ANNEX F

Influence of dose and dose rate on stochastic effects of radiation

CONTENTS

INTRODUCTION

1. Radiation-induced malignant disease is the main late somatic effect in human populations exposed to ionizing radiation and the only statistically detectable cause of radiation-induced life shortening at intermediate to low doses. In this dose range the incidence of radiation-induced cancer appears to increase with increasing dose, and the probabilistic nature of the relationship between dose and risk of the disease has led to the use of the term "stochastic" for this type of effect. Quantitative information on the risk of cancer in human populations exposed to ionizing radiation at present comes largely from information available from populations that have been exposed at intermediate to high doses and dose rates. In general, however, for assessing the consequences of environmental and occupational exposure to radiation, risks need to be known for exposures to low doses delivered at low dose rates. Some information is now starting to become available from epidemiological studies on occupationally exposed groups, although at present the results of such studies have substantial statistical uncertainties associated with them. It is likely that for the immediate future quantitative risk estimates will continue to be based on the higher dose/dose-rate studies, although increasingly low-dose studies will provide support for these values.

2. 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 cancer induction in exposed individuals. The two features of the dose response that are most important for evaluation of the risk at low doses are the possible presence of a threshold dose, below which the effects could not occur, and the shape of the dose response. Both these factors have been considered in earlier reports of the Committee.

3. Proving or disproving a threshold on the basis of epidemiological studies or studies on tumour induction in experimental animals is, for most tumour types, likely to be impossible due to statistical uncertainties in both the spontaneous and induced incidences of the disease. Therefore, on the assumption that cellular targets can be altered by single ionizing events, that such damage is unlikely to be error-free and that it may ultimately give rise to a tumour, it is normally assumed that there is no threshold for the neoplastic response. This working hypothesis is consistent with many, but not all observations of induced cancer rates found in animal experiments as well as with observations in epidemiological studies and is considered in some detail in Annex E, "Mechanisms of radiation oncogenesis".

4. Tumour induction resulting from exposures to ionizing radiation has been systematically examined in studies with various species and strains of animals and

for specific tumour types. Physical factors such as dose, dose rate, dose fractionation and radiation quality, as well as biological factors such as age, gender and species which can modify the tumour yield, have been considered. In the majority of cases, the dose-effect relationships obtained are complex, showing first a rise with increasing radiation doses, a peak or plateau at intermediate doses and in many cases a final decline in incidence at high doses. In a few cases the spontaneous incidence of tumours changes very little with increasing radiation dose, and in some studies where there is a high spontaneous incidence this has resulted in a negative correlation with increasing dose at high doses.

5. The Committee noted in the UNSCEAR 1977 Report [U4] a considerable variability in the net incidence of various tumour types at intermediate to high doses, both between different species and, within species, between inbred strains. In many cases a particular tumour could be induced by irradiation in only one or two strains of a given species, raising questions as to whether such tumours represented adequate models of the corresponding human diseases. Similar doubts also applied to some observed forms of dose-response relationships that differed from species to species, although in other cases consistent patterns were found. For these reasons it was concluded that the absolute excess of radiation-induced tumours per unit of dose could not, as a general rule, be extrapolated between species.

6. Despite these reservations, a number of general conclusions were drawn by the Committee in 1977 [U4] relating to the preponderance of tumour types that show an increasing incidence with increasing dose up to a maximum, with a subsequent decline at higher doses. A number of common features in the dose-response data obtained from experimental animals appeared to be consistent with radiobiological effects occurring in single cells, such as cell killing, induction of mutations and chromosome aberrations:

(a) a decrease in the dose rate of low-LET radiation leads, in general, to a decrease of tumour yield, following some inverse function of the exposure time;
(b) high-LET radiation is generally more efficient than low-LET radiation for tumour induction, and the tumour yield often shows little dependence on dose protraction and dose fractionation;
(c) the relative biological effectiveness (RBE) of high-LET compared with low-LET radiation changes with the dose, reflecting the patterns of the dose-response curves for low- and high-LET radiation. At high sublethal doses (>1 Gy) RBE values as low as 1 have been found, but for various tumour types the RBE generally increases with decreasing dose, approaching a maximum at low doses.

These patterns of dose response for low- and high-LET radiation are illustrated in Figure I.

7. It was generally concluded in the UNSCEAR 1977 Report ([U4], Annex G, paragraph 311) that the risk per unit dose of low-LET radiation at low doses and/or dose rates was unlikely to be higher but could be substantially lower than the values derived by linear extrapolation to the range of a few tens of milligray from observations made above 1 Gy. Reduction factors from 2 to 20 were reported between the highest and lowest dose rates tested (1 to 10^{-4} Gy min^{-1}) and between single and extremely fractionated and protracted doses for various animal strains and tumour types.

8. It was specifically noted by the Committee that the Life Span Study of the survivors of the atomic bombings in Japan followed to 1972 gave a risk of leukaemia of 3.5 10^{-3} Gy^{-1} at a mean kerma of 3.3 Gy and 1.8 10^{-3} Gy^{-1} at a mean kerma of 1 Gy, suggesting a reduction factor of 2 for the risk coefficient at the lower dose as compared with that at the higher dose ([U4], Annex G, paragraphs 317 and 318). The Committee in its final estimate of risk adopted a reduction factor of 2.5 for estimating risks at low doses and low dose rates when extrapolating from high dose and dose-rate studies. The Committee also emphasized that this reduction factor was derived essentially from mortalities induced at doses of the order of 1 Gy and that larger reduction factors may be appropriate for assessing risks from occupational or environmental exposure.

9. On the basis of these conclusions by the Committee [U4], the International Commission on Radiological Protection in 1977 [I1] adopted a reduction factor of 2 for assessing the risk of fatalities from radiation-induced leukaemia and solid cancers for radiological protection purposes from high dose and high dose rate studies.

10. In the UNSCEAR 1982 Report [U3] the Committee reviewed information on causes of death in exposed human and animal populations. It concluded that the overwhelming body of evidence showed that at intermediate and low doses, above about 1 Gy (low-LET) life shortening was essentially caused by an increased incidence of tumours. When the contribution to life shortening by these excess tumours was subtracted from total life shortening, there was no evidence of other non-specific mechanisms being involved. The Committee also examined the effect of radiation quality, as well as dose and dose rate, on life-span shortening and reported some conflicting results. For a given total dose, the chronic exposure of mice to both x and gamma rays was less effective than

acute exposure in causing life shortening, suggesting a dose-rate effect on tumour induction. In mice given single acute doses of low-LET radiation, however, the dose response found in different studies varied widely. By pooling many series of studies, an apparently linear relationship was obtained, which might imply no dose-rate dependence. In practice, however, the data could also be fitted with a linear-quadratic relationship, which would be consistent with the observation of a dose-rate effect.

11. In the case of neutron exposure, some studies reported that fractionated or protracted exposures of animals resulted in reduced life shortening compared with single exposures; other studies reported the reverse. The Committee concluded that such variations in response could be due to differences in dose-effect relationships for different tumours in various strains and species. Thus, although life shortening following exposure to high-LET radiation appeared to be fully explained by a higher incidence of tumours, the effect of dose rate on tumour induction as a cause of death was not clear. Further investigations into the effects of dose and dose rate on life shortening in animals exposed to high-LET radiation were needed.

12. In the UNSCEAR 1986 Report [U2], the Committee reviewed evidence at the subcellular and cellular levels relevant to assessing the possible nature of the dose-response relationships for cancer initiation by radiation. It also examined how the initiation of cancerous clones and their progression to clinical tumours may affect the shape of the dose-response relationship. Finally, it examined various models of cancer induction and tested them for compatibility with epidemiological and experimental findings. This provided the basis for some general conclusions on the shape of the dose response and on the uncertainties involved in the assessment of risks at low doses.

13. Three basic non-threshold models of the effect of radiation as a function of dose were considered with respect to both cellular effects and to cancer induction: the linear, the linear-quadratic and the pure quadratic models. Notwithstanding some exceptions, these relationships provided a general framework for a variety of end-points at the cellular level as well as for tumour induction in experimental animals and human populations. The Committee concluded that the vast majority of dose-response curves for induction of point mutations and chromosomal aberrations by low-LET radiation could be represented by a linear-quadratic model at low to intermediate doses; for high-LET radiation, after correction for cell killing, a linear model usually applied. Linearity of the dose response for somatic mutations and terminal chromosomal deletions in some cell lines was noted even for low-LET radiation, although such findings were relatively infrequent.

14. Cell transformation *in vitro* can be regarded as a simplified model of certain stages of radiation carcinogenesis. Cells exposed *in vitro* to low-LET radiation the day after seeding in culture are transformed according to complex kinetics that cannot always be fitted to models used for other cellular effects such as cell killing and the induction of chromosome aberrations. Moreover, dose fractionation (at total doses <1.5 Gy) in some cases enhances transformation frequency, which is inconsistent with a linear-quadratic dependence unless the dose-squared coefficient is negative. In reviewing this material for the UNSCEAR 1986 Report [U2], the Committee felt that further research was needed to elucidate such phenomena, but it was generally considered that these *in vitro* systems gave anomalous results owing to atypical conditions of cellular growth during the early periods after establishment of the culture. Irradiation of non-dividing cells, or cells under exponential conditions of growth, which may be more typical of asynchronously dividing cell populations *in vivo,* produces results that are more consistent with those obtained for other cellular effects. For example, high-dose-rate gamma-irradiation had resulted in a greater transformation frequency per unit dose than low dose-rate exposure. The Committee also noted that in some studies transformation following dose fractionation or dose protraction of high-LET neutron exposure was enhanced at low to intermediate doses, compared with high doses and high dose rates [U2]. In view of the limited extent of such data and the uncertainties regarding the mechanisms involved, further work was needed before these studies could be properly interpreted.

15. The Committee considered that experimental findings on radiation-induced tumours in animals, mainly rats and mice, published since the UNSCEAR 1977 Report [U4] generally supported the view that dose-response relationships for low-LET radiation tended to be curvilinear and concave upward at low dose rates, although for mammary tumours in rats a linear dose response with little dose-fractionation and dose-rate dependence had been obtained. For tumour induction in animals following neutron-irradiation, the response often gave a nearly linear response at low doses, with little dependence on dose rate. In some cases enhancement upon dose fractionation (and possibly dose protraction) had been noted, and at doses above about 0.1 Gy or so the dose-response curve for acute exposure tended to become concave downward. Under such conditions a linear extrapolation to risks at low doses from information at intermediate or high doses and dose rates would underestimate the risk of tumour induction to a variable degree.

16. Review of dose-response relationships for radiation-induced tumours in man indicated that for low-LET radiation in some cases (leukaemia and

cancer of the thyroid, lung and breast), the data available were consistent with linear or linear-quadratic models. For breast cancer, linearity was considered more probable as the incidence was little affected by dose fractionation. From this review [U2], the Committee concluded that for low-LET radiation linear extrapolation downwards from effects measured at doses of about 2 Gy would not overestimate the risk of breast cancer and, possibly, thyroid cancer and would slightly overestimate the risk of leukaemia. There were insufficient data on lung cancer to permit any assessment of the effect of dose rate on tumour induction. On the basis of data on the incidence of bone sarcomas in experimental animals after intakes of beta-emitting radionuclides, it was considered that linear extrapolation could overestimate the risk of their occurrence at low doses. Dose-response curves for radionuclides with long effective half-times do, however, present great difficulty in interpretation [N9].

17. For radiation-induced cancers of most other organs, only data from experimental animals were available on dose-response relationships. For low-LET radiation, linear-quadratic dose-response relationships are commonly found, with pronounced dose-rate and dose-fractionation effects at intermediate doses. The Committee concluded in the UNSCEAR 1986 Report [U2] that if similar curves applied to cancers in man, a linear extrapolation of risk coefficients from acute doses in the intermediate dose region to low doses and low dose rates would very likely overestimate the real risk, suggesting that a reduction factor of up to 5 might apply.

18. For high-LET radiation, human information for lung cancer and bone sarcoma induction was reviewed [U2]. Although the data were limited, they suggested that for lung cancer induction in miners exposed to radon and its decay products, the response was linear initially; at high exposures, however, because of flattening of the response, linear extrapolation could underestimate the risk. The incidence of bone sarcomas after internal contamination by radium isotopes was interpreted as being distorted by a pronounced inverse relationship between accumulated dose and latent period.

19. On the basis of epidemiological studies and experimental investigations it was recommended in the UNSCEAR 1988 Report [U1] that a reduction factor was needed to modify the risks of cancer calculated from exposures to low-LET radiation at high doses and high dose rates for application to low doses and low dose rates, suggesting that an appropriate value for most cancers would lie in the range 2-10, although no specific values were recommended ([U1], Annex F, paragraph 607). For exposure to high-LET radiation, no dose or dose-rate reduction factor was considered necessary for assessing risks at low doses and low dose rates. The Committee indicated that this was a topic that it would consider in its future programme of work.

20. A number of other organizations have considered the effect of dose and dose rate on tumour induction. These include the United States National Council on Radiation Protection and Measurements (NCRP), the Committee on Biological Effects of Ionizing Radiation (BEIR) of the National Research Council of the United States, the United States Nuclear Regulatory Commission (NRC), the International Commission on Radiological Protection (ICRP) and the National Radiological Protection Board (NRPB) of the United Kingdom. Their estimates of reduction factors for calculating cancer risks at low doses and low dose rates are given in Table 1.

21. In 1980, the NCRP reviewed the influence of dose and its distribution in time on dose-response relationships for tumour induction resulting from exposure to low-LET radiation. It was concluded, largely on the basis of animal studies, that the number of cancers induced at low doses and low dose rates are likely to be lower than they are at high doses and high dose rates by a reduction factor in the range 2-10 [N1]. The BEIR V Committee reached similar conclusions on values for the reduction factor that could be obtained from animal studies [C1]. The NCRP [N1] used at that time the term "dose-rate effectiveness factor (DREF)" for this reduction factor, which has also been referred to as "linear extrapolation overestimation factor (LEOF)" and a "low dose extrapolation factor (LDEF)" [P2, P3]. The NCRP [N1] also concluded that human data were insufficient to allow the shape of the dose-response curve to be established or to provide a basis for confident judgements about any diminution in health risks at low doses and dose rates. In view of the complexity and wide spectrum of tumorigenic responses to radiation found in experimental animals, as well as the lack of information on the detailed mechanisms of such responses in animals or man, more specific reduction factors for either individual tumour types or total tumour incidence were not given.

22. In its 1990 recommendations [I2], the ICRP drew attention to the fact that theoretical considerations, experimental results in animals and other biological organisms, and even some limited human experience suggested that cancer induction per unit dose at low doses and low dose rates of low-LET radiation should be less than that observed after high doses and high dose rates. In making a determination of the appropriate value of a reduction factor to be used for radiation protection purposes, the ICRP considered the following:

(a) the wide range of reduction factors obtained in animal experiments (2-10), which may have been obtained for a broader range of doses than human data and therefore may include higher values than are relevant;
(b) the results of statistical analyses of the data on survivors of the atomic bombings in Japan, which do not seem to allow for a reduction factor of much more than about 2;
(c) the human evidence that shows little effect of dose fractionation for some tumour types, with others indicating possible effects of up to 3 or 4 at most;
(d) reduction factors adopted by other organizations for risk estimation at low doses and low dose rates.

23. Based on these considerations, the ICRP adopted in its 1990 recommendations a reduction factor of 2, "recognizing that the choice is somewhat arbitrary and may be conservative". It was recognized that this recommendation on the reduction factor "can be expected to change if new, more definitive information becomes available in the future". In these recommendations the ICRP called this reduction factor the dose and dose-rate effectiveness factor (DDREF).

24. The Committee has identified the need to keep under review information relevant to the assessment of risks at low doses and low dose rates. This Annex reviews data on dose and dose-rate effects for both high- and low-LET radiation with the aim of improving the basis for estimating risks at low doses and low dose rates. It considers first the role of biophysical models in understanding the response of cells to radiation of different qualities and their application in assessing the effect of dose and dose rate on cellular responses. Experimental data on the effect of dose rate in both experimental animals and cells in culture are then reviewed, with emphasis on studies of the effects of low-LET radiation. Relevant epidemiological data are also summarized.

25. Previous UNSCEAR reports have proposed both doses and dose rates at which reduction factors would be expected to apply. Thus in the UNSCEAR 1986 Report [U2] low doses were taken to be those up to 0.2 Gy of low-LET radiation, while those above 2 Gy were regarded as high doses, with intermediate doses lying between these values. Low and high dose rates were taken to be <0.05 mGy min^{-1} and >0.05 Gy min^{-1}, respectively, with intermediate rates between these two extremes. These upper limits on low doses and low dose rates are substantially higher than those that might be expected to prevail in most cases of human exposure. Thus, the ICRP in 1990 recommended an average annual dose limit for workers of 20 mGy (low-LET) [I2]. The average annual dose limit recommended for members of the public is 1 mGy (low-LET) in addition to exposures to natural background radiation [I2].

26. In practice, the majority of workers receive doses much lower than the recommended dose limits, and actual exposure rates are low (see Annex D, "Occupational radiation exposures"). There will, however, be some individuals (e.g. radiographers in hospitals) exposed over short periods of the working day to substantially higher dose rates than the average, although total doses are low. Lifetime doses for a few workers may also be high even though dose rates are low. Information is, therefore, needed on both total doses and dose rates for which the application of a reduction factor is appropriate. Chapter IV examines the physical, experimental and epidemiological basis for the choice of doses and dose rates below which reduction factors might be expected to apply. The choice of the appropriate unit of time over which to assess dose rate is not straightforward. The experimental data reviewed in this Annex cover a wide range of doses, dose rates and exposure conditions. Cellular studies typically involve irradiation times of minutes to hours, while animal studies can involve exposures of days or weeks. The Committee considers that for assessing the risks of stochastic effects in human populations exposure rates should, in general, be averaged over about an hour, which is in line with the repair time of DNA (deoxyribonucleic acid). However, for consistency and to facilitate the comparison of experiments carried out under different exposure conditions, dose rates are given in this Annex in terms of Gy min^{-1} or mGy min^{-1} as far as is possible.

I. DOSE RESPONSE FROM RADIATION EXPOSURE

A. THEORETICAL CONSIDERATIONS

27. Damage to DNA (deoxyribonucleic acid), which carries the genetic information in chromosomes in the cell nucleus, is considered to be the main initiating event through which radiation causes cancer as well as hereditary disease. Present knowledge on the stages in tumour development is described in Annex E, "Mechanisms of radiation oncogenesis". Damage to the DNA of cells has been directly observed experimentally at absorbed doses in excess of about 1 Gy. The DNA molecule has a double helix structure, and

damage in many forms is observable, including single- and double-strand breaks and base damage [C5, H26, M4, T20]. Damage may be detected, but with greater difficulty, at lower doses (0.05-0.1 Gy) [B13]. Damage to chromosomes in human cells can be observed, either at metaphase or interphase [C9, C10], and has been observed in human peripheral blood lymphocytes at doses down to about 0.02 Gy [L13, L14].

28. The effects of radiation on cellular components are thought to occur either through the direct interaction of ionizing particles with DNA molecules or through the action of free radicals or other chemical products produced by the interaction of radiation with neighbouring molecules. Other more indirect mechanisms have also been proposed. Cells are able to repair both single- and double-strand breaks in DNA over a period of a few hours [B13, M4, M15], but sometimes misrepair can occur. Such damage is thought to be the cause of chromosomal aberrations and may also be the origin of both mutational and cancerous transformations as well as death of the cell [G1, R1, U2, Y2]. Spontaneous single-strand damage can also occur in the absence of radiation or other identifiable insults [B41, L23, S39, V3, V9], but this is unlikely to extend to the full range of types of double-strand, clustered damage that radiation can produce [G21, W9].

29. It is commonly presumed that mutational events in germ cells are due to single biological changes but that carcinogenesis is a multi-stage process in which radiation can induce one or more of the stages involving DNA damage [U1, U2]. Guidance as to likely dose and dose-rate effects may therefore be sought from radiobiological data on the cellular effects that result from DNA damage. General mechanistic concepts derived from these data have had a considerable influence on attempts to understand and extrapolate available data on carcinogenesis. It should be recognized, however, that the cellular data are mostly for single-stage radiation effects, principally related to initiation, and that they therefore represent only a part of the complex process of carcinogenesis.

