficients for childhood cancer following diagnostic radiology in pregnancy. J. Radiol. Prot. 10: 199-203 (1990).

M7 Moolgavkar, S.H. and D.J. Venzon. Two-event models for carcinogenesis: incidence curves for childhood and adult tumours. Math. Biosci. 47: 55-77 (1979).

M8 Muirhead, C.R. and G.W. Kneale. Prenatal irradiation and childhood cancer. J. Radiol. Prot. 9: 209-212 (1989).

M9 MacMahon, B. Prenatal x-ray exposure and childhood cancer. J. Natl. Cancer Inst. 28: 1173-1191 (1962).

M10 Monson, R.R. and B. MacMahon. Prenatal x-ray exposure and cancer in children. p. 97-105 in: Radiation Carcinogenesis: Epidemiology and Biological Significance (J.D. Boice and J.F. Fraumeni, eds.). Raven Press, New York, 1984.

M11 Mays, C.W. and R.D. Lloyd. Bone sarcoma risk from ^{90}Sr. p. 352-375 in: Biomedical Implications of Radiostrontium Exposure (M. Goldman and L.K. Bustad, eds.). CONF-710201 (1972).

M12 Muirhead, C.R. and S.C. Darby. Modelling the relative and absolute risks of radiation-induced cancers. J. R. Stat. Soc., Ser. A 150: 83-118 (1987).

M13 Moolgavkar, S.H., E.G. Luebeck, D. Krewski et al. Radon, cigarette smoke, and lung cancer: a re-analysis of the Colorado plateau uranium miners' data. Epidemiology 4: 204-217 (1993).

M14 Mill, A.J., D. Frankenberg, D. Bettega et al. Transformation of C3H 10T½ cells by low doses of ionising radiation: a collaborative study by six European laboratories strongly supporting a linear dose-response relationship. J. Radiol. Prot. 18: 79-100 (1998).

M15 Miller, R.C., C.R. Geard, D.J. Brenner et al. Neutron-energy-dependent oncogenic transformation of C3H 10T½ mouse cells. Radiat. Res. 117: 114-127 (1989).

M16 Miller, R.C., C.R. Geard and S.G. Martin. Neutron induced cell cycle-dependent oncogenic transformation of C3H 10T½ cells. Radiat. Res. 142: 270-275 (1995).

M17 Mays, C.W. and R.D. Lloyd. Bone sarcoma incidence vs alpha particle dose. p. 409-430 in: Radiobiology of Plutonium (B.J. Stover and W.S.S Jee, eds.). The J.W. Press, University of Utah, 1972.

M18 Muirhead, C.R., R. Cox, J.W. Stather et al. Estimates of late radiation risks to the UK population. Docs. NRPB 4(4): 15-157 (1993).

M19 Muirhead, C.R. and M.P. Little. Evidence for curvature in the cancer incidence dose-response curve in the Japanese atomic bomb survivors. p. 156-159 in: Health Effects of Low Dose Radiation. British Nuclear Energy Society, London, 1997.

M20 Marshall, C.J. and E. Nigg (eds.). Oncogenes and cell proliferation. Curr. Opin. Genet. Dev. 7(7): (1998).

M21 McCormick, F. Activators and effectors of ras p21 proteins. Curr. Opin. Genet. Dev. 4: 71-76 (1994).

M22 Murray, A.W. Cell cycle checkpoints. Curr. Opin. Cell Biol. 6: 872-876 (1994).

M23 Mitelman, F., F. Mertens and B. Johansson. A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. Nature Genet. 15: 417-474 (1997).

M24 Makos, M., B.D. Nelkin, M.I. Weman et al. Distinct hypermethylation patterns occur at altered chromosome loci in human lung and colon cancer. Proc. Natl. Acad. Sci. U.S.A. 89: 1929-1933 (1992).

M25 Mizuno, T., S. Kyoizumi, T. Suzuki et al. Continued expression of a tissue specific activated oncogene in the early steps of radiation-induced human thyroid carcinogenesis. Oncogene 15: 1455-1460 (1997).

M26 Matsumoto, M., J. Takeda, N. Inoue et al. A novel protein that participates in non-self discrimination of malignant cells by homologous complement. Nature Med. 3: 1266-1269 (1997).

M27 Moolgavkar, S.H. The multistage theory of carcinogenesis and the age distribution of cancer in man. J. Natl. Cancer Inst. 61: 49-52 (1978).

M28 Moolgavkar, S.H. and E.G. Luebeck. Multistage carcinogenesis: population-based model for colon cancer. J. Natl. Cancer Inst. 84: 610-618 (1992).

M29 Moolgavkar, S.H., F.T. Cross, G. Luebeck et al. A two-mutation model for radon-induced lung tumors in rats. Radiat. Res. 121: 28-37 (1990).

M30 Moolgavkar, S.H., A. Dewanji and D.J. Venzon. A stochastic two-stage model for cancer risk assessment. I. The hazard function and the probability of tumor. Risk Anal. 8: 383-392 (1988).

M31 Miyaki, M. Imprinting and colorectal cancer. Nature Med. 4: 1236-1237 (1998).

M32 Mothersill, M. (ed). Genomic Instability. Int. J. Radiat. Biol. 74: 661-770 (1998).

M33 Muto, M., Y. Chen, E. Kubo et al. Analysis of early initiating events in radiation-induced thymic lymphomagenesis. Jpn. J. Cancer Res. 87: 247-257 (1996).

M34 Muckerheide, J. Low Level Radiation Health Effects: Compiling The Data. Radiation, Science and Health Inc., 1998.