30. It is usually assumed that the primary mutagenic and carcinogenic effects of radiation arise as relatively rare stochastic consequences of damage to individuals cells. Insult from ionizing radiation is always delivered in the form of separate charged particles traversing the cells, each leaving behind a "track" of ionized molecules. Each discrete track consists of the stochastic spatial array of initial ionizations and excitations of molecules in the cell along the path of a primary charged particle and all its secondaries as they pass through the cell in $\leq 10^{-12}$ s [P9, P10]. The pattern of ionizations in each track is governed by cross-sections (probabilities) for individual molecular interactions. Each track is therefore different but has statistical features characteristic of the radiation. On the nanometre scale of DNA and radical diffusion distances in cells, many of the individual ionizations are likely to occur alone and far from any others in the same track, especially for low-LET radiations. However, many other ionizations occur in clusters of dimensions comparable to those of DNA. This clustering is particularly marked for high-LET radiations, but it is also common in tracks of low-LET radiations, largely because of the likelihood of low-energy secondary electrons being produced within the cell [B25, G6, G16, M27, N10]. Because the radiation insult is always in the form of discrete tracks, the radiobiological process may be described in terms of damage to particular target material, using concepts of target theory. In its general form, target theory assumes that the observed all-or-nothing effect is caused by one or more radiation tracks passing through the cell and directly or indirectly causing specific damage to critical components within it. Almost all biophysical models of radiation action incorporate at least some essential concepts of target theory. Model descriptions of the possible radiobiological mechanisms are usually constructed on selected assumptions and deductions [E2, G3, G5]. An approach based on the general concepts of target theory can describe essential elements of the mechanism of radiation insult in an approximately model-independent way. It can indicate how biological processes may modify the simplest responses and how there may be a dependence on physical parameters such as dose rate. This description could apply to any single radiation-induced stage of the multi-stage process of carcinogenesis and to some combinations of stages. Within this general description many specific models can and have been constructed, based on their own specific mechanistic or phenomenological assumptions (see [B33, C5, G17, G22, H23, K5, K6, M34, M39, R12, T21]).

1. Single-hit target theory

31. In the simplest form of target theory, a direct "hit" of any type (i.e. one or more ionizations) in a critical component by a radiation track is assumed to lead, with certainty, to the observable effect in that cell. In this case, the frequency of affected cells in an irradiated population of cells should increase with dose according to the probability of a cell receiving one or more critical hits. Assuming that the hits occur randomly according to a Poisson distribution in a homogeneous population, then the frequency, f, of cells with one or more hits is

$$f = 1 - e^{-n} = 1 - e^{-\lambda D} \qquad (1)$$

where n is the mean number of critical hits per cell at dose D and λ is the mean number of critical hits per

cell per unit dose. For small n (that is, low frequency effects and/or low doses), the dose response is approximately linear, with

$$f \approx \lambda D \qquad (2)$$

At higher frequencies the dose response takes the form of equation 1, which saturates at high frequency because, after the first critical hit in a cell, subsequent hits in it cannot lead to additional effect. If a negative effect is being measured, such as frequency of surviving cells (that is, cells without a critical hit), then the dose response is

$$f^1 = 1 - f = e^{-\lambda D} \qquad (3)$$

where f^1 is the frequency of cells without a critical hit. This very simple form of target theory, where every elementary hit is biologically effective, may be applicable to the inactivation of many molecules in the dry state and to some viruses, but it is not, in general, appropriate for micro-organisms and mammalian cells, because of their well-established capacity to repair radiation damage and the consequent modification of dose response.

32. More refined forms of single-hit target theory could include variable probability of effect depending on the type ("severity") of a hit and on the cellular reparability of the damage and could also include extension of the size of the target for indirect effects. Provided that the tracks act totally independently of one another in regard to each of these processes, the dose response should still conform to equations 1-3 because the final effective damage should still be randomly distributed among the cells [L3]. The numerical value of λ should now be modified to reflect the combined probability, per cell and per unit dose, of all these single-track processes leading to final effective damage. Indirect effects should increase the value of λ, while biochemical repair should reduce it. Therefore, experimental observation of linear or exponential dose response does not, of itself, indicate that damage cannot be modified and/or repaired by the cell.

2. Multi-track effects

33. Further extension of concepts of target theory can consider additional contributions from two or more tracks, which may modify the probability of effect due to the damage from single tracks alone. Since this modification may be positive or negative, it may introduce corresponding visible curvature to the dose response. Ways in which multiple tracks could increase the probability of effect include the following:

(a) reduction in efficiency of cellular repair of individual points of damage by increasing the overall burden of damage (for example, by partial saturation of the repair process [G17, S40, W10] or induction of damage-fixation processes);
(b) interaction or interference between points of damage to make them less repairable [C5, C28, K6] (for example, formation of exchange events within or between chromosomes [H23]);
(c) production of a series of other independent changes that together increase the overall probability of the final effect (for example, to cause a single-stage effect [K5] or to cause multiple stages in full neoplastic transformation [M39]).

By contrast, decreases in the probability of effect by multiple tracks could occur by the following means:

(d) enhancement of cellular repair (for example, induction of additional repair capabilities [B37, G5, O3, P8]);
(e) elimination of some of the cells from the population by transferring them to a state in which the effect cannot be expressed (for example, by loss of cell viability).

Other processes, such as multi-track perturbation of the cell cycle, have the potential either to increase or to decrease the probability of effect. The reduction of dose rate increases the time intervals between tracks and therefore is likely to alter the contributions from these multiple-track processes.

34. Simple mathematical extension of equations 1-3, now to include multi-track effects, may be made by means of a general polynomial. Those equations are therefore replaced in general by

$$f = 1 - e^{-(\alpha_1 D + \alpha_2 D^2 \ldots)} \qquad (4)$$

For low-frequency effects, the equation is

$$f \approx \alpha_1 D + \alpha_2 D^2 \ldots \qquad (5)$$

and for negative effects it is

$$f^1 = 1 - f = e^{-(\beta_1 D + \beta_2 D^2 \ldots)} \qquad (6)$$

designating the coefficients as β_1 and β_2 to denote that these are negative effects.

35. Attempts to interpret and apply the coefficients α_1, α_2, ... and β_1, β_2, ... must usually rely on particular assumptions of radiobiological mechanisms. Many investigations, including experiments, theory and model formulations, are aimed at identifying the assumptions that may be most reasonable under given circumstances. Without such mechanistic considerations, the coefficients provide no more than fitted values, which may be valid only in the limited range of the experimental data themselves. Quite different mechanistic assumptions can lead to equations such as

equations 4-6, either directly or as the first terms of polynomial expansion approximations. For example, a dose-squared term can arise directly from two tracks damaging separate chromosomes, which then undergo an exchange interaction, or from two tracks creating deletions in complementary chromosomes, causing the loss of both alleles of a gene. By contrast an apparent dose-squared term can arise from multiple tracks increasing the overall burden of damage in a cell and thereby partially saturating a repair system and reducing the probability of repair of particular damage from any one track [G17, S40, W10]. The reliability of extrapolations to low doses, below the range of the fitted data, may depend substantially on the appropriateness of the mechanistic assumptions for these single cellular effects as well as their relevance to carcinogenesis. For example, for exchange aberrations there would be a clear expectation of a substantial linear term to the lowest doses owing to the ability of a single track to damage two separate chromosomes; such a one-track occurrence would be much less probable for a deletion of two identical alleles, but much more probable for a single deletion, which alone may be adequate to enhance carcinogenesis. In the case of saturable repair, extrapolation to low doses would depend largely on the competition between repair and fixation/misrepair processes and whether any types of damage are essentially unrepairable.

36. When the equation is applied to describe a low-frequency effect, such as carcinogenesis or mutagenesis, arising from a given initial population of cells, it may be convenient to separate out the influence of radiation-induced loss of cell viability by replacing equation 5 with

$$f \approx (\alpha_1 D + \alpha_2 D^2 + ...)S(D) \qquad (7)$$

where S(D) is the fraction of cells which survive dose D. S(D) itself may be described by the form of equation 6. With high-LET radiation, it may be necessary to consider also correlations between induction of the initial carcinogenic damage and loss of cell viability by the same radiation track [G15]. Additional non-linearity may arise if adjacent cells can be involved in control of the growth of an altered cell.

37. It is frequently found that only the first two terms of the polynomials in equations 4-7 are needed to describe the experimental data. Most effects on cells (e.g. chromosome aberrations) resulting from low to intermediate doses are fitted, therefore, to a linear-quadratic equation without including powers of dose greater than D^2. This simplification may be reasonable for radiobiological mechanisms underlying some of the possible multi-track processes described in paragraph 33, particularly under processes (a) and (b) and especially if only two-track interactions occur. A two-term polynomial is unlikely, however, to be adequate to describe and interpolate over the full dose response, if it includes processes (c) and (d). From reviews of published data it can be deduced that considerable differences are observed between cells of different origins with respect to the values of α_1 and α_2 [B6, B7, T6]. For a dose response that can be fitted with only the first two terms in the polynomials in equations 4-7 the quotient α_1/α_2 equals the dose at which the linear and quadratic components contribute equally to the observed cellular damage.

38. An example of the type of response of equation 7 is provided by observations of myeloid leukaemia frequency in male CBA/H mice after 10 different doses of x rays in the range 0.25-6 Gy inclusive, delivered at 0.5 Gy min^{-1} [M14] (Figure II). Median survival in all groups was similar, and there was essentially no association between induction period and dose. The results were fitted by a four-term polynomial of the form

$$(\alpha_1 D + \alpha_2 D^2)\, e^{-(\beta_1 D + \beta_2 D^2)} \qquad (8)$$

and four simplifications of it. The only functions with all parameters significantly greater than zero were:

$$\alpha_2 D^2\, e^{-\beta_1 D} \quad \text{and} \quad \alpha_1 D\, e^{-\beta_2 D^2} \qquad (9)$$

The latter function was rejected because no cell survival response depending solely on D^2 is known. The observed data could therefore be well fitted by the function

$$\alpha_2 D^2\, e^{-\beta_1 D} \qquad (10)$$

although none of the alternative functions could be rejected on statistical grounds. A similar dose response for myeloid leukaemia induction in CBA mice was reported by Di Majo [D2].

3. Low doses and low dose rates: microdosimetric considerations

39. For a homogeneous cell population, the dose response for an effect arising solely from a single track interacting independently with cellular target(s) should conform with equation 1 and should be simply linear with dose (equation 2) if the frequency of effect is small. It should extend linearly down to zero dose, with no threshold, because reducing the dose simply reduces the number of tracks proportionately and, consequently, the frequency of effect. The dose response should be independent of dose rate, because the time interval between tracks is irrelevant if the tracks are acting totally independently. There may, of course, be many other interactions that are adequately repaired and do not manifest themselves as damage.

40. If the cell population is inhomogeneous, with subpopulations of differing sensitivity, the dose response for single-track effects in each subpopulation should follow the form of equation 1, and the overall response should therefore show a decreasing sensitivity with increasing dose. Any other deviations from the form of equation 1 must be due to the effect of multiple tracks in some way or another. These deviations, however, need not be obviously apparent over all portions of the dose response. Hence, apparent linearity over an experimentally accessible portion of the dose response does not guarantee that only single-track processes are involved in that region or that extrapolation to lower dose is linear. In general, it is expected that multi-track processes may depend on dose rate as the mean time interval between tracks is varied. Referring to the example above, simple expectations are that a reduction of dose rate would reduce the effectiveness of radiation acting via processes (a) and (b) (paragraph 33). Predictions for the other processes are less clear, because they are likely to depend on the timings of the particular processes in relation to the intervals between tracks and the overall irradiation time. For most single-stage processes, it may be expected that at very low dose rates multi-track effects will become negligible, because the tracks become effectively independent in time; in this limit the dose response should conform to equations 1-3.

41. Available experimental and epidemiological data on radiation carcinogenesis can be considered in terms of three regions of the dose response on the basis of fundamental microdosimetric considerations assuming that the cell nucleus is the relevant sensitive volume to define the limit of possible multi-track effects. These are illustrated in Figure III by schematic dose-response curves, consistent with the form of equation 7, for frequency of an effect such as a type of tumour induced by gamma rays, neutrons or alpha particles. The upper part of the Figure shows the response plotted against dose on a linear scale. The lower part shows the identical curves plotted on a logarithmic scale to magnify the lower dose region; on this part a separate dose axis is provided for each radiation type. The logarithmic plot also marks on a common axis the mean number of tracks per cell nucleus (assuming spherical nuclei of 8 μm diameter for these illustrations). In this way, the correspondence between dose and number of tracks can be read off for each radiation. This correspondence has been calculated [C25, G5] by established microdosimetric methods based on experimental and theoretical data [B42, C24, G2, G4, Z2]. In this Figure the dose scale is divided into three approximate dose regions, as described below.

42. *Dose region I (low-dose region).* In this dose region there are so few radiation tracks that a single cell (or nucleus) is very unlikely to be traversed by more than one track. In this region of "definite" single-track action (less than ~0.2 mGy for ^{60}Co gamma rays; see Section IV.A), the dose response for single-cell effects is almost bound to be linear and independent of dose rate. This is because varying the dose proportionately varies the number of cells singly traversed, and varying the dose rate varies only the time between these independent events. These simple expectations would be violated only if the rare multi-traversals greatly enhanced the probability of effect, such as may be the case if radiation carcinogenesis requires two radiation-induced stages well separated in time. There are no epidemiological or experimental data in or near this region for low-LET radiation, although a few may approach it for high-LET alpha particles and neutrons [C25, D5]. There is, therefore, little direct information about how a cell or a tissue may respond to the damage from a single radiation track.

43. This is, however, the region of main concern in radiation protection. For example, a worker who has received uniform whole-body gamma-ray exposure spread over a year equal to an annual equivalent dose limit of 50 mSv [I1], corresponding to an absorbed dose of 50 mGy (Q = 1), will have received over the full year an average of about 50 electron tracks through each cell nucleus in his body. Multi-track processes should then be relevant only if they operate over long periods of time comparable at least to the times between tracks (days). If, instead, the irradiation is uniform with only 1 MeV neutrons (and ignoring attenuation, energy degradation or gamma-ray production in the body), then the 50 mSv limit corresponds to 5 mGy of neutrons (Q = 10) and an average of about 0.05 directly ionizing tracks (mostly high-LET recoil protons from the indirectly ionizing neutrons) through each cell nucleus during the year. These tracks must clearly act independently unless multi-track processes persist over very long periods of time, extending to many years. Exposure is seldom uniform in the body or in time, and cell nuclei have a variety of sizes and shapes. Nevertheless, the dose and dose-rate region of main practical relevance in radiation protection (0-50 mSv per year) is characterized by small average numbers of tracks per cell with long intervals of time between them. Effects are, therefore, likely to be dominated by individual track events, acting alone. This dominance will be even greater with the introduction of the ICRP recommendations of 1990 [I2] which propose an average annual limit of equivalent dose of 20 mSv and increased radiation weighting factors for neutrons (>100 keV to 2 MeV, w_R = 20).

44. For the purposes of this microdosimetric criterion for a low dose, the cell nucleus (approximated here as an 8 μm diameter sphere) has been considered to be the sensitive volume in which multiple tracks may be

able to influence the effects of one another. This choice is based on the assumption that radiation carcinogenesis is due to radiation damage to the nuclear DNA of a single cell and that biochemical processes, including repair and misrepair, may operate over the full dimensions of the nucleus. If influence can extend over larger distances, say from tracks in the cell cytoplasm or in adjacent cells, then the microdosimetric criterion for a low dose would need to be decreased. Conversely, if smaller regions can act totally autonomously in respect of initial radiation damage and its repair or misrepair, then the criterion would be increased. In the extreme, if each short (say, 6 base pair) segment of DNA were totally autonomous for damage and repair, then the microdosimetric criterion for a low dose of low-LET radiation would be as large as 10^9 mGy [G6]. This is clearly much too large compared to the doses at which multi-track processes have been observed experimentally by curvature of dose-response or dose-rate dependence in cellular and animal systems (Chapter II). Criteria for designating low doses and low dose rates are discussed further in Chapter IV.

45. *Dose region II (intermediate-dose region).* In this dose region it is commonly assumed that tracks act independently if a linear term (α_1) is obtainable by curve-fitting to equations such as 4-7. However, for most of the epidemiological and experimental animal data used for dose-response curve fitting, the lowest dose at which a significant effect is obtained is usually towards the higher doses of this dose region, when individual cells may, in fact, have been traversed by considerable numbers of tracks.

46. 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 efficiency of these processes and, therefore, the expression of the ultimate biological damage of each individual track. This region of the dose response, then, should be independent of dose rate. On these assumptions, it is conventional to interpolate linearly from this region to zero dose in order to deduce the effectiveness of low doses and low dose rates of radiation, dose region I. Such interpolation is based on the coefficient α_1 in equations 4-7 and on the assumption that it remains unchanged even to very low doses and very low dose rates in dose region I. There are a number of radiobiological studies, mostly with cells *in vitro*, but also from animals exposed at different dose rates, which suggest that this common assumption is not universally valid (see Sections I.A.4, I.A.5, II.A. and II.B).

47. *Dose region III (high-dose region).* In this dose region, there are often clearly observable multi-track processes causing upward or downward curvature of the dose response, including cooperative effects and also competing processes such as cell killing. Dependence on dose rate is, therefore, usually to be expected because of time dependence in the multi-track process. Mechanisms in this dose region need to be adequately understood and described if such data are to be used for curve-fitting and extrapolation, together with data from dose region II, to the low doses and low dose rates of prime relevance in radiation protection.

4. Radiation quality and relative biological effectiveness

48. A very wide range of radiobiological data on the doses required to produce a given level of effect have shown that high-LET radiation, including neutrons and alpha particles, is more effective than low-LET radiation [S12]. This greater effectiveness is usually particularly marked in the regions of intermediate and low dose, which implies that the individual high-LET tracks have a greater probability of effect than a very much larger number of low-LET tracks. Thus the concentration of energy deposition within the high-LET tracks more than compensates for the reduced number of tracks per unit dose.

49. The relative biological effectiveness (RBE) values of particular relevance in radiation protection are those that apply in the true low-dose region I, in which tracks are most likely to act individually. At these minimally low doses the RBE of a given radiation should be constant and independent of dose and dose rate, because varying the dose for both high- and low-LET radiation varies only the number of cells that are traversed by single tracks. This RBE, at minimal doses, could in principle be calculated by direct comparison of measured effectiveness per unit dose of neutrons and low-LET radiation in the low-dose region I, or from experimental measurements of the effectiveness of single tracks of the radiation. Current experimental methods have not been able to achieve this.

50. Instead, it is conventional to assume that the RBE at minimal doses is also the maximum RBE and that it can be estimated as the ratio of the α_1 values of the two radiations, determined by fitting equations such as 4-7 to the available data at intermediate and high doses. This method assumes that the multiple tracks in the intermediate-dose region do not influence the effectiveness of each other at all and, consequently, that the α_1 values are constant down to zero dose and independent of dose rate. This assumption is best supported for high-LET radiation, for which *in vitro* radiobiological data usually show strongly linear dose responses that vary little with dose rate, with some data approaching the true single-track region. Notable exceptions have been reported, however, in

cellular, animal and human systems (see, e.g. [C22, C25, D5, F9, F10, J2, K8, H9, M28, R13, S13, T7, T8, U16]). The assumption of constant α_1, independent of dose rate, for low-LET reference radiation is also called into question by data from numerous studies on cellular, and some animal, systems (e.g. [B34, C12, C20, F1, F10, F13, F4, I8, K8, M16, M33, O4, S3, S14, S32, T2, W6]). The general approach of estimating risks of high-LET radiations by means of RBE values would not be applicable to effects that were qualitatively different for, or unique to, high-LET radiations. There are indications that such unique effects may arise in some cellular systems, including the induction of sister chromatid exchanges by irradiation of human lymphocytes before stimulation [A11, A4, S42] and the radiation induction of chromosomal instabilities in haemopoietic stem cells [K9]. There are also indications of qualitative differences in early cellular changes during the development of mammary tumours in mice [U25] and in other *in vivo* effects [H33].

5. Deviations from conventional expectations

51. The conventional approach to estimating both absolute biological effectiveness and relative biological effectiveness at minimal doses is based on the assumption of constant α_1 values from dose region II down to zero dose and independence of dose rate. There are, in principle, many ways in which this may not be the case.

52. For single-cell effects, the assumption may not hold if there are significant multi-track processes in the intermediate-dose region. Such processes could include, for example, the induction of multiple independent steps in radiation carcinogenesis, cellular damage-fixation processes and the induction of enhanced repair by small numbers of tracks. There is strong evidence of induction of repair or amplification of gene products in microbes [S41] and some such indications in mammalian cells [L15, W6]. Possibilities that have been suggested to explain observed dose-rate dependence of neutron-induced cell transformation include promotion by multiple tracks or enhancement of misrepair [H10], variations of cell sensitivity with time [B30, R5] and induction or enhancement of repair [G5].

53. The general approach described in this Annex would also need appreciable modification if the biological effect of interest required damage to more than one cell or if it is influenced by damage to additional cells. For example, van Bekkum et al. [V1] and Mole [M14] have hypothesized that radiation tumorigenesis involves the transfer of DNA from one radiation-inactivated cell to an adjacent radiation-damaged cell. In this case the true low-dose region I of action by individual tracks alone would correspond to even lower doses than in Figure III, because the volume containing the target would need to be enlarged to include adjacent cells. This two-cell hypothesis could be experimentally testable with epithermal neutrons, whose individual proton-recoil tracks are too short to hit the nuclei of two adjacent cells [G5].

54. Some of the above processes allow, in principle, for the possibility of a true threshold in the dose-effect curve, especially for low-LET radiation. The most basic, although not sufficient, condition for a true dose threshold is that any single track of the radiation should be totally unable to produce the effect. Thus, no biological effect would be observed in the true low-dose region (region I), where cells are hit only by single tracks. There is little experimental evidence to demonstrate such a situation, although collaborative studies in six laboratories on the induction of unstable chromosomal aberrations in blood lymphocytes given acute doses of x rays of 0, 3, 5, 6, 10, 20, 30, 50 and 300 mGy were able to demonstrate significant increases in aberration yield at doses greater than 20 mGy. Below 20 mGy the observed dicentric yield was generally lower than in controls, but not significantly so. Excess acentric aberrations and centric rings, on the other hand, were higher than in controls, although the increase was generally not significant. It was concluded that even though these studies involved scoring chromosome aberrations in a total of about 300,000 metaphases some variation was observed between the different laboratories involved, and the lack of statistical precision did not allow linear or threshold models at doses below 20 mGy to be distinguished [L14]. Data on the induction of stable chromosome aberrations in blood lymphocytes from individuals of various ages have also been reported [L1] for doses in the range 50 to 500 mGy. In lymphocytes from newborns chromosome aberrations increased roughly in proportion to the dose. In young adults, however, aberrations were not detected at doses of 50 and 100 mGy and for adults not even at 200 mGy. The difference in detection of aberrations was attributed to a high background of aberrations in older ages, compared with the newborn. Of the aberrations examined, one-break terminal deletions were the best indicators of exposure at low doses.

B. MULTI-STAGE MODELS OF CARCINOGENESIS

1. Multi-stage models

55. In order to become fully malignant, a cell needs to undergo a number of phenotypic changes (see Annex E, "Mechanisms of radiation oncogenesis").

Evidence from diverse sources suggests that changes can be considered as occurring in many stages. This Section describes quantitative models that have been previously developed for multi-stage carcinogenesis. The true nature of the individual biological changes is considered in more detail in Annex E.

56. The first stochastic multi-stage model for the development of full malignancy from a normal cell was proposed in 1954 by Armitage and Doll to account for observations that the age-specific incidence rates for many adult carcinomas were proportional to a power of age [A8]. According to this model, which has been widely used in risk assessment, a normal cell can undergo progressive deterioration in a finite number of stages to reach full malignancy. The authors later proposed a two-stage model in which cells multiply exponentially after undergoing the first change and become malignant after the second change [A9]. A similar two-stage model was proposed for carcinogenesis in animals [N8].

57. None of the above models take into account the growth and development of the normal tissue. A model that does include growth and differentiation was proposed by Knudsen et al. for embryonal tumours [H24, K7]. This model has subsequently been developed to a form that is claimed to give a good qualitative description of the age-specific incidence curves of all human tumours [M17, M38] and an excellent quantitative fit to incidence data for several human tumours that were tested [M42]. The working hypothesis underlying this model is based on a genetic regulatory schema postulated by Comings in 1973 [C27]. However, the formalism and parameters of the model are not dependent on the particular biological identities of the critical targets and changes. According to the schema all cells contain genes, termed "oncogenes", capable of coding for transforming factors that can release the cell from normal growth constraints. The oncogenes are expressed during histogenesis and tissue renewal and are normally controlled by diploid pairs of regulatory genes, termed "anti-oncogenes". A cell acquires the malignant phenotype when an oncogene is expressed at an appropriately high level, owing either to inactivation of both of the appropriate pair of anti-oncogenes or by direct activation of the oncogene itself. This latter may occur, for example, if the oncogene becomes positioned adjacent to a promoter as a result of chromosomal rearrangement or viral insertion. This two-stage model presupposes that human tumours most commonly arise by mutations of the anti-oncogenes. Evidence for this process of carcinogenesis comes from, among other things, analyses of familial tumours, such as childhood retinoblastoma and Wilms' tumour and adult familial polyposis carcinoma of the colon. Studies of these tumours indicate that an inactivated anti-oncogene can be inherited, which means that it is present in this stage in all cells of the individual, greatly increasing the potential for malignancy to develop. Nevertheless, in such an individual at least one other event is necessary for malignancy. In a normal individual, whose cells carry only normal pairs of anti-oncogenes, at least two changes should be necessary. It is recognized that this two-stage model may not apply to some tumours that are due to direct oncogene activation and that may be characterized by specific chromosome rearrangements, possibly including lymphoma and leukaemia [M42].

58. In this two-stage model, agents that act as mutagens, to increase the probability of inactivating either one or both of the anti-oncogenes, may be regarded as tumour "initiators". Tumour "promoters" may be assumed to modify cell kinetics and in particular to encourage clonal expansion by greater mitotic activity of cells that have undergone the first-stage change, thereby increasing the chances that at least one of them subsequently undergoes the second change and hence becomes malignant [M41, M42].

59. To apply these multi-stage models to environmental mutagens, one or more of the rates of mutation, or of other changes, may be made a function of dose [M38]. Dose rate or duration of exposure would also need to be considered in relation to the kinetics of the normal and changed cells [M39]. When the two-stage model was fitted to data on lung cancer in mice exposed to a single acute dose of gamma rays, the results were consistent with the hypothesis that brief exposure to radiation acts by enhancing the rate of the first mutation in a proportion of the cells [C21]. It might be expected that subsequent exposure, either by protraction or as a later brief second exposure, would also be capable of inducing by chance the second mutation in those few cells that had undergone the first mutation.

60. A two-stage model of induction of osteosarcoma by alpha particles was formulated by Marshall and Groer [M34] to fit the entire dose-time-response data from radiation in man and dog. The model assumed two alpha-particle-induced initiation events and a subsequent promotion event not related to radiation. Competition by alpha-particle killing of cells was included. The model predicted that the tumour rate should become independent of dose rate at less than 10 mGy d^{-1} and that over the lower dose range of the available data the rate would be proportional to dose squared and at high doses become independent of dose (plateau). On the assumption that the two initiation events arise from damage to two different structures in the cell (rather than to one structure that must be damaged at two separate times), it was concluded that at doses of less than ~400-1,000 mGy the tumour rate

would be predominantly due to a linear component of dose, because both structures are damaged by a single alpha-particle track [M34, M35]. In an earlier two-stage model for radiation carcinogenesis, Burch [B35] assumed that the two changes (regarded as chromosome breaks) needed to be caused by radiation at different times and, as a consequence, the tumour rate at low doses depended purely on the dose squared.

61. The net effect of protracting of radiation exposure would generally be difficult to predict from multi-stage models, because it would depend on a complex combination of the effect of dose rate on each individual mutagenic or other change; the cell kinetics, and therefore cell numbers, between the changes; and whether or not there is a preferred or required temporal relationship between the changes themselves. Even within the relative simplicity of a two-stage model, clear expectations for dose and dose-rate dependence would require determining numerous parameters of the model, including their radiation dependence (dose, dose rate, quality), for the particular cancer [L24]. There is clearly scope, in principle, for expectations of reduced effectiveness at reduced dose rates, owing for example to reduced mutation rates at each stage or to selective disadvantage in growth kinetics for cells that have undergone the first change relative to normal cells. Conversely, there is also scope for expectations of increased effectiveness at reduced dose rates, due for example to increased rates of mutation at each stage (by analogy, perhaps, with the increased transformation and mutation rates reported with high-LET radiation in some *in vitro* systems [H9, J2, M28, R13]) or to selective advantage and clonal expansion of cells that have undergone the first change. The range of possible expectations becomes even wider in the likely event that carcinogenesis depends on more than two stages, particularly if radiation as well as other environmental or spontaneous factors can play a role in a number of these changes.

2. Thresholds in the dose-effect relationships

62. A necessary, but not sufficient, condition for an absolute threshold in the radiation dose-effect relationship is that any single track produced by the radiation is totally incapable of producing the biological effect. This absolute criterion can be considered at three levels of changes in the carcinogenic process: the initial elementary physico-chemical changes to biological molecules, the repairability and combinations (if any) of molecular damage required to produce single-stage cellular changes, and the combinations (if any) of separate cellular changes required for a cell to reach full malignancy. Even when the criterion does apply, multi-track effects may be sufficient to preclude a true threshold, although the dose response should then tend to zero slope as the dose tends to zero.

63. Biophysical analyses based on Monte Carlo simulations of radiation track structure show clearly that all types of ionizing radiation should be capable of producing, by single-track action, a variety of damage to DNA, including double-strand breaks alone or in combination with associated damage to the DNA and adjacent proteins [C26, G6, G20]. In essence, this is because all ionizing radiation produces low-energy secondary electrons, and these can cause localized clusters of atomic ionizations and excitations over the dimensions of the DNA helix. Hence, for these types of early molecular damage there can be no real prospect of a threshold in the dose-response relationship for any ionizing radiation. This statement is even more categorical for high-LET radiation, which is capable of producing even greater clusters of ionizations and excitations over the dimensions of DNA and its higher-order structures (see Table 2 [G6, G20]).

64. Expectations of a dose threshold for cellular effects depend on the assumptions that are made regarding cellular repair and the combinations of molecular damage that are required to cause the effect. Very many different mechanistic biophysical models have been proposed to explain radiation-induced cellular effects such as cell inactivation, mutation and chromosome aberrations. Some of these models have been summarized by Goodhead [G3, G19]. There has developed from these models a near-consensus that the biologically critical damage by which single tracks can lead to cellular effect is dominated by local properties of the track structure over dimensions of 0.1-50 nm. The mechanistic models variously assume that the cellular effect is the result of the following: DNA double-strand breaks either singly [C5, C23, R12], both singly and in pairs [P7] or in larger numbers [G22]; pairs of DNA single-strand breaks [R15] or simple damage to pairs of unspecified atoms such that the damage to each is due to single ionizations or excitations only, independent of radiation quality [K6, Z1]; pairs of unspecified chromatin damage [H23, V4]; localized clusters of radiation damage in unspecified molecular targets, either singly or in targets of dimensions similar to DNA [G16, G20] or nucleosomes [G18, G20] depending on radiation quality, or singly and in pairs in targets of unspecified dimensions [C28]; unspecified single or double lesions, probably in DNA, but qualitatively similar independent of radiation quality [T21]; multiple (two or more) ionizations in small structured targets [B36]; or damage to DNA and associated nuclear membrane [A7]. In all these mechanistic models a single radiation track from any radiation is capable of producing the full damage and hence the cellular effect.

65. In agreement with these mechanistic models, track structure analyses, as well as simple linear extrapolations to low dose of measured biochemical damage, indicate that a single track, even from the lowest-LET radiation, has a finite probability of producing one, or more than one, double-strand break in a cell (Table 3). Hence, cellular consequences of a double-strand break or of interactions between them should be possible even at the lowest doses or dose rates. This expectation would be contradicted for low-LET radiation only if cellular repair of small numbers of double-strand breaks, even with associated damage, were totally efficient in all the cells. There is no evidence to demonstrate this, but existing experimental assays are not able to test it extensively due to limited resolution of types and quantities of damage.

66. In addition to mechanistic models of cellular effect, as above, there are current phenomenological models based on correlation of effect with patterns of radiation energy deposition over much larger distances of ~1μm [B33, F12, H25, K5]. Even these approaches, with one exception [H25, K5], agree that a single radiation track can produce the cellular effect. The one exception agrees for high-LET radiation, but it assumes that for low-LET radiation the damage from a number of tracks has to accumulate before any cellular effect is possible. This assumption leads to an initial slope of zero, although not a true threshold. Experimental support for this assumption is lacking.

67. Despite their very different assumptions and almost without exception, these biophysical models lead to the common view that a single track of any ionizing radiation is capable of producing cellular changes, including mutations and chromosome aberrations. On this basis no absolute dose threshold would be expected for the individual cellular changes responsible for individual stages of the carcinogenic process. The difficulty of experimentally proving, or disproving, this expected total lack of a dose threshold for single cellular changes is complicated by the possibilities of adaptation or induced repair after small numbers of tracks [C20, I8, M33, O3, O4, P8, S3, S32, W6]. However, unless such "adaptation" is so fast that it can act with total efficiency on the very first track itself, it would not be able to introduce a true dose threshold, although it might complicate the shape of the dose response at slightly higher doses and also its dose-rate dependence.

68. The final level at which an absolute dose threshold might exist is at the two (or more) stages of two-stage (or multi-stage) carcinogenesis. The simplest such situation would arise if the malignancy required that radiation should bring about both changes and that they should be well separated in time. Then, one track would be totally unable to achieve this, and so even would any single, brief exposure. If the exposure were protracted or repeated, or if the time separation were not required, but if a single track were still incapable, then the slope of the dose response would tend to zero as the dose tended to zero (as, for example, in a pure dose-squared dependence). Although this would not imply a true threshold, the risk would become vanishingly small at the lowest doses. There are, however, many ways in which these requirements could, in principle, be violated and thereby introduce a finite slope without a threshold or vanishing risk. These include:

(a) if malignancy could result from the two essential changes occurring at the same time from a single track, especially if it were a high-LET track;
(b) if one or other of the two changes could occur spontaneously, or as a consequence of other environmental factors, so that only one radiation-induced change was necessary, as suggested, for example, when the two-stage model was fitted to lung tumours in mice after brief exposure to radiation [C21]; the occurrence of spontaneous tumours does also indicate that all the changes can occur without radiation;
(c) if the cells of an individual already had one change due to inheritance so that only one radiation change was sufficient for malignancy;
(d) if the malignancy could result from a single radiation-activated oncogene instead of solely from a pair of inactivated anti-oncogenes;

69. In view of these many possibilities, it would be difficult to conclude on theoretical grounds that a true threshold should be expected even from multi-stage mechanisms of carcinogenesis, unless there were clear evidence that it was necessary for more than one time-separated change to be caused by radiation alone. The multitude of animal and human data showing an increase in tumours after a single brief exposure to radiation and also the occurrence of spontaneous tumours in the absence of radiation, implies that these restrictions do not apply in general. These theoretical considerations cannot preclude the possibility of particular situations where the probability of an effect at low doses may be very small, and even practically negligible, compared with that at higher doses. This topic is considered further in Annex E, "Mechanisms of radiation oncogenesis".

C. MECHANISMS OF DOSE-RATE EFFECTS IN MAMMALIAN CELLS

70. For low-LET radiation, dose rate has been shown to be a major factor in the response of mammalian cells. Since the early days of cellular radiobiology, the sparing effects of dose protraction have been interpreted as reflecting increased repair of induced cellular damage. The magnitude of dose-rate effect for cell

inactivation varies between different cell strains; this is reflected usually, but not always, by the extent of the shoulder on acute dose-response curves [H2].

1. Repair of DNA damage

71. There is strong evidence from a range of *in vitro* cellular studies that the most significant detrimental effects of radiation derive from its ability to damage DNA in mammalian cells (see Annex E, "Mechanisms of radiation oncogenesis"), and if this is the case then it can be assumed that the fidelity of repair of induced DNA damage is a major determinant of the dose-rate effect, although there are many other factors involved in the subsequent development of a tumour following the initial DNA lesion. Direct evidence on this issue has been obtained through studies with radiosensitive mutants of mammalian cells that carry defects in DNA processing.

72. Ataxia-telangiectasia (AT) is an autosomal recessive genetic disease with complex clinical manifestations [M10]. Radiotherapeutic observations provide clear evidence of the *in vivo* sensitivity of ataxia-telangiectasia patients to low-LET radiation. Studies *in vitro* show ataxia-telangiectasia radiosensitivity to have a cellular basis, and for acute doses AT cells show a two- to threefold increase in their sensitivity to the lethal and clastogenic effects of low-LET radiation [L5, T5]. However, most importantly, the ataxia-telangiectasia mutation(s) almost completely abolishes both the capacity of cells to repair x-ray-induced potentially lethal damage and any sparing effect of gamma-ray dose protraction. The effect of the ataxia-telangiectasia mutation(s) on human cellular radiosensitivity was most dramatic after chronic gamma-ray exposure at a dose rate of 2 mGy min^{-1}; where after an accumulated dose of 2 Gy, the number of unrepaired lethal lesions in a normal cell strain was ~0.3 per cell, while the corresponding value for ataxia-telangiectasia strains was ~5.0 [C12]. These data, together with those on potentially lethal damage repair after acute doses, have been used to argue that the rate at which cells sustain radiation damage is a major factor in the efficiency of repair and that ataxia-telangiectasia cells are blocked in a major radiation repair pathway [C12]. Biochemical studies so far appear to have failed to identify a consistent DNA-repair defect in ataxia-telangiectasia cases, including DNA double-strand break rejoining [L5, T4]. However, using a molecular assay based on the cell-mediated rejoining of restriction endonuclease induced DNA double-strand breaks in plasmid DNA substrates, some evidence for reduced fidelity of double-strand break rejoining has been obtained in the Sv40-transformed ataxia-telangiectasia cell line [C13, T4]. However, further studies failed to observe a similar effect with a related plasmid [G11], suggesting that an apparent effect on overall transfection frequency is related not only to repair deficiency but also to sensitivity of potential transfectants to the selective agent. A reduction in repair fidelity has also been reported in a radiosensitive mutant of V79 Chinese hamster cells [D1], but there are still no data on dose-rate effects in this mutant. It might be expected that inaccurate repair of double-strand breaks *in vivo* might lead to increased ionizing radiation mutability. Ataxia-telangiectasia cells, however, show normal spontaneous or ultraviolet mutability, and although they show increased chromosome rearrangements following exposure to ionizing radiation, they show either a reduced mutability or an increased incidence of mutation similar to normal cells [G11, T22]. In Annex E, "Mechanisms of radiation oncogenesis", it is noted that cell mutagenesis and DNA repair data may be used to argue that oncogenic initiation following ionizing radiation may occur more frequently through DNA deletions and/or rearrangements than through point mutations. There is, however, insufficient evidence at present on this important aspect of oncogenic initiation.

73. A correlation between reduced dose-rate effects for cell inactivation and deficiency in DNA double-strand break repair has also been established in radiosensitive mutants of CHO Chinese hamster cells [K1, T3] and L5178Y mouse lymphoma cells [B10, E4, E5, W5], further strengthening the link between dose-rate effects and the repair of a specific radiation-induced DNA lesion. In addition, some of the above data also indicate that the fitted initial slopes, α_1, of dose-effect curves are not constant and may be modified by cellular repair processes.

74. The extent to which radiation-induced DNA damage may be correctly repaired at very low doses and very low dose rates is beyond the resolution of current experimental techniques. If DNA double-strand breaks are critical lesions determining a range of cellular responses, including perhaps neoplastic transformation, then it may be that wholly accurate cellular repair is unlikely even at the very low lesion abundance expected after low dose and low-dose-rate irradiation [T5].

75. Radiation-induced molecular damage to both DNA strands at the same point has a finite probability of generating a scission in the initial DNA substrate, with nucleotide base modifications on both strands. Repair enzyme activity may remove these but, in doing so, it will create a secondary substrate that cannot be returned to its original undamaged form without the presence of the necessary template [F3]. In the absence of such aids to repair, the lesions will tend to be misrepaired, producing intrachromosomal dele-

tions or interchromosomal translocations that are the hallmarks of radiation damage in mammalian cells (see Annex E, "Mechanisms of radiation oncogenesis", and [E1]). The existence of such radiation-induced double-strand DNA lesions, which may be extremely difficult to repair correctly, would imply the absence of threshold for initial damage to DNA, even when there are very few double-strand breaks, and hence absence of thresholds for stable changes to individual cells.