M35 Miller, R.C. and E.J. Hall. X-ray dose fractionation and oncogenic transformations in cultured mouse embryo cells. Nature 272: 58-60 (1978).

M36 Miller, R.C., E.J. Hall and H.H. Rossi. Oncogenic transformation of mammalian cells in vitro with split doses of x-rays. Proc. Natl. Acad. Sci. U.S.A. 76: 5755-5758 (1979).

M37 Miller, R.C., D.J. Brenner, C.R. Geard et al. Oncogenic transformation by fractionated doses of neutrons. Radiat. Res. 114: 589-598 (1988).

M38 Miller, R.C., C.R. Geard, D.J. Brenner et al. The effects of temporal distribution of dose on neutron-induced transformation. p. 357-362 in: Cell Transformation and Radiation Induced Cancer (K.H. Chadwick et al., eds.). Adam Hilger, Bristol, 1989.

M39 Maisin, J.R., A. Wambersie, G.B. Gerber et al. The effects of a fractionated gamma irradiation on life shortening and disease incidence in BALB/c mice. Radiat. Res. 94: 359-373 (1983).

M40 Miller, R.C., G. Randers-Pehrson, C.R. Geard et al. The oncogenic transforming potential of the passage of single α particles through mammalian cell nuclei. Proc. Natl. Acad. Sci. U.S.A. 96: 19-22 (1999).

M41 Metting, N.F., S.T. Palayoor, R.M. Macklis et al. Induction of mutations by Bismuth-212 α particles at two genetic loci in human b-lymphoblasts. Radiat. Res. 132: 339-345 (1992).

M42 Moiseenko, V.V., A.A. Edwards and H. Nikjoo. Modelling the kinetics of chromosome exchange formation in human cells exposed to ionising radiation. Radiat. Environ. Biophys. 35: 31-35 (1996).

M43 Moiseenko, V.V., A.A. Edwards, H. Nikjoo et al. The influence of track structure on the understanding of relative biological effectiveness for induction of chromosomal exchanges in human lymphocytes. Radiat. Res. 147: 208-214 (1997).

M44 McDowell, E., J.S. McLaughlin, D.K. Merenyl et al. The respiratory epithelium histogenesis of lung carcinoma in the human. J. Natl. Cancer Inst. 2: 587-606 (1978).

M45 Marsh, J.W. and A. Birchall. Sensitivity analysis of the weighted equivalent lung dose per unit exposure from radon progeny. Radiat. Prot. Dosim. (2000, in press).

M46 Mole, R.H. Leukaemia induction in man by radionuclides and some relevant experimental and human observations. p. 1-13 in: The Radiobiology of Radium and Thorotrast (W. Gossner et al., eds.). Urban and Schwarzenberg, Munich, 1986.

M47 Muirhead, C.R., A.A. Goodill, R.G.E. Haylock et al. Occupational radiation exposure and mortality: second analysis of the National Registry for Radiation Workers. J. Radiol. Prot. 19: 3-26 (1999).

M48 Morlier, J.P., M. Morin, J. Chameaud et al. Importance du rôle du débit de dose sur l'apparition des cancers chez le rat après inhalation de radon. C.R. Acad. Sci., Ser. 3 (315): 436-466 (1992).

M49 Mothersill, C. and C. Seymour. Medium from irradiated human epithelial cells but not human fibroblasts reduces the clonogenic survival of unirradiated cells. Int. J. Radiat. Biol. 71: 421-427 (1997).

M50 Mothersill, C. and C. Seymour. Cell to cell contact during gamma irradiation is not required to induce a bystander effect in normal human keratinocytes: evidence for release during irrradiation of a signal controlling survival into the medium. Radiat. Res. 149: 256-262 (1998).

M51 Manti, L., M. Jamali, K.M. Prise et al. Genetic instability in Chinese hamster cells after exposure to X-rays or alpha particles of different mean linear energy transfer. Radiat. Res. 147: 22-28 (1997).

M52 Mitchel, R.E.J., J.S. Jackson, R.A. McCann et al. The adaptive response modifies latency for radiation-induced myeloid leukaemia in CBA/H mice. Radiat. Res. 152: 273-279 (1999).

M53 Maisin, J.R., G.B. Gerber, J. Vankerkom et al. Survival and diseases in C57BL mice exposed to X rays or 3.1 MeV neutrons at an age of 7 or 21 days. Radiat. Res. 146: 453-460 (1996).

M54 Makinodan, T. and S.J. James. T cell potentiation by low dose ionizing radiation: Possible mechanisms. Health Phys. 59: 29-34 (1990).

N1 National Council on Radiation Protection and Measurements. Influence of dose and its distribution in time on dose-response relationships for low-LET radiation. NCRP Report No. 64 (1980).

N2 Nasmyth, K. Viewpoint: putting the cell cycle in order. Science 274: 1643-1645 (1996).

N3 Novelli, M.R., J.A. Williamson, I.P.M. Tomlinson et al. Polyclonal origin of colonic adenomas in an XO/XY patient with FAP. Science 272: 1187-1190 (1996).

N4 Nakamura, Y. Cleaning up on β-catenin. Nature Med. 3: 499-500 (1997).

N5 Naik, P., J. Karrim and D. Hanahan. The rise and fall of apoptosis during multistage tumorigenesis. Genes Dev. 10: 2105-2116 (1996).

N6 National Council on Radiation Protection and Measurements. The relative biological effectiveness of radiations of different quality. NCRP Report No. 104 (1990).

N7 Natarajan, A.T., S.J. Santos, F. Darroudi et al. ^{137}Cesium-induced chromosome aberrations analyzed by fluorescence in situ hybridization: eight years follow up of the Goiânia radiation accident victims. Mutat. Res. 400: 299-312 (1998).