76. This postulate may be contrasted with that for ultraviolet, where there is experimental evidence that biologically critical cellular damage arises as a consequence of the induction of ultraviolet photoproducts that principally affect the nucleotide bases on one strand of the DNA duplex. Ultraviolet-modified bases may be excised from the damaged strand by DNA repair complexes, leaving a gapped strand that may then be accurately filled with the appropriate nucleotides using the coding sequence of the undamaged strand as a template [F3, M2].

77. From a mechanistic standpoint such single-strand damage excision processes, which also act on many chemically induced DNA base adducts, may be regarded as potentially error-free [M2], although even here mistakes in copying may occur. Thus, although in principle the efficient (subsaturation) operation of single-strand excision processes in cells could result in wholly accurate repair and a dose-effect relationship with a threshold at low doses, in practice such thresholds are unlikely to exist for the initial damage to DNA from ionizing radiation. Apparent low-dose thresholds for the ultraviolet-inactivation and mutation of cultured human cells have, however, been demonstrated [M2].

2. Effect of dose rate

78. A number of studies have been reported on the influence of dose rate from low-LET radiation on cell mutagenesis. In cultured rodent cells, radiation mutagenesis may be considerably reduced by dose protraction [T2, T5]. In contrast, in a human lymphoblast system, continuous exposure to radiation from tritiated water [L6] or from daily exposure to x-ray doses <0.1 Gy [G11] failed to produce any reduction in induced mutation frequency. This response may not, however, be characteristic of the response that would be obtained for normal cells *in vivo*. In human TK6 cells, the *hprt* and *tk* mutation frequencies after acute x-irradiation and continuous gamma-irradiation (0.45 and 4.5 mGy min^{-1}) showed linear responses and no dose-rate dependency [K4]. The dose rate of 0.45 mGy min^{-1} is one of the lowest used for mutation studies of cells in culture. While it is possible that these observations highlight a real difference between human and rodent cells in a low-dose radiation response and in the potential for repair, there are complex issues regarding dose-rate effects on cell mutagenesis that need to be considered [T5].

79. For the induction of unstable chromosome aberrations (dicentrics and acentric rings) in human lymphocytes by low-dose and low-dose-rate radiation, there has been considerable interlaboratory variation in aberration yield, so the magnitude of any dose-rate effect at low doses is not clear [L14]. It has been concluded, however, that at low doses, taking all data together, aberration yield is probably linear with dose and independent of dose rate [E1]. Recently, however, observations on the existence of a radiation-induced adaptive response in human lymphocytes have raised questions about the response at low doses. In these experiments it has been shown, for example, that lymphocytes exposed to an x-ray dose of 0.01 Gy (corresponding to an average of 10 tracks per cell) become adapted so that only about half as many chromatid deletions are induced by a subsequent challenge with high doses (e.g. [W7]). The mechanisms and generality of this potentially important post-irradiation response have yet to be established, but it has been shown that cellular protein synthesis is necessary for the development of the adaptive response and that a dose of 0.01 Gy from x rays reproducibly induces the synthesis of a number of cellular proteins (putative repair enzymes) not found in unirradiated lymphocytes [W7]. The effect of radiation on cellular processes has recently been reviewed by Wolff [W4].

80. A number of models have been published that ascribe the repair of radiation damage in the quadratic region of the dose response to a reduction in sublethal or submutagenic damage. Thus, Leenhouts et al. [L20] modelled cellular damage and its repair in terms of induced DNA double-strand breaks; these may be reduced in number in a cell either by the repair of single-strand breaks or by the repair of double-strand breaks, which might not always be perfect. On this basis, three regions of the dose response can be distinguished: an acute dose-rate region (>60 Gy h^{-1}), where exposures are very short compared with the repair rate of sublethal or submutagenic damage and where a linear quadratic dose-effect relationship is measured; a region of protracted dose rate, where the radiation effect decreases with decreasing dose rate; and a region of lower dose rates, where the repair of sublethal damage is essentially complete and the dose-rate effect is essentially negligible. These different regions will not necessarily be the same for all cell types. Similar patterns of response could be obtained, however, if the feature of cellular response giving rise to the quadratic component included a component that could be attributed to the saturation of repair processes.

D. SUMMARY

81. Guidance on expected effects at low doses and low dose rates can be sought from the quantitative models that have been developed to describe the available radiobiological and epidemiological data. Radiobiological data for effects on single cells under a variety of conditions have led to the development of many quantitative models, mechanistic or phenomenological, for single radiation-induced changes in the cells. Multi-stage models of radiation carcinogenesis, based on epidemiological or animal data, assume that a series of two or more changes is required before a cell becomes malignant and that radiation can induce at least some of these changes. The biophysical concepts underlying the different models can be described in terms of general features of target theory, because the insult of ionizing radiation is always in the form of finite numbers of discrete tracks. In this way fundamental expectation can be sought on the nature of overall dose responses, their dependence on dose rate and their features at the low doses that are of greatest practical relevance. Radiation carcinogenesis involves complex changes after the initial cellular damage. The cellular effects and concepts of appropriate models have been emphasized in this Chapter. Other aspects, including organ effects, are considered later in this Annex.

82. Dose responses can be subdivided into regions. In region I, a negligible proportion of cells (or cell nuclei) are intersected by more than one track and hence dose responses for single-stage effects can be confidently expected to be linear and independent of dose rate. In region II, many tracks intersect each cell (or nucleus), but multi-track effects may not be observed in the experimental data, so independent single-track action is commonly assumed, although true linearity and dose-rate independence hinge on the validity of this assumption. In region III, multi-track effects are clearly visible as non-linearity of dose response, and hence dose-rate dependence, is likely. The simpler forms of the dose-response relationship can be expanded as a general polynomial, with only the dose and dose-squared terms being required to fit most experimental data, although sometimes a separate factor is added to account for competing effects of cell killing at higher doses. The induction of an effect can then be represented by an expression of the following form:

$$I(D) = (\alpha_1 D + \alpha_2 D^2)\, e^{-(\beta_1 D + \beta_2 D^2)} \quad (11)$$

in which α_1 and α_2 are coefficients for the linear and quadratic terms for the radiation response and β_1 and β_2 are linear and quadratic terms for cell killing. It is generally assumed that at sufficiently low doses, α_1 will be constant and independent of dose rate. In this approach it is common to regard the fitted linear coefficient as being constant and fully representative of the response extrapolated down to minimally low dose and dose rate. However, there are in the literature data from numerous studies that violate this simple expectation, for both low-LET and high-LET radiation. Many of these imply that multi-track effects can occur in the intermediate-dose region (II) and that even when the dose response appears linear it may be dose-rate dependent and non-linear at lower doses.

83. Low-dose and low-dose-rate expectations based on multi-stage processes of carcinogenesis depend crucially on the detail of the radiation dependence of the individual stages and on the tissue kinetics. Expectations could, in principle, readily range between two opposite extremes. On the one hand a linear term could be absent entirely, implying vanishing risk as the dose tends to zero, as should be the case if two (or more) time-separated radiation steps were required. On the other hand, there could be, right down to the lowest doses, a clear linear term that even increases with decreasing dose rate, as may occur if either of the stages can occur spontaneously and if there is clonal expansion between them.

84. Consideration has also been given to the possible existence of a true dose threshold in the response to radiation. It is highly unlikely that a dose threshold exists for the initial molecular damage to DNA, because a single track from any ionizing radiation has a finite probability of producing a sizable cluster of atomic damage directly in, or near, the DNA. Only if the resulting molecular damage, plus any additional associated damage from the same track, were always repaired with total efficiency could there be the possibility of a dose threshold for consequent cellular effects. Almost all of the many biophysical models of radiation action assume that there is no such threshold for single-stage changes in cells. Multi-stage models of carcinogenesis could lead to expectations of a dose threshold, or a response with no linear term, under particular, highly restricted sets of assumptions. Available data imply that these restrictions do not apply in general to all tumours, although they may in some particular cases. These fundamental considerations cannot preclude practical situations where the possibility of effects at low doses may be very small or where significant tissue damage is necessary for particular types of tumour to develop.

II. DOSE-RESPONSE RELATIONSHIPS IN EXPERIMENTAL SYSTEMS

85. To provide an experimental basis for assessing the effects of dose rate on cancer induction in man, information is available from a number of sources. Tumour induction in animals provides the main source, but both the transformation of cells in culture and the induction of somatic and germ cell mutations are also valuable for assessing the influence of dose and dose rate on the initiating event(s) resulting from damage to DNA. The following Sections review information from these areas of research that are relevant to considerations of dose-rate effects for cancer induction by both low- and high-LET radiation.

86. In the UNSCEAR 1986 Report [U2] information on dose-response relationships for mutations, chromosomal aberrations in mammalian cells, cell transformation and radiation-induced cancer were reviewed. Three basic non-threshold models were considered for both cellular effects of radiation and for cancer induction: linear, linear-quadratic and pure quadratic models (Figure IV). It was concluded that for most experiments and end-points the prevailing form of the dose-response relationship at intermediate to high doses of low-LET radiation is concave upward and can be represented by an equation of the form

$$I(D) = (\alpha_0 + \alpha_1 D + \alpha_2 D^2) S(D) \qquad (12)$$

in which α_0 is the spontaneous incidence, α_1 and α_2 are coefficients for the linear and quadratic terms for the specific cellular response and S(D) is the probability of survival of transformed cells having received the absorbed dose D. The probability of survival may be expressed as

$$S(D) = e^{-(\beta_1 D + \beta_2 D^2)} \qquad (13)$$

where β_1 and β_2 are coefficients of the linear and quadratic terms of cell killing. For mammalian cells exposed to low-LET radiation, values of the parameter α_1/α_2 for mutations and chromosome aberrations (equivalent to the dose at which the linear and quadratic terms contribute equally to the response) cluster around 1 Gy (geometric mean, 1.27 Gy) while values of the parameter β_1/β_2 for cell sterilization are generally much higher, in the range 2-10 Gy (geometric mean 7.76 Gy) [B27]. The difference appears to be due mainly to higher values of the linear term for cell killing, in accordance with conclusions that, at least at low doses, the loss of proliferative capacity of cells is caused by damage that is not all observable as chromosomal changes at mitosis [B31, B38]. Some may be associated with less severe damage [B13].

87. An example was given in the UNSCEAR 1986 Report [U2] of how the range of survival parameters for cell lines of varying sensitivity for cell killing would affect the shape of the dose-response curve for tumour induction, and hence the reliability of extrapolation from risks obtained at intermediate doses to the low doses that are generally of practical concern. In this analysis [U2], the Committee selected two values of the α_1/α_2 quotient for tumour yield (or mutation/aberration yield) applying to x rays and gamma rays: 0.5 Gy and 2.0 Gy. For survival characteristics, the bone marrow stem cell was selected as the most sensitive. Its survival curve is described by $\beta_1 = 0.4\ Gy^{-1}$ and $\beta_2 = 0.08\ Gy^{-2}$ [B5]. For the least sensitive cell, a hypothetical cell line was assumed with survival parameters $\beta_1 = 0.1\ Gy^{-1}$ and $\beta_2 = 0.08\ Gy^{-2}$ [B5]. To normalize the data, a lifetime cumulative incidence at 3 Gy of 150 cases per 10,000 population ($150\ 10^{-4}\ Gy^{-1}$) was assumed. The results in terms of the cumulative tumour incidence from doses of 1 mGy to 4 Gy for all combinations of α_1/α_2 and β_1/β_2 are plotted in the upper part of Figure V. In the lower plot of Figure V, the data have been redrawn giving relative risks normalized to the same value of the α_1 coefficient.

88. From this analysis, three conclusions can be drawn. First, the sensitivity to cell killing has a more pronounced effect on the shape of the dose-response relationships than the α_1/α_2 quotient. Secondly, for the cells most sensitive to killing (S_{min}, minimal survival), the relationship is concave downward, with maxima at 2-2.5 Gy. Since such curves are not observed for human cancers after exposure to low-LET radiation (i.e. reaching maximum values at doses of about 2 Gy), it seems likely that the assumed sensitivity is too high for *in vivo* irradiation. This would be consistent with the lower sensitivity of single cells irradiated *in situ*. Thirdly, for the cells least susceptible to killing (S_{max}, maximum survival), the overestimate of the tumour yield per unit dose at low doses by linear extrapolation from 1-2 Gy down to 0.001-0.01 Gy ranges from 3.0-2.5 at $\alpha_1/\alpha_2 = 0.5$ Gy, to 1.2-1.3 at $\alpha_1/\alpha_2 = 2$ Gy, respectively.

89. The maximum overestimation of the risk results from totally neglecting cell killing. In such a case the overestimate of the risk at low doses from risks observed at high doses, D, can be calculated from equation 12:

$$DDREF = (\alpha_1 D + \alpha_2 D^2)/\alpha_1 D = 1 + (\alpha_2/\alpha_1) D \qquad (14)$$

The extent of overestimation of the risk corresponds to the dose and dose-rate effectiveness factor (DDREF) of ICRP [12].

90. Linear extrapolation from 3, 2 and 1 Gy down to a low dose of, say, 0.01 Gy for cellular systems with a range of α_1/α_2 quotients between 0.5 Gy and 2 Gy would thus involve the overestimates of radiation effect shown in the Table below. Thus, for a cell response with an α_1/α_2 quotient of 1.0 Gy, if the risk is assessed at 2 Gy then linear extrapolation to assess the risk at low doses will overestimate the risk coefficient by a factor of about 3. If the risk is assessed at 3 Gy, however, the DDREF would be 4. In practice, the available epidemiological and experimental data on tumour incidence generally do not allow reliable estimates to be made of α_1 and α_2, and tumour incidence data up to about 2 Gy are frequently compatible with linear or linear-quadratic models, although a variety of dose-response curves have been obtained (Figures VII-XV). This type of modelling approach does, however, indicate that tumour induction rates at low doses, when based on information obtained at intermediate doses, will, in the absence of significant cell killing, tend to be overestimated by linear extrapolation.

α_1/α_2 (Gy)	DDREF		
	3 Gy	2 Gy	1 Gy
0.5	7	5	3
1.0	4	3	2
2.0	2.5	2	1.5

91. Of concern for radiation protection is how cellular damage by ionizing radiation manifests itself as long-term effects, with cancer induction and hereditary disease being the main effects of concern at low doses and low dose rates. The induction of hereditary disease by ionizing radiation may be readily explained in terms of damage to the genetic material that is manifested in future generations. For cancer induction in both animals and humans the situation is more complex, because tumours in somatic tissue can arise many years after exposure to radiation, following development through a succession of events. Experimentally, cancer caused by exposure to radiation or other agents appears to be the result of a multi-stage process. In the liver, skin, oesophagus, colon and other complex epithelia the cancer induction process can be considered to consist of three stages: initiation, promotion and progression, which are described in Annex E, "Mechanisms of radiation oncogenesis". An initiating event can result from a single exposure to a genotoxic carcinogen that alters a cell or a group of cells, giving a potential for cancer to develop. This damage may be repaired but it may also be irreversible, although the cell and its progeny may never develop to form a tumour. This initial damage may conform to single- or multi-hit models. Subsequent exposure to tumour promoters permits the neoplastic changes to be expressed in initiated cells, with the result that tumours develop. Further stimulation may therefore aid the progression of the tumour. Some chemical agents act as initiators, some as promoters and others as both [A3]. Radiation can act in a dual capacity, as cancers may appear many years after exposure to radiation without any further radiation stimulus, however, at relatively high doses, radiation damage to surrounding tissue may also play a promotional role in cancer development. Many other environmental factors, including hormones, immunological factors or cigarette smoke, may also play a promoting role after an initiating event.

92. The problems in assessing risks of cancer for exposures to low-LET radiation at low doses and low dose rates, when human data are available mainly at high doses and high dose rates, were summarized by the NCRP [N1]. The dose-response relationships are illustrated in Figure I, which gives schematically data points and possible dose-response curves for cancer incidence. Frequently, as in this example, data points are only available at relatively high doses. The approach commonly used in risk assessment is to fit a linear dose-response relationship to the data (curve B), a procedure that is usually considered to give an upper limit to the risk at low doses [C4, I1, U4]. If this linear relationship is due to single tracks acting independently, then the effect per unit dose (the slope of the line, α_{H}, or risk coefficient at high doses and high dose rates) would be expected to be independent of dose magnitude and dose rate. In practice, however, this is not generally the case, and experimental data suggest that a linear-quadratic relationship (curve A) will frequently provide a better fit to the data, implying that damage is the result not only of single interactions but also of other, more complex interactions. Other explanations for the quadratic function in the response are also possible, such as saturation of repair processes [T16]. With a progressive lowering of the dose and/or the dose rate, allowing more opportunity for repair of damage and less opportunity for interacting events, a point may ultimately be reached at which damage is produced as a result of single events acting alone, giving a linear response (curve D, slope α_L) with the effect proportional to dose. A similar response would be obtained

by lowering the dose rate alone, as even at high total doses, lesions accumulate more slowly. Thus, experimentally, the effect per unit absorbed dose at low dose rates (even at high total doses) would be expected to become progressively less as the dose rate is lowered. Hence, the limiting slope (α_L of Figure I) would be reached either by reducing the dose to very low values where the effect is independent of dose rate or by reducing the dose rate to very low values where the effect is dependent only on the total dose. On this basis, even fractionated exposures will not necessarily give slopes approaching α_L, as the overall dose response will depend on both the total dose and the dose rate per fraction.

93. In practice, because of statistical limitations it is extremely difficult to detect radiation-induced effects in the low dose range (<0.1 Gy) at any dose rate; thus, there are uncertainties in the determination of the limiting slope, α_L, in both animal and human studies. The initial slope of the dose-response curve can be more readily examined by changing the dose rate, as can be done in studies with experimental animals. In many experiments, however, even at low or intermediate dose rates, the limiting slope may not be reached, and a dose response in between the two slopes α_H and α_L is obtained with slope α_{Exp}. Despite this limitation, animal experiments provide the best indication of the extent to which lowering the dose rate of low-LET radiation, even at intermediate or high total doses, can reduce the effectiveness of radiation in inducing cancer. They therefore provide the most useful guidance on the extrapolation of risks observed at high doses and high dose rates to the low doses and low dose rates generally of concern in radiological protection.

94. The ratio of the slope of the no-threshold, "apparently" linear fit to the high-dose and high-dose-rate data (α_H) to the slope of the linear fit to the low-dose-rate data (α_L or α_{Exp}) has been used as a measure of the dose and dose-rate effectiveness factor. The terms dose-rate effectiveness factor (DREF) [N1], linear extrapolation overestimation factor (LEOF) and low dose extrapolation factor (LDEF) [P2, P3] have also been used for this reduction factor. In this Annex, the term dose and dose-rate effectiveness factor (DDREF) (or, more simply, relative effectiveness) will be used for comparing the response at different dose rates.

95. The data on tumour induction in experimental animals that are most directly useful for the derivation of DDREFs for man are, surprisingly, very restricted in their extent. Significant effects have been obtained mainly with intermediate or relatively high doses, although at very different dose rates. Thus, only limited evaluation of the shape of the overall dose-response curve has been achieved, as is also true for epidemiological studies. In general, a significant increase in tumour incidence in experimental animals is found at doses of about 0.2 Gy and above (see Section II.A). Some radiation-induced cancers have been detected in human populations at relatively low doses. Human data on cancer induction relevant to considerations of dose and dose-rate effects from low-LET radiation are reviewed briefly in Chapter III.

A. TUMORIGENESIS IN EXPERIMENTAL ANIMALS

1. Radiation-induced life shortening

96. Extensive studies in experimental animals have reported radiation-induced life shortening as a result of whole-body external irradiation and as the result of intakes of radionuclides. This is a precise biological end-point that reflects the earlier onset of lethal diseases, an increased incidence of early occurring diseases or a combination of the two. To understand the effects of radiation on life-span it is important to know the underlying cause of death, although this is often difficult and in some cases impossible, as death may be the result of a variety of causes acting together. This is particularly the case in older animals, in which multiple lesions are often present. In contrast, in younger animals a specific pathological lesion can frequently be identified.

97. Life shortening is an effect that must be estimated by comparing irradiated and non-irradiated populations. The different ways of describing and expressing the effect quantitatively have been reviewed in the UNSCEAR 1982 Report [U3]. The mean or median life-span, the per cent cumulative mortality or the age-specific mortality rate may all be regarded as compounded expressions of specific and non-specific causes, acting within each individual to decrease fitness and ultimately to cause death.

98. There has been considerable discussion in the published literature about the specificity or non-specificity of life shortening in experimental animals exposed to ionizing radiation. Life shortening must ultimately be due to an underlying cause, and the lack of specific information frequently results from the lack of detailed pathology. A "specific" cause of death has therefore been taken to mean that irradiated animals die earlier than controls and show a different spectrum of diseases or causes of death. Since not all diseases are readily induced by radiation, interest has centred on whether or not radiation may produce life shortening by inducing tumours and how much of the observed shortening can be accounted for by neoplastic diseases. The words "specific" and "non-specific" have generally been taken to indicate neoplastic and non-neoplastic contributions to life shortening.

99. Life shortening by radiation was comprehensively reviewed in the UNSCEAR 1982 Report [U3]. Although life shortening can only be assessed on the basis of death, an end-point that can be defined precisely in time, it is usually more informative to know the cause of death, as most irradiated animals die of diseases that are unrelated to radiation exposure, complicating the identification of the terminal pathological syndromes. Some of the difficulties in interpreting much of the work were summarized in that Report [U3]. These included the lack of careful pathological observations on the animals at death or of a refined multifactorial analysis, particularly in earlier studies. Some studies at low doses have even reported life lengthening, although any increase has generally not been statistically significant. Another problem is that even when good pathology is available, information is usually collected at death, when it is impossible to assess the contribution of each specific cause to life shortening, since there is no reason to presume that all causes are equally accelerated by irradiation. While serial sacrifice experiments might provide this information, they are time-consuming and expensive. The additional effort required to implement this technique is considerable and, as a consequence, such information is uncommon in the literature.

100. Radiation-induced life shortening appears to have been first described in the rat by Russ et al. [R9] and in the mouse by Henshaw [H18]. They reported that irradiated animals had a shorter life-span and appeared to age more rapidly than non-irradiated controls. These and other studies led to the view that the life-shortening action of radiation was due to its enhancement of natural ageing processes. Early reviews of mammalian radiation injury and lethality by Brues et al. [B28] and Sacher [S18] recognized that single acute exposures to radiation tended to displace the Gompertz age mortality function upward and chronic exposure throughout life increased the slope of this function.

101. The concept that radiation-induced life shortening might be equivalent to aging was criticized by Mole [M44], who considered that this view had arisen largely as a result of observations on surviving animals given single large doses in the lethal range. The similarities and differences between natural ageing and radiation-induced life shortening were considered by Comfort [C16]. His review was a significant attempt to differentiate between the various biological effects observed. Neary [N3, N4] regarded theories of ageing as belonging to one of two main groups: those interpreting ageing as due to random events in a population of supposedly uniform individuals and those examining the individual and its component cells. He proposed a theory that ageing proceeds in two successive stages, induction and development, each characterized by appropriate parameters. Experiments reported later from the Soviet Union [V2, V3, V5, V6] tended to show that induction consists of the spontaneous occurrence of lesions in cellular DNA and that development (promotion) results from the activation of endogenous viral genomes by chemical carcinogens or radiation.

102. The first experimental series that allowed analysis of specific causes of death were those of Upton et al. in 1960 [U27] in RF mice. The authors could not, however, establish any clear-cut relationship between life shortening and the incidence of tumours, as the dose-response relationships for different tumour types varied: some increased with dose and some decreased. These data gave some support to the view that radiation could cause non-specific life shortening. A statistical evaluation of these data by Walburg in 1975 [W8], using a method that allowed for competing probabilities of death, indicated that life shortening, which was clearly apparent when all deaths were considered together, disappeared when tumours were excluded from the analysis. Table 4 shows the mean age at death adjusted for competing probabilities of death, for deaths from all causes and from all non-neoplastic diseases, of female RF mice exposed to 1 and 3 Gy of ^{60}Co gamma-radiation (0.067 Gy min^{-1}) at 10 weeks of age compared with data from controls. For these mice, myelogeneous leukaemia, thymic lymphosarcoma and endocrine tumours were induced or accelerated by irradiation. When only non-neoplastic causes of death were considered, there was no significant effect on life shortening, and the mean age at death increased in irradiated animals relative to controls.

103. In a series of studies in mice Storer [S33] noted in the dose range 1-5 Gy from x rays a tendency for neoplastic diseases to occur earlier in irradiated than in control mice. In extensive studies by Upton et al. [U21, U28] in male and female RF mice exposed to either fast neutrons or to gamma rays, detailed pathology was not performed, but the authors considered that the death of irradiated animals was characteristically associated with tumours and degenerative diseases of old age, although the induction of neoplasms could not entirely account for life-span shortening. These data were subsequently analysed in more detail by Walburg [W8], who demonstrated that, in the absence of tumour induction, life shortening was negligible, at least for exposure to gamma rays in the dose range of 1-3 Gy.

104. A number of more recent publications have also addressed the question of life-span shortening in mice. In general, the conclusions have been that for doses of up to a few gray, life-span shortening is due to an increase in tumour incidence. Thus, Grahn et al. [G23] showed that at doses up to 4 Gy life shortening was due to excess tumour mortality, although at higher

doses decreased life expectancy was not accompanied by a parallel increase in tumour incidence. Maisin [M45] attributed life shortening in BALB/c and C57BL mice at intermediate doses essentially to thymic lymphoma and at higher doses to glomerulosclerosis. Similar conclusions have been reached by other authors [L21, L22] in studies with rats; and the same is true for dogs in the series of Andersen et al. [A5], according to an analysis by Walburg [W8].

105. For exposures to high-LET radiation similar conclusions can be drawn. In an analysis of causes of death in B6CF1 mice exposed to single and fractionated doses of fission neutrons Thompson and Grahn [T12] concluded that practically all (>90%) of the excess mortality could be attributed to tumour deaths.

106. From his comprehensive review of published data, Walburg [W8] concluded that at the low to intermediate doses of practical interest in radiation protection, life shortening after irradiation may principally be explained by the induction or acceleration of neoplastic diseases. This conclusion was supported by Storer [S19], although it was recognized that at higher doses other mechanisms were involved in early radiation damage.

107. The majority of comprehensive studies that give quantitative information on the effects of dose, dose fractionation and dose rate on life-span shortening 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} [G8]. 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} [M5]. The effects of acute single doses on life-span shortening in other species are summarized in the UNSCEAR 1982 Report [U3].

108. The sensitivity to tumour induction has also been shown to depend on age at exposure as well as the gender of the animals. Thus, the lifetime excess of neoplasia in Sprague-Dawley rats following exposure to gamma rays from ^{60}Co decreased by a factor of about 10 in 9-month-old rats as compared to animals irradiated *in utero*, and the spectrum of tumours was different. The higher incidence of tumours observed in the fetal-exposed group appeared to be mainly due to the high sensitivities of the central nervous system and gonads during organogenesis. Differences in tumour incidences were observed between male and female rats and between the incidences of primary cancers and benign tumours in the different groups of animals [M29].

109. Partial-body irradiation is much less effective than whole-body irradiation in causing life shortening. Thus for female ddY/SLC mice following head or lower body exposure to doses of 1.9 Gy from x rays, life shortening was 23 and 26 days Gy^{-1}, respectively, with almost no further life shortening up to 7.6 Gy. After irradiation of the trunk with 1.9 Gy, life shortening was 38 days Gy^{-1}, with a further increase of 6 days Gy^{-1} at doses up to 7.6 Gy. In contrast, for whole-body exposures between 0.95 and 5.7 Gy, the mean survival time decreased linearly with increasing dose, with a loss of life expectancy of 37 days Gy^{-1} [S37]. Extensive studies on the effects of incorporated radionuclides have also shown a reduction in life-span as a result of tumour induction resulting from intakes of radionuclides. Some of these studies are described later in this Chapter in sections which relate to effects on specific organs and tissues. It is noteworthy that in a number of studies, where non-fatal tumours are induced in particular organs and tissues, this does not necessarily lead to a loss of life-span (e.g. [M24]).

110. *Summary.* It may be concluded on the basis of a number of studies that, although irradiated animals do experience, on the average, a shorter life-span than non-irradiated controls, the hypothesis that life shortening at low to intermediate doses up to a few gray of low-LET radiation is due to the same causes of death as is normal in the animals (although appearing earlier in time) is not substantiated by experimental evidence. In general, life shortening as a result of exposure to ionizing radiation arises largely as a result of an acceleration or higher incidence of fatal tumours in irradiated populations. At higher doses that are well into the lethal range, a non-specific component of life shortening becomes apparent from cellular damage to the blood vasculature and other tissues. This does not imply that dose-response relationships for tumour induction and life shortening are directly comparable, because even for the same radiation dose, the mean latent period of some tumours in a given species can be influenced by a number of factors including the dose, dose rate, gender and age at exposure. Furthermore, some tumours that may be induced are non-fatal and do not influence life-span. If the induction of fatal tumours is the main influence on life shortening, however, then a comparison of survival following various patterns of exposure should provide some indication of the effects of dose, dose rate and dose fractionation on tumour induction.

(a) Dose fractionation

111. In the UNSCEAR 1982 Report [U3], the Committee reviewed data on fractionated exposures in which a given dose of whole-body irradiation was split into a series of doses given in two or more fractions. The dose per fraction, fractionation interval and total time of irradiation are all interacting variables that cannot normally be separated. Frequently, therefore, the comparison is between a single exposure given acutely and the same dose given in fractions over a period of time. Fractionation of a given dose into two equal or unequal fractions at an interval of about a day or less has, in general, not altered life shortening significantly, although such a dose-fractionation schedule can decrease acute effects significantly [G8, M21]. Longer dose-fractionation intervals have been more comprehensively studied. In some cases survival has been unaltered or only slightly prolonged by fractionation [G8, K2, L16, U18]; in others it was slightly shortened [A5, C15, M22]. Many of the differences are perhaps due to differences from one animal strain to another in sensitivity to the induction of various tumour types, although a number of studies have also demonstrated how the spectrum of diseases that can result in life shortening may be influenced by the pattern of radiation exposure. Thus, Cole et al. [C15] examined the influence of dose fractionation on the life-span of LAF1 female mice. Animals were exposed either to an acute dose of 6 Gy (250 kVp x rays) or to about 7 Gy given in two, four or eight equal fractions separated by 8 weeks, 19 days or 8 days, respectively. Irradiation shortened survival in all the groups compared with controls, but the greatest effect was seen in the group given eight fractions, for which the mean age at death was about 15 months compared to about 21 months in the group given a single exposure. This was attributed to an increased incidence of leukaemia in the eight-fraction group (39%) compared with the single fraction group (13%) and the controls (29%). It was noteworthy that nephrosclerosis, which was the main cause of death in the group given a single dose (53% incidence) was very much reduced in the group given eight fractions (5%).

112. Dose fractionation at progressively longer periods of time seems to decrease the effects of radiation, but again the variability in results obtained has been considerable [A1, A5, G8, M5, M20, S17]. Ainsworth et al. [A1] reported that exposing both male and female B6CF1 mice to fractionated doses of ^{60}Co gamma rays (8.4 Gy total in 24 equal fractions over 23 weeks) produced an approximately threefold "sparing" effect compared to a similar (7.9 Gy) acute dose (corresponding to life shortening of 45 days Gy^{-1} for acute exposure and 18 days Gy^{-1} for fractionated exposure) in both sexes. In contrast, Grahn et al. [G8] compared the effects of 4.5 and 7 Gy from ^{60}Co gamma rays given as acute exposures or as two fractions separated by time intervals between 3 hours and 28 days. No significant effect of dose fractionation on life shortening was found, and the incidence of leukaemia was not altered in any consistent way. In a preliminary experiment, Silini et al. [S16] compared the effects of a single dose of 5 Gy from 250 kVp x rays on adult male C3H mice with the same dose given as two fractions (1.5 and 3.5 Gy) at different intervals of time (4-48 hours). The results suggested an increase in survival time with increasing fractionation interval, but there was considerable variability in the results obtained. The 50% cumulative mortality for the acutely exposed animals was 450 days; for the animals given fractionated exposures at an interval of 36 hours, it was 520 days.

113. A series of papers have been published by Thomson et al. [T7, T10, T12] that compare survival of male B6CF1 mice following single and fractionated exposures. Mice were exposed to ^{60}Co gamma rays either as single exposures or as 24 or 60 weekly fractions. With single exposures the average loss of life-span was 38.5 ± 2.9 days Gy^{-1}, whereas with 24 weekly fractions it was 22.6 ± 2.2 days Gy^{-1} and with 60 weekly fractions 17.5 ± 3.3 days Gy^{-1}. This study therefore showed that prolonged dose fractionation had a significant effect on life-span, reducing the effectiveness of the radiation by a factor of about 2. Table 5 summarizes some early results on the effect of dose fractionation on survival in rodents and beagle dogs.

114. Maisin and his colleagues have reported a series of studies on the effect of dose fractionation on survival in C57Bl and BALB/c mice. A preliminary study compared the survival of C57Bl mice given either a single exposure to x rays (3.5 or 6.5 Gy) or four equal fractions delivered at weekly intervals, with total doses from 2 to 15 Gy [M19]. Although the results for the two patterns of exposure were not strictly comparable, as the cumulative doses were not the same, the data suggested that life shortening after a fractionated exposure was slightly greater (~20%) than after an acute exposure. The disease spectrum was also different for single exposure and fractionated exposure. Thus the incidence of thymic lymphoma nearly doubled with dose fractionation and that of reticulum cell sarcoma B increased even more, while other diseases decreased in incidence.

115. Maisin et al. [M5, M47] reported a more comprehensive study in male BALB/c mice exposed to ^{137}Cs gamma rays (3 Gy min^{-1}) given either as single or fractionated exposures (10 fractions at 24 hour intervals) in the range 0.25-6 Gy. A significant shortening in life-span ($p < 0.05$) was obtained from a dose of 1 Gy for single exposures and from 2 Gy for fractionated exposures. Both patterns of exposure gave

nearly the same linear dependence of survival on dose with a life shortening of 46.2 ± 4.3 days Gy^{-1} for single exposures and 38.1 ± 3.1 days Gy^{-1} for fractionated exposures (Figure VI). After a single exposure malignant tumours were the principal cause of death in the dose range up to about 2-4 Gy; deterministic effects in the lung and kidney were preponderant at higher doses. In general, the total incidence of malignant tumours increased with dose after fractionated exposures, compared with controls, but decreased after single exposure. The difference between the two groups was significant ($p < 0.05$), although neither differed significantly from the controls. This was partly accounted for by an increase in the proportion of animals with two or more tumours after fractionated exposures in the higher dose groups [M25, M47]. The main exception to this trend was thymic lymphoma, where the incidence remained constant after fractionated exposure. This finding is contrary to observations in other studies, as thymic lymphoma incidence can be substantially increased by dose fractionation [K3, M19] and may be explained by the lower doses per fraction used in this study. Deterministic effects (e.g. lung pneumonitis and kidney damage) appeared, however, to diminish significantly with fractionation, and this may have allowed more tumours to develop. In this strain of mice there is, however, a high spontaneous tumour incidence (>60%) which could have influenced the results obtained and which also limits detailed analysis of the results.

116. In a more recent study [M20, M47], 12-week-old C57BlCnb mice, for which there is a lower spontaneous cancer incidence, particularly with respect to thymic lymphoma and lung cancer, were given either single or fractionated exposures (10 fractions separated by 24 hours or 8 fractions separated by 3 hours) to ^{137}Cs gamma rays (3 Gy min^{-1}), with total doses from 0.25 to 6 Gy. The data on tumour incidence and non-cancerous late degenerative changes in the lungs and kidneys were evaluated by the Kaplan-Meier procedure, using cause of death and probable cause of death as criteria. In general, survival appeared to be a linear function of the dose received in all the experimental groups, although survival was longer with fractionated than with single exposures (Table 6). Survival of the control animals was shorter than in the previous studies with C57Bl mice and may have resulted from the use of specific-pathogen-free animals that are more sensitive to non-cancerous late degenerative changes in the lung. Life shortening, calculated by linear weighted regression on dose of the values obtained by the Kaplan-Meier calculation for survival time, amounted to 31.1 ± 2.6 days Gy^{-1} for a single exposure, 19.6 ± 2.9 days Gy^{-1} for a 10-fraction exposure, and 16.5 ± 3.4 days Gy^{-1} for an 8-fraction gamma exposure. Malignant tumours, particularly leukaemia and including thymoma, as well as non-cancerous late degenerative changes were the principal causes of life shortening after a single high-dose exposure to gamma rays. Fractionated exposures, in particular eight fractions delivered 3 hours apart, appeared to result in an earlier and more frequent appearance of leukaemia and solid tumours in the range 1-2 Gy, a finding similar to that obtained with BALB/c mice [M5]. Since the average life-span is longer after fractionation, earlier death after single exposure may be attributed to the development of non-cancerous late degenerative lesions. It was noteworthy, however, that following single exposures the incidence of all tumours except thymoma was significantly less in the low dose groups (0.25-2 Gy) than in the controls (Table 6). This was a significant factor in the observation of an enhanced tumour incidence for animals given fractionated exposures compared with controls.

117. *Summary.* Studies on experimental animals, mainly mice, have shown no clear trend in effects of dose fractionation on life-span. The results from a number of studies suggest that, when compared with the effects of acute exposures, the effects of dose fractionation are small and, at least for exposure times of up to about a month, simple additivity of the injury from each increment of dose can be assumed. In general, fractionated doses were given at the same dose rate as acute exposures. For fractionation over a longer time period there is a tendency to a longer life-span with a longer interval between the doses. A reduction in life-span shortening by a factor of ~2, compared with acute exposure, was obtained in one study in which the dose was given as 60 weekly fractions.

(b) Protracted exposures

118. There are far fewer studies of the effect of dose rate on life-span in experimental animals. The majority of studies have been undertaken in mice, although some work has also been reported with rats [R9], rabbits [B26], and beagle dogs [F13]. A number of early studies were described in the UNSCEAR 1982 Report [U3], but they relate mainly to early effects of radiation and do not provide any insight into the effects of dose rate on tumour induction.

119. In a series of studies by Bustad et al. [B29] hybrid male mice (C57BLx101) were exposed for 8 hours daily, between the ages of 6 and 58 weeks, to either 1 mGy h^{-1} or 2 mGy h^{-1} from ^{60}Co gamma-radiation giving total doses of 2.9 and 4.8 Gy. The animals were then maintained for their normal life-span. The average life-span for two subgroups of animals exposed to 1 mGy h^{-1} was about 863 days and for two further subgroups exposed to 2 mGy h^{-1} it was about 875 days. The life-span of the control

animals was about 920 days. Although there were some differences in the survival times between the different subgroups of irradiated animals and the controls, no significant increase in tumour incidence was observed in the irradiated animals.

120. Mole et al. [M23] exposed female CBA mice to gamma rays to give daily doses ranging from 0.03 to 0.5 Gy for progressively longer times (from four weeks to the duration of life). The shape of the cumulative mortality curve depended systematically on the particular level of daily exposure and on the cumulative dose, except possibly at the lowest daily dose. However, the total doses received by the animals were high: 0.6-72 Gy, causing substantial tissue damage, and many of the animals died of acute effects. It was concluded that lower total doses were needed for examining the relationship between dose rate and the late effects of exposure. The experimental results are described in detail in the UNSCEAR 1982 Report [U3].

121. More comprehensive studies that are more directly relevant to the effect of dose rate on life-span and tumour induction were undertaken over many years by Grahn et al. [G8, G9]. This work with mice has been summarized in a series of publications. A number of mouse strains and hybrids were exposed for 8 hours daily to gamma rays in doses ranging from 0.003 to 0.56 Gy per day. Exposures began when the mice were 100 days old and continued throughout life [G8, G9, S1]. The results were analysed in terms of the mean survival time after the initiation of the exposures, designated mean after survival (MAS). Thus, the MAS equalled the mean age at death minus 100 days. There was good consistency in the degree of life shortening for cumulative doses above a few gray when expressed as the MAS, between and among the strains as a function of daily dose. The MAS declined exponentially with increasing daily dose, D (in gray), and could be represented adequately by

$$\text{MAS (treated)} = \text{MAS (controls)}\ e^{-\alpha D} \quad (15)$$

At the lowest daily doses, no consistent life shortening was found, and in some groups there appeared to be life lengthening. This was possibly due to any effect of radiation being lost due to variation between the animals (e.g. see Table 4).