N8 Nakanishi, M., K. Tanaka, T. Shintani et al. Chromosomal instability in actue myelocytic leukaemia and myelodysplastic syndrome patients among atomic bomb survivors. J. Radiat. Res. 40: 159-167 (1999).

N9 Nikjoo, H., S. Uehara and D.J. Brenner. Track structure calculations in radiobiology: How can we improve them and what can they do? p. 3-10 in: Microdosimetry: An Interdisciplinary Approach (D.T. Goodhead, P. O'Neill and H.G. Menzel, eds.). Royal Society of Chemistry, Cambridge, 1997.

N10 Nagasawa, H. and J.B. Little. Induction of sister chromatid exchanges by extremely low doses of α-particles. Cancer Res. 52: 6394-6396 (1992).

N11 Nagasawa, H. and J.B. Little. Unexpected sensitivity to the induction of mutations by very low doses of alpha particle radiation: evidence for a bystander effect. Radiat. Res. 152: 552-557 (1999).

O1 Oren, M. Relationship of p53 to the control of apoptotic cell death. Semin. Cancer Biol. 5: 221-227 (1994).

O2 Orkin, S.H. Development of the haemopoietic system. Curr. Opin. Genet. Dev. 6: 597-02 (1996).

O3 Ootsuyama, A. and H.A. Tanooka. One hundred percent tumour induction in mouse skin after repeated β irradiation in a limited dose range. Radiat. Res. 115: 488-494 (1988).

O4 Ojeda, F., H.A. Diehl and H. Folch. Radiation-induced membrane damage and programmed cell death: possible interrelationships. Scanning Microsc. 8: 645-651 (1994).

O5 Oghiso, Y., Y. Yamada, I. Haruzo et al. Differential dose responses of pulmonary tumor types in the rat after inhalation of plutonium dioxide aerosols. J. Radiat. Res. 39: 61-72 (1998).

O6 Olivieri, G., J. Bodycote and S. Wolff. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science 223: 594-597 (1984).

O7 Okayasu, R., K. Suetomi, Y. Yu et al. A defect in DNA repair associates with the high incidence of solid tumour formation in Balb/c mice. Cancer Res. (2000, in press).

O8 Ootsuyama, A. and H. Tanooka. Threshold-like dose of local β irradiation repeated throughout the life span of mice for induction of skin and bone tumours. Radiat. Res. 125: 98-101 (1991).

O9 Otake, M. and W.J. Schull. Radiation-related small head sizes among prenatally exposed A-bomb survivors. Int. J. Radiat. Biol. 63: 255-270 (1993).

O10 Otake, M., W.J. Schull and H. Yoshimaru. A review of radiation-related brain damage in the prenatally exposed atomic bomb survivors. RERF CR 4-89 (1990).

P1 Pierce, D.A. and M. Vaeth. The shape of the cancer mortality dose-response curve for atomic bomb survivors. Radiat. Res. 126: 36-42 (1991).

P2 Pierce, D.A., Y. Shimizu, D.L. Preston et al. Studies of the mortality of A-bomb survivors, Report 12, Part I. Cancer: 1950-1990. Radiat. Res. 146: 1-27 (1996).

P3 Preston, D.L., S. Kusumi, M. Tomonaga et al. Cancer incidence in atomic bomb survivors. Part III: leukaemia, lymphoma and multiple myeloma, 1950-1987. Radiat. Res. 137: S68-S97 (1994).

P4 Pottern, L.M., M. Kaplan, P. Larsen et al. Thyroid nodularity after childhood irradiation for lymphoid hyperplasia: a comparison of questionnaire and clinical findings. J. Clin. Epidemiol. 43: 449-460 (1990).

P5 Powell, S.M., N. Zilz, Y. Beazer-Barclay et al. *APC* mutations occur early during colorectal tumorigenesis. Nature 359: 235-237 (1992).

P6 Pritchard, C. and M. McMahon. Raf revealed in life or death decisions. Nature Genet. 16: 214-215 (1997).

P7 Pierce, D.A., D.O. Stram and M. Vaeth. Allowing for random errors in radiation dose estimates for the atomic bomb survivor data. Radiat. Res. 123: 275-284 (1990).

P8 Papworth, D.G. and E.V. Hulse. Dose-response models for the radiation-induction of skin tumours in mice. Int. J. Radiat. Biol. 44: 423-431 (1983).

P9 Patterson, H.W. Setting standards for radiation protection: the process appraised. Health Phys. 72: 450-457 (1997).

P10 Peto, R. Epidemiology, multistage models, and short-term mutagenicity tests. p. 1403-1428 in: Origins of Human Cancer (H.H. Hiatt and J.A. Winsten, eds.). Cold Spring Harbor Laboratory, Cold Spring Harbor, 1977.

P11 Pierce, D.A., Y. Shimizu, D.L. Preston et al. Response to the letter of M.P. Little (letter). Radiat. Res. 148: 400-401 (1997).

P12 Pirrotta, V. PcG complexes and chromatin silencing. Curr. Opin. Genet. Dev. 7: 249-258 (1997).

P13 Paretzke, H.G. Physical aspects of radiation quality. p. 514-522 in: Low Dose Radiation: Biological Bases of Risk Assessment (K.F. Baverstock and J.W. Stather, eds.). Taylor and Francis, London, 1989.

P14 Pierce, D.A. and M. Vaeth. Cancer risk estimation from the A-bomb survivors: extrapolation to low doses, use of relative risk models and other uncertainties. p. 54-69 in: Low Dose Radiation: Biological Bases of Risk Assessment (K.F. Baverstock and J.W. Stather, eds.). Taylor and Francis, London, 1989.