122. This information on life shortening in mice exposed at low dose rates has been compared with that at high dose rates [N1]. Since animals exposed at low dose rates were exposed until death, the total dose accumulated by each animal depended on its survival time. Thus, a wide range of total doses is represented in the population exposed at any given dose regimen. However, by calculating mean loss of life-span (in days), in terms of the mean accumulated dose to death, it was shown that in the low dose range, life shortening amounted to 4 days Gy^{-1}. This may be compared with the results of 10 studies summarized by Grahn et al. [G8], which gave an average of about 35 days Gy^{-1} (range: 15-81 days Gy^{-1}) for acute exposures, and those reported for BALB/c mice [M5], which gave 46.2 ± 4.3 days Gy^{-1} at acute doses down to 0.25 Gy.

123. These data suggest that for radiation-induced life shortening either single brief exposures to low-LET radiation or fractionated exposures at high dose rates are about 8-10 times as effective as the same total dose given in a long protracted exposure at low dose rate. In a review of some of these data and allowing for uncertainties, including the effect of age-dependent decreases in sensitivity with increasing age, it was concluded by NCRP [N1] that protracted exposures may be considered to be one fifth to one tenth as effective in the mouse as single, high-dose-rate exposures (at total doses >0.5 Gy), assuming linearity for life shortening in both cases.

124. The above analyses assumed a linear dose response, with no threshold, for doses above 0.5 Gy at high dose rate. Storer et al. [S13] have, however, reported non-linear dose responses for life shortening in female RFM mice. Groups of mice were exposed to either 0.45 Gy min^{-1} or 0.06 mGy min^{-1} from a ^{137}Cs gamma-ray source to give a range of doses from 0.1 to 4 Gy and 0.5 to 4 Gy, respectively. Life shortening was calculated by subtracting the mean survival time in each experimental group from the mean survival time of the appropriate control. The dose-response curve for female RFM mice exposed to ^{137}Cs gamma rays between 0.1 and 4 Gy at high dose rate (0.45 Gy min^{-1}) showed that significant life shortening occurred at doses of 0.25 Gy and above. There was a rapid rise in life shortening with doses up to 0.5 Gy, followed by what appeared to be a generally linear upward trend with a much shallower slope in the range 0.5-4 Gy (Figure VII). In the region up to 0.5 Gy, the relationship between life-shortening and dose could be described by a dose-squared model ($p > 0.80$) or a linear-quadratic model, with the quadratic component predominating above about 0.04 Gy. These mice appear to be more sensitive than other mouse strains. Storer et al. [S13] have speculated that a contributory factor may have been the barrier environment in which the mice were maintained, as Upton et al. [U20] found less life shortening in conventionally housed RFM female mice. For female mice exposed at the intermediate dose rate (0.06 mGy min^{-1}), there was a significant reduction in life-span compared with controls at all doses examined (0.5-4 Gy), and a linear relationship adequately described the dose response ($p > 0.5$), with the intercept being not significantly different from that for controls (Figure VII). The weighted regression line to the intermediate dose-rate

data at total doses above 0.5 Gy could be described by the equation Y = 37.5D, where Y represents the days of life shortening and D is the dose, in gray. For the high-dose-rate data a weighted linear regression could be fitted, giving an intercept of 57.5 days. The equation of the line was Y = 57.5 + 46.3D. It was concluded that the main difference in response at high and intermediate dose rates was an upward displacement of the regression line at high dose rate, reflecting an increased sensitivity at doses up to about 0.5 Gy. At total doses of 1-2 Gy, protraction of the dose reduced life shortening by about one half and at lower doses by a factor of 2 to 3.

125. Thomson et al. [T8] have published information on the survival of male B6CF1 mice exposed for 22 hours per day, 5 days per week, to ^{60}Co gamma radiation at dose rates of 14-126 μGy min^{-1} for 23 weeks, giving total doses between 2.1 and 19 Gy, or at 14-63 μGy min^{-1} for 59 weeks, giving total doses between 5.3 and 25 Gy. For deaths from all causes, linear dose-response curves were obtained with slopes, corresponding to days of life lost per gray, of 15.8 ± 1.6 and 7.7 ± 0.2 for exposures of 23 and 59 weeks, respectively. These values were not significantly altered if the analysis was restricted to those mice dying with tumours, as about 90% of the radiation-specific mortality was tumour-related.

126. Thomson et al. [T8] compared the data they obtained in their study with data previously published [T7, T10, T12] on mice exposed either to single acute (20-minute) exposures or to 24 or 60 fractions given once weekly (20- or 45-minute exposures). The life shortening coefficients for single, fractionated and continuous gamma exposures, expressed as days of life lost Gy^{-1}, are shown in Table 7. Dividing the total dose into 24 once-weekly fractions (total exposure time: 18 hours) reduced the effectiveness of the radiation by about 40% (22.6 days lost Gy^{-1} compared with 38.5 days lost Gy^{-1} for acute exposure). Giving the same total dose almost continuously (total exposure time: 2,530 hours) over 23 weeks reduced the effectiveness by a further 30%. The effect of dose protraction was more pronounced if fractionated and continuous exposures were carried out over about 60 weeks. Forty-five hours of fractionated exposure had about 45% of the effect of acute exposure, and 6,490 hours of almost continuous exposure had only 20% of the effect of the single exposure. Also shown in Table 7 are the days of life lost per weekly fraction for the different exposures. Thus the maximum reduction in effect is obtained by comparing acute exposure with the effect of continuous exposure over 59 weeks, when the effectiveness is reduced by a factor of about 5. However, this comparison will tend to overestimate the effects of protraction, as a fraction of the radiation exposure will not have contributed to tumour initiation, although it could have influenced tumour development. However, the extent of this effect, if any, is difficult to quantify. For comparing the effectiveness of different patterns of exposure it may, therefore, be more appropriate to compare the effect of continuous and fractionated exposures given over the same period of time. On this basis, a reduction in effect in the range of 1.4-2.3 is obtained.

127. In an extended analysis of the data on life shortening obtained in mice exposed to acute or protracted exposure to low doses (less than a few gray) of low-LET radiation, Scott et al. [S34] have developed a model based on the assumption that life shortening from late effects is caused mainly by radiation-induced tumours. The state-vector model adopted for the analysis was kinetic in nature, with a two-step process leading to partition of the irradiated population into two groups: a group with radiation-induced tumours, in which it was assumed that mean survival is relatively independent of the radiation dose, although the incidence in the population is dose-related and a group without induced tumours, in which the mean survival time is nearly identical to an unirradiated control population. The results based on the model were in reasonable agreement with the available experimental data and were consistent with curvilinear dose-response relationships for acute exposures as well as with a reduced effect after fractionated exposure to ^{60}Co gamma rays.

128. The effect of dose and dose rate on life-span has also been examined in rats [M43]. Male Sprague-Dawley rats (3 months old) were exposed to ^{60}Co gamma rays to give 2.83 Gy (304 rats, 1.34 mGy h^{-1}), 1 Gy (505 rats, 78 mGy h^{-1}) and 3 Gy (120 rats, 78 mGy h^{-1}). The mean survival time of the controls (837 ± 147 days) was greater than that of the two groups of animals given about 3 Gy, but there was no difference between the animals given high dose-rate exposures (life-span: 738 ± 160 days) and those exposed at the lower dose rate (726 ± 160 days).

129. There are few studies that have examined the effect of dose rate on life-span and tumour induction in large animals. Carnes and Fritz [C30] have reported the results of a comprehensive study in young adult beagle dogs exposed to ^{60}Co gamma rays to give total accumulated doses of 4.5, 10.5, 15 and 30 Gy at dose rates of 38, 75, 128 and 263 mGy d^{-1}. Hazard models were used to identify trends in mortality associated with radiation exposure. The probability of an acute death (related to haematopoietic aplasia) was positively associated with the total dose received and the dose rate. For late effects, although there was good evidence of an increase in tumour mortality relative to the controls in all the irradiated groups, no relationship was found between tumour mortality and dose rate. There was, however, a clear relationship between

tumour mortality and cumulative dose. This lack of a dose-rate effect may be a consequence of the relatively small range of dose rates used (38-263 mGy d^{-1}); in the majority of rodent studies in which such an effect was observed, the high and low dose-rate exposures varied by a factor of 100 or more (Section II.A.2).

130. *Summary.* Experimental studies in mice have demonstrated that with protracted exposures over a period of a few months to a year there is less life shortening by factors of 2 to 5 compared with exposures at high dose rates. In two studies in rats and beagle dogs no evidence was found for an effect of dose rate on tumour mortality. This lack of an effect may be a consequence of the fact that the dose rates used in these studies varied by factors of about 60 (rats) and 7 (dogs), while in the mouse studies they varied by a factor of 100 or more. It is noteworthy that not all tumours are a cause of life shortening.

(c) High-LET radiation

131. Since the 1970s, the Argonne National Laboratory has carried out a series of experiments to examine the effect on life shortening of brief, fractionated and protracted neutron exposures. Early results by Ainsworth et al. [A1] indicated that fractionated fission neutron exposures induced more life shortening in male B6CF1 mice than did single exposures. Thus, with a single dose of 2.4 Gy the mean survival time was 636 ± 13 days, whereas with fractionated exposures (various schedules) survival was 553 ± 6 days (controls: 838 ± 13 days). At a lower dose of 0.8 Gy, life shortening was also reduced, but there was less difference between the two treatment schedules, although the data still suggested that life shortening was greatest with fractionated exposures.

132. Data published before 1981 suggested that regardless of the mode of exposure (single, fractionated or chronic) the RBE could be expressed by the relationship $RBE = AD^B$, where the value of B was approximately -0.5 and that of A (the RBE value at 10 mGy) ranged from 10 to 80, depending on a number of factors, including the instantaneous dose rate of the reference low-LET radiation [T7, T10]. This observation was compatible with suggestions that the RBE increased over a wide range of doses as the inverse of the square root of the neutron dose, to values in excess of 100 [R10].

133. Later studies at the Argonne National Laboratory showed, however, that when total doses are low and the doses per fraction small, there is no significant difference in life shortening between fractionated and single exposures [C18, T18]. When the effects of single brief exposures of male and female B6CF1 mice to 0.83 MeV fission neutrons giving total doses from 10 to 400 mGy [T15] were compared with the effects of 60 equal, once weekly exposures giving doses of from 20 to 400 mGy [T12], the dose-response curves were linear and of similar slope between 0 and 300 mGy. Based on a linear-quadratic fit to the data for female mice, the days of life lost were 46 Gy^{-1} for single exposures and 44 Gy^{-1} for the 60-week fractionated exposure. Data for male mice gave similar results but were less extensive [T12]. At a dose level of about 400 mGy the dose-response curve for single exposures starts to become less steep and to separate from that for fractionated exposures. Overall, a significant effect of exposure pattern was observed at neutron doses in the range 400-600 mGy. Significant augmentation of radiation damage with dose protraction was observed in both sexes from doses above ~600 mGy. No difference in the dose-response curves for mice given 24 equal once-weekly or 60 equal once-weekly exposures was obtained. Although for exposure to neutrons the dose response at intermediate to low doses was linear and independent of dose pattern, this was not the case for exposures to gamma rays. As a consequence, RBE values from 6 to 43 were obtained, depending on the protraction period (1 day, 24 weeks or 60 weeks) [C18]. At low doses there is likely to be a limiting value for the RBE when the dose-response curves for both the neutron and the reference (gamma) radiation are linear. For single low doses, this has been calculated to be 15.0 ± 5.1 for $B6CF_1$ mice [T15]. In a supplementary analysis it was shown that practically all of the excess mortality resulting from radiation exposure (93% ± 8%) could be attributed to tumour deaths [T12].

134. Results obtained by Storer et al. [S13, S27] at Oak Ridge National Laboratory, using RFM and BALB/c mice were similar to those obtained at Argonne National Laboratory. Thus, female BALB/c mice were given total neutron doses between 25 mGy and 2 Gy in a single brief exposure or in equal fractions at either 1- or 30-day intervals. The neutrons were those of a slightly degraded ^{235}U-fission spectrum. After single or fractionated exposures, the extent of life shortening increased rapidly over the 0-0.5 Gy range and then began to plateau. While no significant increase in effectiveness of dose fractionation on life shortening was observed at total doses below 0.5 Gy, between 0.5 and 2 Gy there was an increase. With protracted neutron exposures using a moderated ^{252}Cf source giving dose rates ranging from 1 to 100 mGy d^{-1}, with total doses between 25 and 400 mGy, again no increase in effectiveness on life shortening was observed at doses below 0.5 Gy. It was also concluded that life shortening resulted primarily from an increased incidence and/or an early onset of malignant neoplasms, particularly in the low to moderate dose range [S27].

135. Maisin et al. [M20] have reported that fractionated exposures to high-energy neutrons (d(50MeV)Be; 8 fractions, 8 hours apart) appeared to have a slightly but not significantly greater effect than single exposures on life shortening in male C57Bl mice at doses up to 1.65 Gy. There appeared to be no significant difference in tumour incidence in the two groups, although in animals exposed to 1.65 Gy malignant tumours appeared earlier with fractionated than with single exposures. Life shortening could be described by a linear function of dose up to 3 Gy.

136. The results of these studies on a number of strains of mice are all reasonably consistent and suggest that the dose response for life shortening following exposure to high-LET radiation is a linear function of dose, at least for total doses up to about 0.5 Gy, and that neither dose fractionation nor dose protraction has much effect.

137. *Summary.* A number of studies in mice have examined the effects of dose fractionation and protraction of neutron doses on life-span shortening. Most recent studies have shown that when total doses are low (<0.5 Gy) and the dose per fraction is small there is no significant difference between acute and fractionated exposures. Data on protraction effects are rather limited but again suggest that protraction of exposure from high-LET radiation does not alter life-span shortening.

(d) Summary

138. 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, although this could also be influenced by the early appearance of some tumours. There is little suggestion that there is a general increase in other non-specific causes of death. At higher doses, into the lethal range, a non-specific component of life shortening becomes apparent due to cellular damage to the blood vasculature and other tissues. Accordingly, life shortening at low to intermediate doses can be used as a basis for examining the effect of dose fractionation and dose protraction on tumour induction.

139. The majority of comprehensive studies on the effect of dose fractionation of low-LET radiation on life-span have used the mouse as the experimental animal. The effect of dose fractionation appears to be very dependent on the strain of mouse and the spectrum of diseases contributing to the overall death rate. For example, in some strains thymic lymphoma incidence is increased by fractionation [M19]. Where this is a major contributor to the fatality rate, dose fractionation can result in a greater loss of life expectancy than acute exposures. Overall there is no clear trend in the effect of dose fractionation on life-span shortening, and the results from a number of studies suggest that, when compared with acute exposures, the effects of dose fractionation are small and in some studies have given either small increases or decreases in life-span. However, at least for exposure times of about a month, simple additivity of the injury from each dose increment can be assumed. One study in mice has reported that the reduction in survival time with eight fractions given 3 hours apart is half that obtained with an acute exposure, although this was accompanied by an enhanced tumour incidence. For fractionation intervals over a longer time there is a tendency to a longer life-span with an increasing interval between the doses, but the variations observed are generally less than those observed with protracted exposures.

140. When the effects in mice of acute exposures to low-LET radiation are compared with those of protracted irradiation given more or less continuously, it is seen that 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 life-span. The results available suggest, however, that with protracted exposures over a period of a few months to a year the effect on life-span shortening is reduced by factors of between about 2 and 5, compared with exposures at high dose rates. The effects of dose rate on tumour induction and life-span shortening have also been examined in rats and beagle dogs, although no significant differences have been seen. In these studies, however, dose rates varied by a factor of 60 or less, whereas in the studies in mice they varied by a factor of more than 100.

141. A number of early studies suggested that fractionated exposures to high-LET radiation induced more life shortening than single exposures. More recent studies have shown, however, that when total doses are low (<0.5 Gy) and the dose per fraction small, there is no significant difference in life shortening between fractionated and acute exposures. Although the data are less extensive than for low-LET radiation, the available information suggests that protraction of exposure does not affect life-span shortening.

2. Tumour induction

142. Information on radiation-induced tumours in experimental animals was extensively reviewed in the UNSCEAR 1977 Report [U4], in the UNSCEAR 1986 Report [U2], by the NCRP [N1], by Upton [U22] and in a comprehensive monograph on radiation carcinogenesis [U23]. Despite a substantial body of research potentially available for analysis, there are in practice

only a limited number of studies on tumour induction in experimental animals following exposure to low-LET radiation that can help to define the dose-response relationship for cancer induction over a reasonable dose range and to assess the influence of dose rate on tumour response. Important information comes from a series of studies with mice reported by Ullrich and Storer [U11-U16]. Although these investigations covered tumour induction in a number of tissues, it is convenient to discuss the results for different tumour types separately, in the context of other studies. It should be stressed, however, that the experimental animals used in many studies are inbred strains, with patterns of disease that are very different from those found in man. One of the main differences among mouse strains is their varying susceptibilities to both spontaneous and radiation-induced tumours; furthermore, within a given strain, there are frequently sex differences in the incidence and time of onset of specific tumour types. For example, the commonly used BALB/c strain has a very high incidence of spontaneous tumours, and the C57Bl strain has a much lower incidence [M25]. A number of tumour types for which information is available are either not found in man (Harderian gland) or appear to require substantial cell killing for their development (ovarian tumour, thymic lymphoma). For a number of other tumours there may be a human counterpart (myeloid leukaemia and tumours of the lung, the breast, the pituitary and the thyroid), but even here there can be differences in the cell types involved and in the development of the tumour. Furthermore, the development of tumours in both man and animals is subject to the modifying influence of various internal and external environmental factors, all of which can potentially influence dose-response relationships. There are also substantial differences in the rates of turnover of cells and in the life-span of the majority of experimental animals and man. Interpreting the results of animal studies and extrapolating them to man is therefore difficult. Nevertheless, such studies can make an important contribution to understanding the influence on tumour induction of factors such as dose rate and dose fractionation, radiation quality, dose distribution, age, disease and other internal and external agents. The use of animals to provide a basis for understanding factors influencing tumour induction applies not only to radiation but also to other agents such as chemicals and is considered further in Annex E "Mechanisms of radiation oncogenesis".

143. In assessing the influence of dose rate on tumour induction, a particular problem is that the dose-response relationship can vary substantially for different tumour types (Figures VIII-XV) [U22]. As a result, published reports describe the dose response in terms of a wide range of functions. Where data fits are given in the published papers that allow tumour incidences at different dose rates to be compared, the relevant information is given. In other cases, however, it has been necessary to fit the data reported. In general, the approach used has been to fit a linear function to the data obtained up to the highest dose at which there is no apparent influence of cell killing on the tumour yield. Generally this is the case for doses up to 2-3 Gy for acute exposures, but for low dose rates higher doses may be used. In some cases the most appropriate dose ranges for assessing dose and dose-rate effectiveness factors (DDREFs) are not clear from the data. In these cases, data fits have been calculated for a number of dose ranges.

144. The data from experimental animals on the effect of dose rate from low-LET radiation on tumour induction that are described in the following Sections are summarized principally in Tables 8-10. Table 8 gives best estimates of the DDREF for a range of tumour types in different animal species, together with information on the dose rates at which the studies were conducted and the dose ranges used for the calculation of the DDREF. Table 9 gives the results of fitting the data from a number of studies in mice over various dose ranges, and Table 10 examines uncertainties in the calculation of DDREF from studies in male and female mice. Results from a number of individual studies are given in Tables 11-15.

(a) Myeloid leukaemia

145. The effect of dose and dose rate on the induction of myeloid leukaemia has been examined in a number of strains of mice. Upton et al. [U21] compared the effect of a wide range of x- or gamma-ray doses in RF mice in the dose range from 0.25 to ~10 Gy. Male mice were substantially more sensitive than females, with an increased incidence of the disease detectable at 0.25 Gy given at a high dose rate (0.8 Gy min^{-1}, 250 kVp x rays). The incidence passed through a maximum at 3 Gy and declined at higher doses (Figure VIII). At doses up to about 1.5 Gy the incidence of the disease appeared to vary roughly with the square of the dose, although a linear dose response would fit the experimental results up to about 2 Gy. Low-dose-rate irradiation was much less effective than acute exposure. At dose rates of 0.04-0.6 mGy min^{-1}, no significant leukaemogenic effects were evident at a total dose of 1.5 Gy, although a significant increase was found at a dose of about 3 Gy. The induction period, as judged by mean age at death of mice with the disease, varied inversely with the dose and dose rate, suggesting that the disease contributed to the overall reduction in the life-span of the population. The exposures at different dose rates entailed time periods of between a few minutes up to about a month, so age effects are unlikely to have affected

tumour response, and differences between acute and chronic exposures appear to be predominantly due to differences in dose rate.