P15 Pohl-Rüling, J., P. Fischer, O. Haas et al. Effect of low-dose acute X-irradiation on the frequencies of chromosomal aberrations in human peripheral lymphocytes in vitro. Mutat. Res. 110: 71-82 (1983).

P16 Preston, D.L., H. Kato, K. Kopecky et al. Life Span Study, Cancer mortality among A-bomb survivors in Hiroshima and Nagasaki 1950-1982, Report 10, Part 1. RERF TR 1-86 (1986).

P17 Potten, C.S. Stem cells in gastrointestinal epithelium: numbers, characteristics and death. Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci. 353: 821-830 (1998).

P18 Potten, C.S. Communication to the UNSCEAR Secretariat (1999).

P19 Priest, N.D. Risk estimates for high LET alpha-irradiation of skeletal tissues: problems with current methods? p. 423-429 in: Health Effects of Internally Deposited Radionuclides: Emphasis on Radium and Thorium (G. van Kaick, A. Karaoglou and A.M. Kellerer, eds.). World Scientific, London, 1995.

P20 Purrott, R.J., A.A. Edwards, D.C. Lloyd et al. The induction of chromosome aberrations in human lymphocytes by *in vitro* irradiation with α-particles from plutonium-239. Int. J. Radiat. Biol. 38: 277-284 (1980).

P21 Prise, K.M., O.V. Belyakov, M. Folkard et al. Studies of bystander effects in human fibroblasts using a charged particle microbeam. Int. J. Radiat. Biol. 74: 793-798 (1998).

P22 Pierce, D.A. and M.L. Mendelsohn. A model for radiation-related cancer suggested by atomic bomb survivor data. Radiat. Res. 152: 642-654 (1999).

P23 Pohl-Rüling, J., P. Fischer, D.C. Lloyd et al. Chromosomal damage induced in human lymphocytes by low doses of D-T neutrons. Mutat. Res. 173: 267-272 (1986).

R1 Raabe, O.G. Three-dimensional models of risk from internally deposited radionuclides. Internal radiation dosimetry. Health Physics Society, 1994 Summer School (1994).

R2 Rabbitts, T.H. Chromosomal translocations in human cancer. Nature 372: 143-149 (1994).

R3 Raff, M.C. Social controls on cell survival and cell death. Nature 356: 397-399 (1992).

R4 Rowland, R.E., A.F. Stehney and H.F. Lucas. Dose-response relationships for female radium dial workers. Radiat. Res. 76: 368-383 (1978).

R5 Russell, W.L., L.B. Russell and E.M. Kelly. Radiation dose rate and mutation frequency. Science 128: 1546-1550 (1958).

R6 Russell, W.L. and E.M. Kelly. Mutation frequencies in male mice and the estimation of genetic hazards of radiation in man. Proc. Natl. Acad. Sci. U.S.A. 79: 542-544 (1982).

R7 Rundo, J., A.T. Keane and M.A. Essling. Long-term Retention of Radium in Female Former Dial Workers. pages 77-85. CMTP Press Ltd., Lancaster, 1985.

R8 Ron, E., B. Modan, D.L. Preston et al. Thyroid neoplasia following low-dose radiation in childhood. Radiat. Res. 120: 516-531 (1989).

R9 Ron, E., J.H. Lubin, R.E. Shore et al. Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. Radiat. Res. 141: 259-277 (1995).

R10 Ranier, S., A. Johnson, C.J. Dobry et al. Relaxation of imprinting in human cancer. Nature 362: 749-751 (1993).

R11 Roth, S.Y. Something about silencing. Nature Genet. 14: 3-4 (1996).

R12 Rubin, H. Cancer as a dynamic development disorder. Cancer Res. 45: 2935-2942 (1985).

R13 Reznikoff, C.A., J.S. Bertram, D.W. Brankow et al. Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to post confluence inhibition of cell division. Cancer Res. 33: 3239-3249 (1973).

R14 Raabe, O.G., M.R. Culbertson, R.G. White et al. Lifetime radiation effects in beagles injected with ^{226}Ra as young adults. p. 313-318 in: Health Effects of Internally Deposited Radionuclides: Emphasis on Radium and Thorium (G. van Kaick, A. Karaoglou and A.M. Kellerer, eds.). World Scientific, London, 1995.

R15 Ron, E., B. Modan, D. Preston et al. Radiation-induced skin carcinomas of the head and neck. Radiat. Res. 125: 318-325 (1991).

R16 Rowland, R.E. Radium in humans: a review of US studies. ANL/ER-3, UV-408 (1994).

R17 Rowland, R.E. Dose-response relationships for female radium dial workers: a new look. p. 135-143 in: Health Effects of Internally Deposited Radionuclides: Emphasis on Radium and Thorium (G. van Kaick, A. Karaoglou and A.M. Kellerer, eds.). World Scientific, London, 1995.

R18 Rossi, H.H. Microscopic energy distribution in irradiated matter. p. 43 in: Radiation Dosimetry, Volume 1 (F.H. Atix and W.C. Roesch, eds.). Academic Press, New York, 1968.

R19 Russell, W.L. and E.M. Kelly. Mutation frequencies in female mice and the estimation of genetic hazards or radiation in women. Proc. Natl. Acad. Sci. U.S.A. 74: 3523-3527 (1977).

R20 Russell, W.L. The genetic effects of radiation. p. 487-500 in: Peaceful Uses of Atomic Energy. STI/PUB/300, Volume 13. IAEA, Vienna, 1972.

R21 Ramsey, M.J., D.H. Moore II, J.F. Briner et al. The effects of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting. Mutat. Res. 338: 95-106 (1995).

R22 Redpath, J.L. and R.J. Antoniono. Induction of an adaptive response against spontaneous neoplastic transformation *in vitro* by low-dose gamma radiation. Radiat. Res. 149: 517-520 (1998).

S1 Shimizu, Y., H. Kato and W.J. Schull. Studies of the mortality of A-bomb survivors. 9. Mortality, 1950-1985: Part 2: Cancer mortality based on the recently revised doses (DS 86). Radiat. Res. 121: 120-141 (1990).

S2 Shore, R.E., N. Hildreth, P. Ovoretsky et al. Thyroid cancer among persons given x-ray treatment in infancy for an enlarged thymus gland. Am. J. Epidemiol. 137: 1068-1080 (1993).

S3 Skuse, G.R. and J.W. Ludlow. Tumour suppressor genes in disease and therapy. Lancet 345: 902-906 (1995).

S4 Smith, J.R. and O.M. Pereira-Smith. Replicative senescence: implications for *in vivo* ageing and tumour suppression. Science 273: 63-67 (1996).

S5 Simmons, J.A. and D.E. Watt. Radiation Protection Dosimetry - A Radical Reappraisal. Medical Physics Publishing, Madison, Wisconsin, U.S.A., 1999.

S6 Shore, R.E. Issues and epidemiological evidence regarding radiation-induced thyroid cancer. Radiat. Res. 131: 98-111 (1992).

S7 Stewart, A.M., J. Webb and D. Hewitt. A survey of childhood malignancy. Br. Med. J. 1: 1495-1508 (1958).

S8 Shin, C., L.C. Padhy and R.A. Weinberg. Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts. Nature 290: 261-264 (1981).

S9 Schechtman, L.M., E. Kiss, J. McCorvill et al. A method for the amplification of chemically induced transformation in C3H 10T½ clone 8 cells: its use as a potential screening assay. J. Natl. Cancer Inst. 79: 487-498 (1987).

S10 Searle, A.G. Mutation induction in mice. Adv. Radiat. Biol. 4: 131-207 (1974).

S11 Sanders, C.L. and D.L. Lundgren. Pulmonary carcinogenesis in the F344 and Wistar rats after inhalation of plutonium dioxide. Radiat. Res. 144: 206-214 (1995).

S12 Saunders, C.L., K.E. Lauhala and K.E. McDonald. Lifespan studies in rats exposed to $^{239}PuO_2$. III. Survival and lung tumours. Int. J. Radiat. Biol. 64: 417-430 (1993).

S13 Sankaranarayanan, K. Ionising radiation and genetic risks IV. Current methods, estimates of risk of Mendelian disease, human data and lessons from biochemical and molecular studies of mutations. Mutat. Res. 258: 99-122 (1991).

S14 Speicher, M.R., S.G. Ballard and D.C. Ward. Karyotyping human chromosomes by combinational multi-fluor FISH. Nature Genet. 12: 368-375 (1996).

S15 Shibata, D., M.A. Peinado, Y. Ionov et al. Genomic instability in repeated sequences is an early somatic event in colorectal tumorigenesis that persists after transformation. Nature Genet. 6: 273-281 (1994).

S16 Stilgenbaur, S., C. Schuffner, A. Litterst et al. Biallelic mutations in the *ATM* gene in T-prolymphocytic leukaemia. Nature Med. 3: 1155-1159 (1997).

S17 Sidransky, D. Is human *patched* the gatekeeper of common skin cancers? Nature Genet. 14: 7-8 (1996).

S18 Shoemaker, A.R., K.A. Gould, C. Luongo et al. Studies of neoplasia in the Min mouse. Biochim. Biophys. Acta 1332: F25-F48 (1997).

S19 Strong, L.C. and W.R. Williams. The genetic implications of long-term survival of childhood cancer. Am. J. Pediatr. Hematol./Oncol. 9: 99-103 (1987).

S20 Shore, R.E., N. Hildreth, E. Woodard et al. Breast cancer among women given X-ray therapy for acute postpartum mastitis. J. Natl. Cancer Inst. 77: 689-696 (1986).

S21 Stewart, A.M. and G.W. Kneale. Radiation dose effects in relation to obstetric x-rays and childhood cancers. Lancet i: 1185-1188 (1970).

S22 Sugahara, T. A radiation protection system aimed at cancer prevention: a proposal. in: Proceedings of DAE Symposium on Recent Advances in Genetic Epidemiology and Population Monitoring, Madras, India, 1998.

S23 Schiestl, R.H., F. Khogali and N. Carls. Reversion of the mouse *pink-eyed unstable* mutation induced by low doses of x-rays. Science 266: 1573-1576 (1994).

S24 Selby, P.B., S.S. Lee, E.M. Kelly et al. Specific-locus experiments show that female mice exposed near the time of birth to low-LET ionizing radiation exhibit both a low mutational response and a dose rate effect. Mutat. Res. 249: 351-367 (1991).

S25 Shadley, J.D. and S. Wolff. Very low doses of X-rays can cause human lymphocytes to become less susceptible to ionizing radiation. Mutagenesis 2: 95-96 (1987).

S26 Shadley, J.D., V. Afzal and S. Wolff. Characterization of the adaptive response to ionizing radiation induced by low doses of X-rays to human lymphocytes. Radiat. Res. 111: 511-517 (1987).

S27 Sanderson, B.J.S. and A.A. Morley. Exposure of human lymphocytes to ionizing radiation reduces mutagenesis by subsequent ionizing radiation. Mutat. Res. 164: 347-351 (1986).