146. A linear fit to the incidence data up to 1 Gy in male mice [U21] exposed to high dose rates (slope: 14.4% ± 3.2% Gy^{-1}) and to 3.1 Gy following low dose rates (slope: 2.8% ± 0.8% Gy^{-1}) suggests that effectiveness at low dose rates decreased by a factor of 5.1 (Tables 8 and 9). A linear fit to the incidence data up to 3.0 Gy at high dose rate (slope 14.3% ± 1.7% Gy^{-1}) gave a similar result. In an earlier analysis of the high-dose and low-dose incidence data obtained up to 3.0 and 3.3 Gy, respectively, a dose-rate effectiveness factor of 6.7 was reported [N1].

147. In female RF mice the incidence of myeloid leukaemia following acute high dose-rate exposure was highly variable, and no clear dose-response relationship was obtained, although an overall increase in incidence was found at doses of 1 Gy or more at 0.067 Gy min^{-1} (Figure VIII) [U12]. At low dose rates, 0.004-0.7 mGy min^{-1}, the incidence of myeloid leukaemia (~6%) in mice exposed to doses between 1 and 6 Gy was approximately double that in controls (3%), but it showed no trend with increasing dose. The variability in results obtained for mice at high dose rates makes any estimates of dose-rate effect very uncertain. Based on a weighted least-squares fit to the data obtained up to 3 Gy at high dose rates (slope 6.8% ± 2.0% Gy^{-1}) and up to 5.8 Gy at low dose rate (slope 1.04% ± 0.38% Gy^{-1}), a dose-rate effectiveness factor of 6.5 is suggested (Table 9). A somewhat higher dose-rate factor (9.6) is obtained if the high-dose data on myeloid leukaemia incidence are compared with low-dose data over the range 0-6.1 Gy.

148. In contrast to these studies involving variations in dose rate, Upton et al. [U19] also examined the incidence of myeloid leukaemia in male RF mice given fractionated doses (2-3 exposures) of 0.75-1.5 Gy from x rays at high dose rates and found it to be similar to the incidence after single acute exposures. Robinson et al. [R3] re-analysed part of Upton's data on male RF mice [U18, U20, U21], considering those irradiated with 250 kVp x rays up to 4.5 Gy (~2,000 male mice) and correcting for competing risks. They obtained a good fit to the experimental data with a linear-quadratic model having an α_1/α_2 value of 0.5 Gy and a β_1/β_2 value of 2.4 Gy. It appears from these data that the decreased incidence of myeloid leukaemia at low dose rate can be interpreted in terms of an increasing linear component of the response and a diminishing quadratic component. The generalization of these results to other tissues is complicated, however, by the fact that the females are less sensitive than the males; also, it has been shown that the incidence of myeloid leukaemia is influenced by a number of host factors, including genetic background, hormonal status and the environment in which the animals are maintained [U19, U20]. Thus, animals maintained in a germ-free environment are less sensitive to the disease than animals housed in conventional facilities. It is not clear, therefore, whether the data suggesting dose-rate effects can be influenced by environmental factors involved in tumour initiation or expression.

149. Ullrich and Storer [U15] have reported dose-rate effects for myeloid leukaemia induction in 10-week-old specific-pathogen-free RFM/Un female mice exposed at 0.45 Gy min^{-1} or 0.083 Gy d^{-1} to ^{137}Cs gamma rays. Comparative data on dose response were obtained up to 2 Gy. Low-dose-rate exposure was much less effective than high-dose-rate exposure; in fact, no significant increase above control levels was observed at the low dose rate at doses up to 2 Gy. At high dose rate, a significant increase in myeloid leukaemia incidence above control levels was apparent at doses of 0.5 Gy and above, although the difference was only significant at 1.5 Gy or more. Even at 3 Gy the incidence was only 5.2%. Although the data could be fitted with either a linear or linear-quadratic model ($p > 0.5$ and $p > 0.8$, respectively), the dose-squared component was not significant, and linearity predominated over the dose range used in the study [U13]. A linear model fitted to the high-dose-rate data gives an incidence of 1.38% ± 0.12% Gy^{-1}, while that fitted to the low-dose rate data gives −0.050% ± 0.096% Gy^{-1}, reflecting the lack of any significant increase in incidence. The data therefore suggest a dose-rate effectiveness factor of infinity with a lower 95% confidence limit of 9.7.

150. Ullrich and Storer [U13] have also given dose-response data on male RFM mice exposed at 0.45 Gy min^{-1} to total doses between 0.1 and 3 Gy. Myeloid leukaemia incidence was higher than in female mice, and it was notable that the dose response could again be fitted with either a linear or a linear-quadratic model, with the linear component predominating over the dose range used in the study. The ratio of the linear slopes indicates that the sensitivity of male RFM mice to myeloid leukaemia ($I = 0.67 + 6.5D$, where I is the incidence in per cent and D is the dose in gray) is greater than that of female mice ($I = 0.63 + 1.4D$) by a factor of nearly 5.

151. The effect of variation in dose rate on the induction of myeloid leukaemia in male CBA/H mice has been examined by Mole et al. [M13]. This strain of mouse 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 [H17] (see Annex E, "Mechanisms of radiation onco-

genesis"). Groups of mice received exposure to ^{60}Co gamma rays continuously over a four-week period (0.04-0.11 mGy min^{-1}) or single brief exposures five days a week for four successive weeks (0.25 Gy min^{-1}) or a single brief exposure (0.25 Gy min^{-1}). Total doses were 1.5, 3 or 4.5 Gy. The results of the study, summarized in Table 11, demonstrate a dose-dependent increase in incidence of myeloid leukaemia in the acutely exposed animals compared with controls, with a higher incidence than those groups given protracted exposure. However, in the groups in which radiation exposure was spread over a period of four weeks, either continuously or in 20 equal fractions (giving differences in dose rate of several thousand-fold), the incidence was the same, and within the dose range used appeared independent of the total cumulative dose. There is no obvious explanation for this result. Mole et al. [M13] speculated that the critical factor determining leukaemogenic frequency in this experiment was not the instantaneous physical dose rate but some biologically important factor correlated with protraction. Nevertheless, the frequency of leukaemia induction was already reduced by protraction of the dose. As a consequence of the lack of a dose-response relationship for myeloid leukaemia incidence with protracted exposure, in contrast to the results for acute exposure, the factor for reduction in myeloid leukaemia induction at low dose rates varies from 2.2 at 1.5 Gy to 5 at 4.5 Gy (Table 11).

152. *Summary.* These studies have shown that radiation-induced myeloid leukaemia can be induced in RFM and CBA mice, although there are differences in sensitivity between the strains and between both sexes. For dose rates varying by factors ranging from 100 to more than 1,000, DDREFs between about 2 and more than 10 have been obtained for doses in the 1-3 Gy range given at high dose rates, but there is no consistent trend (Table 8).

(b) Lung cancer

153. Information on the effect of dose and dose rate on carcinogenesis in the respiratory tract from low-LET radiation has come mostly from whole-body exposure of animals to x rays and gamma rays. However, comparative data are also available on the effects of inhaled radionuclides with different effective half-times in the lung; these data provide further information on dose-rate effects.

154. The induction of lung adenocarcinomas at high dose rates (0.4 Gy min^{-1}) and low dose rates (0.06 mGy min^{-1}) has been compared in female BALB/c mice [U12, U15] in the dose range 0.5-2 Gy. Tumour induction was less at low dose rates than at high dose rates. After high dose-rate exposure, the age-correlated incidence (%) could be represented by a linear function [$I(D) = 13.4 + 12D$; $p > 0.5$]; at low dose rates a linear function also gave a good fit to the data [$I(D) = 12.5 + 4.3D$; $p > 0.8$]. Since the authors indicated there were no changes in sensitivity with age over the period of irradiation and the data were adjusted for differences in the distribution of ages at death among the various treatment groups, the differences in slope can be considered to reflect differences in effectiveness at the two dose rates and suggest a dose-rate effectiveness factor of 2.8 (Table 8). The data on lung tumour induction in mice were extended in a further study [U24] which provided information on the dose response at high dose rates (0.4 Gy min^{-1}) in the dose range from 0.1 to 2 Gy. Although the tumour incidence data could again be fitted by a linear model [$I(D) = 10.9 + 11D$; $p > 0.70$)], they could also be fitted by a linear-quadratic model [$I(D) = 11.9 + 4D + 4.3D^2$, $p > 0.70$]. In this equation the linear term was very similar to that obtained for low-dose-rate exposures, and it was concluded that the result was in general consistent with a linear-quadratic model in which the linear term is independent of dose rate at high and low dose rates.

155. Recently, Ullrich et al. [U26] tested the predictions of the linear-quadratic model in a series of studies with BALB/c mice using fractionated exposures. The model predicts that fractionating an exposure using high-dose-rate fractions but with a small total dose per fraction, which would lie on the predominantly linear portion of the dose-response curve, would have an effect similar to that obtained with low dose rates. Mice were exposed to total doses of 2 Gy from ^{137}Cs gamma rays given in different daily fractions (0.1, 0.5, and 1 Gy) at high dose rate (0.35 Gy min^{-1}). The linear-quadratic dose-response curve for lung tumour induction gave an α_1/α_2 quotient of 0.93, indicating that at doses of about 0.9 Gy, the α_1 and α_2 terms contribute equally to the tumour response. At doses substantially below this, the linear term should predominate, giving a tumour induction rate similar to that at low dose rates (0.06 mGy min^{-1}) (Figure IX).

156. The results of the study are shown in Table 12, which gives both the observed incidences of lung adenocarcinomas and those calculated from the predictions of a linear-quadratic model, on the assumption that the effects of each dose fraction are additive and independent of each other. The lung tumour incidence following daily fractions of 0.1 Gy (group 3), which would be on the linear component of the response curve, was comparable to that obtained following low-dose-rate exposure (group 2). If the dose per fraction was increased the quadratic term would be expected to make an increasing contribution to the response, with an increase in the tumour incidence per unit dose, as was indeed observed (groups 4 and 5), although not to

the level obtained with a single exposure to 2 Gy at high dose rate (group 1). The results are seen to be consistent with the predictions of the linear-quadratic model, which can therefore be used to assess the effect of dose rate on tumour response at various doses and dose rates. Table 13 gives both total tumour incidence and radiation-induced excess tumours for a range of doses (0.1-3 Gy) administered at both high and low dose rates. The incidence of tumours has not been calculated at doses above 3 Gy, because at higher doses cell killing is expected to become significant. At the lowest dose (0.1 Gy), the linear term dominates and the tumour incidence is largely independent of the dose rate (DDREF = 1.1). At higher doses, however, the quadratic term becomes of increasing importance at high dose rates, giving a DDREF of about 3.2 at 2 Gy and 4.2 at 3 Gy (mean: 3.7). These experimental data illustrate very clearly the extent to which lowering the dose rate can reduce the tumour incidence. They also suggest that for lung adenocarcinoma in mice, a low dose, at which there is no significant effect of dose rate, is in the range 0.1-0.2 Gy.

157. Extensive long-term studies on the effects of inhaled beta- and gamma-emitting radionuclides in dogs have been reported by McClellan et al. [M1]. Groups of about 100 beagle dogs about 13 months old were exposed to aerosols of a range of radionuclides bound in fused aluminosilicate particles and having different half-lives (^{90}Y: 64 hours; ^{91}Y: 58.5 days; ^{144}Ce: 285 days; ^{90}Sr: 28 years) to give a range of initial lung contents. In this insoluble form, the radionuclides are poorly transportable in the lung tissue and do not readily translocate to the blood. The different radioactive half-lives of the nuclides gave effective half-times in the lung ranging from 2.5 days for ^{90}Y to 600 days for ^{90}Sr. As a consequence, very different dose rates were obtained for the same cumulative dose (Figure X). For ^{90}Y, more than 90% of the total dose to the lung was received within two weeks of exposure, for ^{91}Y, about 90% of the dose was received by six months; while for ^{144}Ce and ^{90}Sr, only 77% and 34% of the total doses were received by one year. The dogs are being observed for their active lifetime, and the study is not yet complete. Some dogs exposed to high radiation doses died early with radiation pneumonitis and fibrosis; others died later with lung tumours. Tumours occurred with absorbed doses to lung ranging from 11 to 680 Gy. Preliminary data on the incidence rates of radiation-induced lung tumours have been reported [G10, H12]. The estimated risk coefficients for lung tumour induction in dogs exposed to ^{90}Y, ^{91}Y, ^{144}Ce and ^{90}Sr at times up to more than 10 years after exposure were 0.036, 0.032, 0.011 and 0.013 Gy^{-1}. Thus, the relative risk of lung cancer in dogs exposed at the higher dose rate from ^{90}Y is about three times the risk observed in dogs exposed at low dose rates.

158. *Summary.* Two studies in mice have found an effect of dose and dose rate on lung tumour induction, with DDREFs in the range of 3-4 for doses in the range 2-3 Gy given at high dose rate. Studies in beagle dogs exposed to inhaled, insoluble radionuclides with different effective half-times have given a range of risk coefficients for induced lung cancer that varied by a factor of about 3 between ^{90}Y, which gave the highest dose rate, and ^{90}Sr, which gave the lowest (Tables 8 and 13).

(c) Mammary tumours

159. Mammary carcinogenesis in inbred strains of mice is highly dependent on hormonal, viral, genetic, immunological, dietary and environmental factors [S7, S8]. As a consequence, irradiation may affect mammary carcinogenesis either directly, by affecting the cells of the breast, or indirectly by causing functional changes in the endocrine glands or by activating mammary tumour virus or other viral agents. In some mouse strains there is evidence that ionizing radiation can induce mammary tumours by "abscopal" effects, i.e. tumours can be induced in the mammary tissue irrespective of the area irradiated [B19, S8]. Similar considerations also apply in the rat. Rat mammary tumours can be classified as fibroadenomas and adenocarcinomas [Y3]. The incidence and proportion of these tumour types is very dependent on the strain of rat irradiated.

160. The most extensive experimental data on the induction of mammary tumours by ionizing radiation come from studies in Sprague-Dawley rats. However, in this strain of rat, which is sensitive to the induction of adenocarcinomas by radiation, there is a high spontaneous tumour incidence beyond about 15 months of age, so that in many experimental studies a cut-off period of approximately 12 months is imposed. Near the end of life of these animals the total tumour incidence in controls approaches that seen in irradiated animals, with the result that the absolute excess incidence is increased minimally, if at all. Thus, it may be that the effect of irradiation is to accelerate the appearance of tumours rather than to increase the overall incidence [C14, S8]. For rats of other strains and for other species the incidence of radiation-induced mammary tumours is less than in Sprague-Dawley rats [U11].

161. In Sprague-Dawley rats given whole-body x-irradiation or ^{60}Co gamma-irradiation at 1-2 months of age (0.25-4 Gy), the incidence of tumours at one year increased as a linear function of the dose [B17]. Similar results were obtained by Shellabarger et al. [S6] in the dose range 0.16-2 Gy with ^{60}Co gamma rays. The incidence of mammary tumours in rats

exposed to ^{60}Co at two different dose rates has been reported by Shellabarger et al. [S15]. Groups of rats were exposed at either 0.0003 Gy min^{-1} or 0.1 Gy min^{-1} to give total doses of 0.9 Gy and 2.7 Gy. In animals exposed to 2.7 Gy, the incidence of mammary adenocarcinomas was higher in the high-dose-rate group (8/20, or 40%) than in the low-dose-rate group (4/35, or 11%). With an incidence in controls of 1%-2%, this suggests a dose-rate effectiveness factor of about 4. However, no effect of dose protraction was found in the low-dose group, and for both dose levels, no protraction effect was observed for mammary fibroadenomas or total mammary tumours. The overall incidence of tumours in the animals exposed at low dose rate was also lower than in the animals exposed at high dose rate, largely because of the effect on adenocarcinomas. Shellabarger et al. [S4, S5] have also compared mammary tumour induction in rats given 4-5 Gy from x rays, either in a single exposure or in up to 32 fractions delivered over a 16-week period. No apparent change in the total tumour incidence was observed with dose fractionation, but this may have been because the total dose exceeded the level at which the response reached a maximum with single-exposure irradiation.

162. The incidence of mammary tumours in Sprague-Dawley rats exposed at different dose rates has been reported by Gragtmans et al. [G7]. Groups of approximately 120 SPF rats were either chronically exposed to 200 kVp x rays over a 10-day period, to give doses between 0.3 and 2 Gy, or given acute exposures of 0.6 or 1.8 Gy over one hour. In all dose groups, total tumour incidence was significantly greater than in controls, and by 450 days the average number of tumours per animal exceeded unity for the highest dose groups following both acute exposure (1.52 tumours per animal) and chronic exposure (1.14 per animal) (controls: 0.17 per animal). The best fit to the data up to 450 days was obtained with a linear function with cumulative tumour incidences of 78.3% ± 10.4% Gy^{-1} for acute exposure and 45.5% ± 5.4% Gy^{-1} for chronic exposure. A linear dose response was also obtained for the proportion of animals with tumours at 450 days with parameter values of 40.5% ± 0.4% Gy^{-1} and 24.8% ± 2.4% Gy^{-1} for acute and chronic exposures, respectively. These results indicate dose-rate effectiveness factors in the range 1.6-1.7, which is the same range in which other data on mammary tumour induction fall.

163. The effect of fractionated or single doses of x rays has been reported for WAG/RIJ rats [B23]. The rats were eight weeks old when irradiated and were kept until death. The frequency of fibroadenomas and carcinomas was based on histological examination. Weibull functions were fitted to the dose-response data, and the probability of survival without evidence of a tumour was calculated according to the Kaplan-Meier life-table analysis. The analysis showed that irradiation accelerated the appearance of fibroadenomas and carcinomas. Fractionation of the dose (10 exposures of 0.2 Gy at one month intervals) was only marginally less effective in respect of the effect on appearance time of mammary carcinomas than a single dose of 2 Gy; this study, therefore, provides no evidence for a reduction factor (i.e. DDREF ≈1).

164. The effect of dose rate on mammary tumour induction in mice has been reported by Ullrich and Storer [U12, U15]. In female BALB/c mice exposed to ^{137}Cs gamma rays, groups of mice were exposed at either a low dose rate (0.06 mGy min^{-1}) or a high dose rate (0.45 Gy min^{-1}) to give total cumulative doses of 0.5 Gy or 2 Gy. At both dose levels the incidence of mammary adenocarcinomas could be adequately described by linear relationships and was higher at high dose rates [$I(D) = 7.9 + 6.7D$; $p > 0.5$] than at low dose rates [$I(D) = 7.8 + 3.5D$; $p > 0.25$] (Figure XI). The ratio of the slope constants for mammary tumours suggests a dose-rate effectiveness factor of 1.9.

165. These data on mammary tumour induction were extended in a further study [U24] which provided information on the dose response at high dose rates (0.4 Gy min^{-1}) in the dose range from 0.1 to 2 Gy. The incidence of mammary tumours increased rapidly over the dose range up to 0.25 Gy. At higher doses, although there was some response, it was roughly flat. The high initial sensitivity to tumour induction was surprising in the light of the previous results [U12], which, however, were based on fewer data points and doses no lower than 0.5 Gy. Taken together with the previous data, the data obtained up to a dose of about 0.25 Gy were consistent with a linear-quadratic model of the form $I(D) = 7.7 + 3.5D + 150D^2$. The linear term was similar to that obtained after low-dose-rate exposures.

166. Ullrich et al. [U26] have tested the predictions of this linear-quadratic model in a series of studies with fractionated exposures. The fit to the data gives an α_1/α_2 quotient of 0.023, indicating that doses as low as 0.1 Gy will give a significant contribution from the quadratic component. BALB/c mice were exposed to total doses of 0.25 Gy given as daily fractions of either 0.01 Gy or 0.05 Gy. The incidence of mammary tumours for these two groups and for other groups of mice exposed at high dose rate (0.35 Gy min^{-1}) to give total doses of 0.1-0.25 Gy and at low dose rate (0.07 mGy min^{-1}) to give 0.25 Gy are compared in Table 14 with tumour incidences predicted by the linear-quadratic model. Acute daily fractions of 0.01 Gy gave a tumour incidence similar to that observed following low-dose-rate exposure and in

good agreement with model predictions. In general the results demonstrate that for mammary tumour induction, the effects of dose fractionation can be predicted by the linear-quadratic dose-response model. The response of this strain of mouse appears, however, to be markedly different from that of the rat strains described above, as a substantial dose-rate effect is apparent, with an implied DDREF at 0.25 Gy, the highest dose at which tumour incidence was measured, of about 12 (Table 15). In rats evaluation of the DDREF was made at doses up to about 3 Gy (Table 8).