S28 Sparrow, A.H., A.G. Underbrink and H.H. Rossi. Mutations induced in tradescania by small doses of x rays and neutrons: analysis of dose response curves. Science 176: 916-918 (1972).

S29 Szumiel, I. Review: ionising radiation-induced cell death. Int. J. Radiat. Biol. 66: 329-341 (1994).

S30 Schervish, M.J. Theory of Statistics. Springer-Verlag, New York, 1995.

S31 Shore, R.E., R.E. Albert, M. Reed et al. Skin cancer incidence among children irradiated for ringworm of the scalp. Radiat. Res. 100: 192-204 (1984).

S32 Savage, J.R.K. Sites of radiation induced chromosome exchanges. Curr. Top. Radiat. Res. VI: 129-196 (1970).

S33 Sullivan, M.F., P.L. Hackett, L.A. George et al. Irradiation of the intestine by radioisotopes. Radiat. Res. 13: 343-355 (1960).

S34 Schmutte, C. and R. Fishel. Genomic instability: First step to carcinogenesis. Anticancer Res. 19: 4665-4696 (1999).

S35 Sinclair, W.K. Experimental RBE value of high LET radiations at low doses and the implications for quality factor assignment. Radiat. Prot. Dosim. 13: 319-326 (1985).

S36 Sevan'kaev, A.V., E.A. Zherbin, N.V. Luchnik et al. Cytogenetic effects produced by neutrons in lymphocytes of human peripheral blood in vitro. 1. Dose-response dependence of neutrons of different energies for different types of chromosomal aberrations. Genetika 15: 1046-1060 (1979). (In Russian).

S37 Searle, A.G., C.V. Beechey, D. Green et al. Cytogenetic effects of protracted exposures to alpha particles from ^{239}Pu and to gamma rays from ^{60}Co compared in male mice. Mutat. Res. 41: 297-310 (1976).

S38 Sasaki, S. Influence of the age of mice at exposure to radiation on life-shortening and carcinogenesis. J. Radiat. Res. 2 (Suppl.): 73-85 (1991).

S39 Sasaki, S. and N. Fukuda. Dose-response relationship for induction of solid tumours in female B6C3F$_1$ mice irradiated neonatally with a single dose of gamma rays. J. Radiat. Res. 40: 229-241 (1999).

S40 Silver, A., J. Moody, R. Dunford et al. Molecular mapping of chromosome 2 deletions in murine radiation-induced AML localises a putative tumour suppressor gene to a 1.0 cM region homologous to human chromosome segment 11p 11-12. Genes Chrom. Cancer 24: 95-104 (1999).

S41 Sadekova, S., S. Lehnert, B. Chandrasekar et al. Induction of a PBP74/mortalin/Grp75, a member of the hsp 70 family, by low doses of ionizing radiation. A possible role in induced radioresistance. Int. J. Radiat. Biol. 72: 653-660 (1997).

S42 Sambani, C., H. Thomou and P. Kitsiou. Stimulatory effect of low dose x-irradiation on the expression of the human T lymphocyte CD2 surface antigen. Int. J. Radiat. Biol. 70: 711-717 (1996).

S43 Shankar, B., S. Premachandran, P. Bharambe et al. Modification of immune response by low dose ionizing radiation: role of apoptosis. Immunol. Lett. 68: 237-245 (1999).

T1 Takeichi, M. Cadherins in cancer: implications for invasion and metastasis. Curr. Opin. Cell Biol. 5: 806-811 (1993).

T2 Thacker, J. Radiation-induced mutation in mammalian cells at low doses and dose rates. Adv. Radiat. Biol. 16: 77-117 (1992).

T3 Thomas, D.C. A model for dose rate and duration of exposure effects in radiation carcinogenesis. Environ. Health Perspect. 87: 163-171 (1990).

T4 Thompson, D.E., K. Mabuchi, E. Ron et al. Cancer incidence in atomic bomb survivors. Part II: solid tumors, 1958-1987. Radiat. Res. 137: S17-S67 (1994).

T5 Trosko, J.E., C.L. Chang, B.V. Madhukar et al. Intercellular communication: a paradigm for the interpretation of the initiation/promotion/progression model of carcinogenesis. in: Chemical Carcinogenesis: Modulation and Combination Effects (J.C. Arocs, ed.). Academic Press, New York, 1992.

T6 Tan, W-Y. Stochastic Models of Carcinogenesis. Marcel Dekker, New York, 1991.

T7 Tucker, H.G. A stochastic model for a two-stage theory of carcinogenesis. p. 387-403 in: Fifth Berkeley Symposium on Mathematical Statistics and Probability. University of California Press, Berkeley, 1967.

T8 Tomlinson, I. and W. Bodmer. Selection, the mutation rate and cancer: ensuring that the tail does not wag the dog. Nature Med. 5: 11-12 (1999).

T9 Tao, Z.-F., H. Kato, Y.-R. Zha et al. Study on cancer mortality among the residents in high background radiation area of Yangjiag, China. p. 249-254 in: High Levels of Natural Radiation 96: Radiation Dose and Health Effects (L. Wei et al., eds.). Elsevier, Amsterdam. 1997.

T10 Tirmarche, M., A. Rannou, A. Mollié et al. Epidemiological study of regional cancer mortality in France and natural radiation. Radiat. Prot. Dosim. 24: 479-482 (1988).

T11 Tubiana, M. The report of the French Academy of Sciences: Problems associated with the effects of low doses of ionising radiation. J. Radiol. Prot. 18(4): 243-248 (1998).

T12 Thomas, R.G. Tumorigenesis in the US radium luminizers: how unsafe was this occupation? p. 145-148 in: Health Effects of Internally Deposited Radionuclides: Emphasis on Radium and Thorium (G. van Kaick, A. Karaoglou, A.M. Kellerer, eds.). World Scientific, Singapore, 1995.

T13 Tanooka, H. and A. Ootsuyama. Radiation carcinogenesis in mouse skin and its threshold-like response. J. Radiat. Res. 2 (Suppl.): 195-201 (1991).

T14 Thacker, J. The nature of mutants induced by ionizing radiation in cultured hamster cells. Mutat. Res. 160: 267-275 (1986).

T15 Trump, B.F., E.M. McDowell, F. Glavin et al. The respiratory epithelium III. Histogenesis of epidermoid metaplasia and carcinoma in situ in the human. J. Natl. Cancer Inst. 61: 563-575 (1978).

T16 Thacker, J., A. Stretch and M.A. Stephens. Mutation and inactivation of cultured mammalian cells exposed to beams of accelerated heavy ions. II. Chinese hamster V79 cells. Int. J. Radiat. Biol. 36: 137-148 (1979).

T17 Tao, Z.F., S. Akiba, Y.R. Zha et al. Analysis of data from investigation of cancer mortality in high background radiation area of Yangjiang, China (1987-1995). Chin. J. Radiol. Med. Prot. 19(2): 75-82 (1999).

U2 United Nations. Sources and Effects of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation, 1994 Report to the General Assembly, with scientific annexes. United Nations sales publication E.94.IX.11. United Nations, New York, 1994.

U3 United Nations. Sources and Effects of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation, 1993 Report to the General Assembly, with scientific annexes. United Nations sales publication E.94.IX.2. United Nations, New York, 1993.

U4 United Nations. Sources, Effects and Risks of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation, 1988 Report to the General Assembly, with annexes. United Nations sales publication E.88.IX.7. United Nations, New York, 1988.

U5 United Nations. Genetic and Somatic Effects of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation, 1986 Report to the General Assembly, with annexes. United Nations sales publication E.86.IX.9. United Nations, New York, 1986.

U6 United Nations. Ionizing Radiation: Sources and Biological Effects. United Nations Scientific Committee on the Effects of Atomic Radiation, 1982 Report to the General Assembly, with annexes. United Nations sales publication E.82.IX.8. United Nations, New York, 1982.

U7 United Nations. Sources and Effects of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation, 1977 Report to the General Assembly, with annexes. United Nations sales publication E.77.IX.1. United Nations, New York, 1977.

U8 United Nations. Ionizing Radiation: Levels and Effects. Volume I: Levels, Volume II: Effects. United Nations Scientific Committee on the Effects of Atomic Radiation, 1972 Report to the General Assembly, with annexes. United Nations sales publication E.72.IX.17 and 18. United Nations, New York, 1972.

U14 Ullrich, R.L. Tumour induction in BALB/c female mice after fission neutron or γ-irradiation. Radiat. Res. 93: 506-515 (1983).

U15 Ullrich, R.L., M.C. Jernigan, G.E. Cosgrove et al. The influence of dose and dose rate on the incidence of neoplastic disease in RFM mice after neutron irradiation. Radiat. Res. 68: 115-131 (1976).

U16 Ullrich, R.L. and J.B. Storer. Influence of γ irradiation on the development of neoplastic disease in mice. I. Reticular tissue tumours. Radiat. Res. 80: 303-316 (1979).

U17 Ullrich, R.L. and J.B. Storer. Influence of γ irradiation on the development of neoplastic disease in mice. II. Solid tumours. Radiat. Res. 80: 317-324 (1979).

U18 Ullrich, R.L. and J.B. Storer. Influence of γ irradiation on the development of neoplastic disease in mice. III. Dose-rate effects. Radiat. Res. 80: 325-342 (1979).

U19 Ullrich, R.L., M.C. Jernigan, L.C. Satterfield et al. Radiation carcinogenesis: time-dose relationships. Radiat. Res. 111: 179-184 (1987).

U20 Ullrich, R.L. and R.J. Preston. Myeloid leukaemia in male RFM mice following irradiation with fission spectrum neutrons or γ-rays. Radiat. Res. 109: 165-170 (1987).

U21 Upton, A.C., M.L. Randolph and J.W. Conklin. Late effects of fast neutrons and gamma-rays in mice as influences by the dose rate of irradiation: induction of neoplasia. Radiat. Res. 41: 467-491 (1970).

U22 Upton, A.C. Radiological effects of low doses. Implications for radiological protection. Radiat. Res. 71: 51-74 (1977).

U23 Upton, A.C., R.E. Albert, F.J. Burns et al. Radiation Carcinogenesis. Elsevier, New York, 1986.

U24 Ullrich, R.L. and J.B. Storer. Influence of dose, dose rate and radiation quality on radiation carcinogenesis and life shortening in RFM and BALB/c mice. p. 95-113 in: Late Effects of Ionizing Radiation, Volume II. IAEA, Vienna, 1978.

U25 Ullrich, R.L. and B. Ponnaiya. Radiation-induced instability and its relation to radiation carcinogenesis. Int. J. Radiat. Biol. 74: 747-754 (1998).

U26 Ullrich, R.L., N.D. Bowles, L.C. Satterfield et al. Strain-dependent susceptibility to radiation-induced mammary cancer is a result of differences in epithelial cell sensitivity to transformation. Radiat. Res. 146: 353-355 (1996).

V1 Vogelstein, B. and K.W. Kinzler. The multistep nature of cancer. Trends Genet. 9: 138-141 (1993).

V2 Vogelstein, B. and K.W. Kinzler. The Genetic Basis of Human Cancer. McGraw-Hill, New York, 1998.

V3 van Zwieten, M.J. The Rat as Animal Model in Breast Cancer Research. Martinus Nijhoff, Boston, 1984.

V4 Vral, A., H. Cornelisen, H. Thierens et al. Apoptosis induced by fast neutrons versus ^{60}Co gamma rays in human peripheral blood lymphocytes. Int. J. Radiat. Biol. 73: 289-295 (1998).

V5 Villunger, A. and A. Strasser. The great excape: is immune evasion required for tumour progression? Nature Med. 5: 874-875 (1999).

W1 Wagner, R., E. Schmid and M. Bauchinger. Application of conventional and FPG staining for the analysis of chromosome aberrations induced by low levels of dose in human lymphocytes. Mutat. Res. 109: 65-71 (1983).

W2 Williams, B.O. and T. Jacks. Mechanisms of carcino-genesis and the mutant mouse. Curr. Opin. Genet. Dev. 6: 65-70 (1996).

W3 Weinberg, R.A. Oncogenes and tumour suppressor genes. CA Cancer J. Clin. 44: 160-170 (1994).

W4 Williams, E.D. Thyroid cancer and the Chernobyl accident. in: Health Effects of Low Dose Radiation. British Nuclear Energy Society, London, 1997.

W5 Willerford, D.M., S. Wojciech and F. Alt. Developmental regulation of V(D)J recombination and lymphocyte differentiation. Curr. Opin. Genet. Dev. 6: 603-609 (1996).

W6 Wolff, S. Adaptive responses. p. 103 in: Low Doses of Ionizing Radiation: Biological Effects and Regulatory Control. IAEA, Vienna, 1998.

W7 Ward, J.F. Response to commentary by D. Billen. Radiat. Res. 126: 38-57 (1991).

W8 Williams, J.P., J.E. Coggle, M.W. Charles et al. Skin carcinogenesis in the mouse following uniform and non-uniform beta irradiation. Br. J. Radiol. (Suppl.) 19: 61-64 (1986).

W9 Weiss, H.A., S.C. Darby and R. Doll. Cancer mortality following X-ray treatment for ankylosing spondylitis. Int. J. Cancer 59: 327-338 (1994).

W10 Woloschak, G.E., T. Pauneska, C-M. Chang-Liu et al. Changes in gene expression associated with radiation exposure. p.545-547 in: Radiation Research 1895-1995 (U. Hagen, D. Harder, H. Jung et al., eds). H. Sturtz AG, Wurzburg, 1995.

W11 Walter, S.D., J.W. Meigs and J.F. Heston. The relationship of cancer incidence to terrestrial radiation and population density in Connecticut, 1935-1974. Am. J. Epidemiol. 123: 1-14 (1986).

W12 Wang, J. Statistical analysis of cancer mortality data of high background radiation areas in Yiangjiang. Chin. J. Radiol. Med. Prot. 13: 291-294 and 358 (1993).

W13 Wolff, S. Adaptive responses. Environ. Health Perspect. 106: 277-283 (1998).

W14 Wiencke, J.K., V. Afzal, G. Olivieri et al. Evidence that the [3H]thymidine-induced adaptive response of human lymphocytes to subsequent doses of X-rays involves the induction of a chromosomal repair mechanism. Mutagenesis 1: 375-380 (1986).

W15 Wolff, S., J.K. Wiencke, V. Afzal et al. The adaptive response of human lymphocytes to very low doses of ionizing radiation: A case of specific proteins. p. 446-454 in: Low Dose Radiation: Biological Bases of Risk Assessment (K.F. Baverstock and J.W. Stather, eds.). Taylor and Francis, London, 1989.

Y1 Yoshimoto, Y., R. Delongchamp and K. Mabuchi. *In-utero* exposed atomic bomb survivors: cancer risk update. Lancet 344: 345-346 (1994).

Y2 Yoshimoto, Y., H. Kato and W.J. Schull. Risk of cancer among children exposed *in utero* to A-bomb radiations, 1950-84. Lancet ii: 665-669 (1988).

Y3 Yin, Y., Y. Terauchi, G. Solomon et al. Involvement of p85 in p53-dependent apoptotic response to oxidative stress. Nature 391: 707-710 (1998).

Y4 Yonezawa, M., J. Misonoh and Y. Hosokawa. Two types of x-ray-induced radioresistance in mice - Presence of four dose ranges with distinct biological effects. Mutat. Res. 358: 237-243 (1996).

Y5 Yessner, R. The dynamic histopathologic spectrum of lung cancer. Yale J. Biol. Med. 54: 447-456 (1981).

Y6 Yamamoto, O., T. Seyama, H. Itoh et al. Oral administration of tritiated water (HTO) in mouse. III. Low dose-rate irradiation and threshold dose-rate for radiation risk. Int. J. Radiat. Biol. 73: 535-541 (1998).

Z1 Zimmerman, D. Thyroid neoplasia in children. Curr. Opin. Pediatr. 9: 413-418 (1997).

Z2 Zheng, P., Y. Guo, Q. Niu et al. Proto-oncogene *PML* controls genes devoted to MHC class 1 antigen presentation. Nature 396: 373-376 (1998).

Z3 Zhou, P.K., X.Y. Liu, W.Z. Sun et al. Cultured mouse SR-1 cells exposed to low dose of gamma-rays become less susceptible to the induction of mutagenesis by radiation as well as bleomycin. Mutagenesis 8(2): 109-111 (1993).