167. *Summary.* A number of studies have been published on the effect of dose rate on mammary tumour induction in rats. These studies give DDREFs from less than 2 to about 4 for dose rates varying by a factor of 150 or more and for doses at high dose rate in the range from about 2 to 3 Gy (Table 8). One study in mice gives an implied DDREF based on an assumed linear-quadratic response of about 12 at 0.25 Gy for dose rates varying by a factor of about 5,000, although interpretation of the data is limited by the lack of information at higher doses.

(d) Pituitary tumours

168. The effect of dose rate on the induction of pituitary tumours in RFM mice has been reported by Ullrich and Storer [U14, U15] for female mice exposed at high dose rates to ^{137}Cs gamma-radiation (0.45 Gy min^{-1}) giving total doses of 0.1-3 Gy. The incidence of these tumours with radiation dose was found to increase at doses of 0.5 Gy or higher, although the response was somewhat irregular and did not differ significantly from controls, even at 2 Gy. The incidence remained at approximately control levels (6%-7%) over the range 0-0.25 Gy, increased to 9%-10% at 0.5 Gy, remained at that level over the range to 2 Gy, and increased to 20.9% at 3 Gy. Both a linear model [$I(D) = 5.7 + 4.4D$; $p > 0.2$] and a linear-quadratic model [$I(D) = 6.3 + 0.8D + 0.013D^2$] adequately described the data. Lowering the dose rate to 0.06 mGy min^{-1} resulted in a reduced tumour incidence up to a total dose of 2 Gy; this incidence was best described by a linear model [$I(D) = 6.3 + 0.7D$; $p > 0.95$]. When the linear dose responses fitted at low and high dose rates were compared, low-dose-rate exposures were found to be less effective in inducing pituitary tumours, by a factor of about 6 (Table 8). However, if a linear-quadratic response is assumed after high-dose-rate exposure [$I(D) = 6.3 + 0.8D + 0.013D^2$], then the linear term is similar at both dose rates, suggesting that the primary effect of dose rate is to alter the dose-squared component. Male RFM mice were exposed only at the higher dose rate, and the incidence of pituitary tumours was too low to warrant analysis.

169. *Summary.* Only one study has been published that allows an estimate to be made of a DDREF for the induction of pituitary tumours. In female mice, a value of about 6 can be obtained for doses up to about 3 Gy given at high dose rate and for dose rates that differ by a factor of about 8,000.

(e) Thyroid tumours

170. Thyroid cancer in animals can be induced by iodine deficiency, chemical carcinogens, and goitrogens, and exposure to ionizing radiation. Information is available from human populations on the effects of both external radiation and internal radiation from intakes of iodine isotopes; it suggests that ^{131}I is less carcinogenic than external radiation (see Chapter III), although whether this is due solely to dose-rate effects or to other factors as well is not clear. In principle, animal studies should be able to provide information on the relative effects of external radiation and ^{131}I, but in practice the reported results present some difficulties in interpretation, and there are species differences in the way thyroid cancer is expressed.

171. Doniac [D4] reviewed a series of studies in rats that could be used to compare the tumorigenic effects of x rays and ^{131}I. Results from three studies suggested that the carcinogenic effect of 11 Gy from acute x-ray exposure was comparable to that of 1.1 MBq of ^{131}I, which would give a dose to the thyroid of about 100 Gy. The dose rates are substantial, and significant cell killing might be expected, although higher doses of ^{131}I are needed to cause atrophy. With this proviso, the data suggest that protracted irradiation from ^{131}I is less damaging, by a factor of about 10, than an acute dose of x rays.

172. Walinder [W2] compared the carcinogenicity of x rays and ^{131}I in adult CBA mice. His results indicated that ^{131}I was one fourth to one tenth as effective as x rays for the production of thyroid adenomas and carcinomas; doses from x rays were 15 Gy and from ^{131}I, 64-160 Gy. He also found that at somewhat lower doses (10 Gy from x rays and 22-110 Gy from ^{131}I), ^{131}I was one half to one tenth as effective as x rays.

173. Whether this difference in effect is due solely to differences in dose rate is difficult to determine, as a number of factors influence the dosimetry of ^{131}I [C8, D4, J1]. As a consequence of the small mass of the thyroid in the rat or mouse, considerable beta-radiation is lost from the peripheral portions and the isthmus. Thyroid cells near the surface may receive as little as 50% of the dose to the central cells, an effect that becomes more important as the gland size decreases. Unlike the dose from external radiation, the dose from

intakes of ^{131}I may be heterogeneously distributed owing to variation in uptake between follicles, although Walinder et al. [W1] have reported measurements of the dose distribution for ^{131}I and ^{132}I in mouse thyroid and found it to be generally uniform, but with decreases at the thyroid edges, as would be expected for a uniform concentration. Walinder et al. [W1] also observed a similar effectiveness of ^{132}I ($T_{½}$ ~ 2.2 h) and x rays on the thyroid in the inhibition of goitrogen-stimulated growth in the CBA mouse and that ^{131}I was one half to one tenth as effective as x rays. Book et al. [B14] observed in the Sprague-Dawley rat a difference in effectiveness of ^{131}I to ^{132}I of about 1:9, in terms of average thyroid dose, for the suppression of thyroid gland weight increase stimulated by goitrogen. The dose distribution in the thyroid gland is similar for the two radionuclides, and the observed difference in radiation is likely to be due to differences in dose rate. Liu et al. [L2] found that the tumour incidence was lower following exposure to ^{131}I than ^{132}I. Although this difference may have been partly due to higher radiation doses from ^{131}I, the dose rate may have also been a factor in the response. Since ^{131}I uptake by the thyroid in laboratory animals varies with environmental temperature and the dietary content of stable iodide, the administration of similar amounts in separate experiments in different laboratories may give rise to varying doses. The usual assumption, for dosimetric purposes, of a single exponential function for loss of activity from the gland may also result in some uncertainty in the calculated doses, although by a factor of less than 2 [C8]. Despite these uncertainties, it seems likely that the differences in the incidence of cancer resulting from intakes of ^{131}I and from exposure to external radiation cannot be readily explained by differences in dose distribution.

174. In a subsequent study, Lee et al. [L4] compared tumour induction in six-week-old female Long Evans rats given ^{131}I or localized x-irradiation of the thyroid. Three groups of 300 rats were injected intraperitoneally with 18, 70 and 200 kBq of sodium ^{131}I-iodide, giving thyroid doses of 0.8, 3.3 and 8.5 Gy (maximum dose rates: 0.17, 0.69 and 1.6 mGy min^{-1}). Three further groups received localized (collimated) x-ray exposures of the thyroid gland giving doses of 0.94, 4.5 and 10.6 Gy (dose rate: 2.8 Gy min^{-1}). Six hundred animals were kept as controls. All the animals surviving to two years (~62%) were killed, and a six-month minimum latent period for radiogenic thyroid cancer was assumed. The doses from ^{131}I in this study were considerably lower than those in the earlier ones. Exposure to ^{131}I was found to be about 40% as effective as x-irradiation at the highest dose for the production of adenomas, but there was no significant difference from x rays at the lower doses. For the production of thyroid carcinomas the two radiations appear to be of equal effectiveness at all three doses, although the statistics were such that the results do not exclude a two- to threefold difference in the effectiveness of x rays and ^{131}I.

175. These differences in effectiveness observed in the studies by Lee et al. [L4] and the earlier rat studies are not easy to explain. They may reflect differences in the doses used, in the ages of animals or in the strains of rats. Female Long Evans rats are also more sensitive than males, which has been attributed to hormonal fluctuations. The results of Lee et al. [L4] provide probably the largest single body of information on thyroid cancer induction by ^{131}I or x rays in an animal model. Furthermore, the dose range was low and more relevant to the assessment of risks from low-level exposures. That study, however, terminated at two years, rather than allowing the animals to live out their natural life-span. This may have prevented the appearance of some late tumours, an important feature, as about two thirds of the animals remained alive at the end of the two-year study.

176. *Summary.* Animal data do not support large differences between ^{131}I and x rays for thyroid cancer induction for doses below about 10 Gy. Early experiments that indicated differences of up to a factor 10 were at doses that would have caused appreciable tissue damage. However, differences in tumour response of a factor of about 3 between ^{131}I and x rays cannot be ruled out.

(f) Liver tumours

177. Di Majo et al. [D3] have reported dose-response relationships for liver tumour induction in $BC3F_1$ male mice. Three-month-old mice were exposed to x rays (0.133 Gy min^{-1}) in graded acute doses from 0.5 Gy to 7 Gy. A significant increase in liver tumours was observed from 2 Gy, and the dose response was best fitted by a pure quadratic response [$I(D) = 11.3 + 1.2D^2$] (Figure XII). Although the animals were not exposed at different dose rates, this pattern of dose response would imply that tumour induction would be reduced at lower dose rates.

(g) Harderian gland tumours

178. The induction of Harderian gland tumours at different dose rates has been examined in RFM mice [U12, U14, U15]. This information is included here for the sake of completeness, although it is noted that there is no human counterpart to this tumour. The data are, however, considered to be relevant to understanding the overall response of tissues to radiation. Low-dose-rate exposures (0.06 mGy min^{-1}) were less effective than high-dose-rate exposures (0.45 Gy

min^{-1}), with incidences at 2 Gy being significantly different for the two treatments (Figure XIII). Dose-response relationships at high dose rates suggested a linear-quadratic model for both males [$I(D) = 1.5 + 0.3D + 0.012D^2$; $p > 0.99$] and females [$I(D) = 1.2 + 1.5D + 0.022D^2$; $p > 0.25$], although linearity could only be excluded with confidence for females ($p < 0.05$). At low dose rates, a linear dose response gave the best fit to the data for female mice [$I(D) = 1.2 + 1.5D$; $p > 0.9$], suggesting, as in the case of pituitary tumours, a similar linear response for low and high dose rates, and that the primary effect of lowering the dose rate was to diminish the dose-squared component. On the basis of a simple linear fit to the high- dose-rate dose-response data [$I(D) = 0.93 + 4.7D$; $p < 0.05$], the results for female mice indicate that tumour incidence at low dose rates is reduced by a factor of about 3 for doses of about 2 Gy (Table 8).

179. *Summary.* The dose-response for Harderian gland tumours in female mice resulting from high-dose rate exposures can be fitted by a linear-quadratic relationship. The data suggest a DDREF for high-dose-rate exposures of about 3 for doses of about 2 Gy and for dose rates varying by a factor of about 8,000.

(h) Ovarian tumours

180. The induction of ovarian tumours in gamma-irradiated mice has been shown to depend on the dose rate [U12, U21, Y4]. Interpretation of some of the data is complicated, however, by a decrease with age in the susceptibility of the mouse ovary to tumorigenesis. Furthermore, since the stimulus for tumorigenesis is believed to involve killing of oocytes and associated changes in hormonal status [U21], this is likely to contribute to observed dose-rate effects.

181. The induction of ovarian tumours by x rays was studied by Ullrich and Storer [U15] using SPF/RFM female mice exposed at 0.45 Gy min^{-1} and 0.06 mGy min^{-1}. After high-dose-rate exposures, a significant increase in tumour incidence relative to controls was observed for doses of 0.25-3 Gy. In the group exposed at the lower dose rate, no significant increase in incidence was seen until 1 Gy, when the incidence was similar to that observed in the groups receiving 0.25 Gy at higher dose rate (Figure XIV). The high-dose-rate data could be adequately described by a linear-quadratic model with a negative linear component [$I(D) = 2.3 + (-23)\ D + 1.8D^2$; $p > 0.25$] 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] [U14]. Linear and quadratic models were rejected. For the low-dose-rate response linear, quadratic and threshold plus quadratic models could be rejected ($p < 0.01$). The two models that appeared to describe the relationship adequately were a linear-quadratic model [$I(D) = 2.3 + (-3.7)D + 0.068D^2$; $p > 0.25$] and a threshold plus linear model [$I(D) = 2.07 + 14.9(D-D^*)$; $p > 0.75$, where the threshold dose, D^*, was estimated to be 0.115 Gy].

182. In female BALB/c mice similar results have been obtained [U15]. Ovarian tumours were readily induced with high-dose-rate exposures (0.40 Gy min^{-1}) to ^{137}Cs gamma rays, giving a 66% incidence at 0.5 Gy, the lowest dose used, compared with a 9.9% incidence at low dose rate (0.06 mGy min^{-1}) for the same total dose (dose-rate effectiveness factor: 6.7, Table 8). As in the case of RFM mice, linearity could be rejected ($p < 0.05$) at low dose rate, and the dose response up to 2 Gy could be described by a linear-quadratic model [$I(D) = 6.0 + 8.3D + 0.05D^2$]. There were insufficient data at high dose rate to define a dose-response function; doses below 0.5 Gy would have been required.

183. This pattern of response for ovarian tumour induction seen in both RFM and BALB/c mice is explained by the mechanism of induction for ovarian cancers, which is considered to involve substantial cell killing. This mechanism will be less effective at low dose rates and may account for the apparent threshold in the response. Linear functions fitted in this Annex to the dose-response data in RFM mice obtained up to 2 Gy for both high- and low-dose-rate exposures [U15] give a crude overall DDREF of 5.5 (range: 4.1-6.8, Table 9), compared with a value of 6.7 for BALB/c mice (Table 8), but because of the mechanisms involved, the extrapolation of these results to man is uncertain.

184. *Summary.* The induction of ovarian tumours has been shown in mice to depend on dose rate. DDREFs of 5.5 and 6.7 have been obtained in RFM and BALB/c mice at doses up to 2 Gy for dose rates varying by a factor of about 8,000. Since the stimulus for tumorigenesis is believed to involve killing of oocytes and associated changes in hormonal status, extrapolation of these results to man is uncertain.

(i) Thymic lymphoma

185. A number of investigators have studied the incidence of thymic lymphoma after radiation exposure. However, many of these studies have been concerned with modifying factors that influence the course of the disease or the sequence of events leading to its development rather than with dose-response relationships. An added complication is that dose-response curves of a threshold type have been reported [M5], indicating that cell killing is important in the induction mechanism.

186. Ullrich and Storer [U12, U15] studied the dose-response relationship and dose-rate effects for exposure to ^{137}Cs gamma rays in 10-week-old female RFM/Un mice. The incidence of thymic lymphoma after high-dose-rate exposure (0.45 Gy min^{-1}) was substantially greater than after low dose rates (0.06 mGy min^{-1}) at all doses for which comparable data were given (0.5, 1 and 2 Gy) (Figure XV). In fact, no significant increase in incidence relative to controls was observed after low-dose-rate irradiation up to a total dose of 1 Gy, whereas at high dose rates a significant increase in incidence was observed at doses of 0.25 Gy. Examination of the relationship between the incidence of thymic lymphoma and the radiation dose at high and low dose rates indicated both quantitative and qualitative differences. The dose response after high-dose-rate exposure appeared to have two components. Up to 0.25 Gy, the incidence of thymic lymphoma increased with the square of the dose, with a second, linear component describing the response over the range 0.5-3 Gy. At the lower dose rate the response was best described by a linear-quadratic model with a shallow (perhaps zero) initial linear slope, and linearity could be rejected. Considering that the mechanism of thymic lymphoma induction is thought to involve cell killing or the possible release of viruses and subsequent target cell viral interactions, it is not surprising that somewhat complex dose and dose-rate response relationships have been obtained.

187. An early analysis by the NCRP [N1] gave a dose-rate effectiveness factor of 6.4 for these data. Alternatively, the high- and low-dose-rate data obtained up to 3 and 2 Gy may be fitted with linear models, giving fits of 16.7% ± 1.8% Gy^{-1} and 2.9% ± 1.9% Gy^{-1}, respectively, corresponding to a dose-rate effectiveness factor of 5.8 (Tables 8 and 9). In males [U13] no dose-response data were obtained at low dose rates, but at high dose rates (0.45 Gy min^{-1}) a significant increase in incidence occurred at doses of 0.25 Gy and above, and the data over the entire dose range up to 3 Gy could be adequately fitted by a linear function (6.7% ± 6.9% Gy^{-1}). Overall, males were less sensitive than females by a factor of about 2.4, reflecting the difference of a factor of about 2 in the incidence in controls.

188. An increased incidence of thymic lymphoma has also been reported by Upton et al. [U21] for male and female RFM mice exposed to x rays. Lymphoid neoplasms occurred in about 4%-10% of controls, and the increase in incidence depended on both the total dose and the dose rate, with a significant increase, relative to controls, at doses of about 2 Gy or more. With decreasing dose rate, the effectiveness of gamma-radiation in inducing tumours declined. A linear dose function fitted to both high-dose-rate (0.8 Gy min^{-1}) and low-dose-rate (0.04-0.6 mGy min^{-1}) incidence data for males suggests a dose-rate effectiveness factor of about 2.6 (Table 8) at doses up to about 4 Gy. For females the data are too variable to infer a dose-rate effectiveness factor.

189. In experiments by Maisin et al. [M5], 12-week-old male mice were exposed to single or fractionated (10 equal doses separated at daily intervals) doses from ^{137}Cs gamma rays (4 Gy min^{-1}) in the dose range 0.25-6 Gy. The dose-response curve for thymic lymphoma was of a threshold type, the incidence in irradiated animals rising above that in controls only at 4 and 6 Gy. Single doses were more effective than fractionated exposures at 4 Gy by a factor of about 2; there was no significant difference in response at 6 Gy.

190. *Summary.* An effect of dose rate on the induction of thymic lymphoma has been demonstrated in RFM male and female mice with DDREFs of about 2.6 and 5.8 for doses of 2 to 4 Gy and for dose rates varying by factors of more than 1,000 (Table 8). As with ovarian tumours there are difficulties in extrapolating these data to man, however, as cell killing appears to be involved in the development of this tumour.

(j) Skin tumours

191. A number of studies have reported that when the radiation dose is fractionated, the incidence of skin tumours decreases in comparison with acute exposures. Hulse et al. [H30] irradiated the skin of three-month-old CBA/H female mice with a ^{204}Tl source. Four different schedules of exposure were used: four equal doses at weekly intervals, four equal doses at monthly intervals, 12 equal doses at weekly intervals and 20 equal doses five days weekly for four weeks. Total doses given were large, 60 Gy or 120 Gy. The tumours occurring after the different irradiation schedules were similar to those seen after single exposures and were mainly dermal tumours at both dose levels. Dividing the total dose into four fractions did not affect tumour yield, whether the exposures were spread over 22 days or 12 weeks. When 20 fractions were given over 25 days, however, the yield was significantly reduced ($p = 0.02$) to about half that with a single exposure. With 12 fractions given over 11 weeks, the yield was non-significantly reduced ($p = 0.09$ for both dose groups; $p = 0.06$ for 60 Gy). It was concluded that a reduction in tumour yield followed multiple fractionation and protraction over several weeks only if the dose per fraction was 5-6 Gy or less. The reduction factor was about 2. For epidermal tumours, of which there were fewer than half the number of dermal tumours, there was a much greater variation in response between the groups. None of the

groups with fractionated exposure had a significantly different tumour yield from the single exposure group, and there was no clear evidence that protraction or fractionation reduced tumour yield. The yield of epidermal tumours was, however, significantly less in the 20-fraction groups (at 6 and 12 Gy total doses) compared with the groups given 4 and 12 weekly fractions.

192. In a series of studies in male CD rats [B40], skin tumour incidence following acute exposure to attenuated 0.7 MeV electrons (1.6-2.4 Gy min^{-1}) was measured at nine doses (20 rats per group) between 5 Gy and 23 Gy. A peaked dose-response curve was obtained, with a maximum tumour incidence at a dose of about 16 Gy. At 10, 14.5 and 23 Gy, the exposures were also split into two equal fractions spaced at intervals of 1, 3 and 6.3 hours. The effect of split doses on tumour yield depended on the position on the dose-response curve. At the lowest split dose the tumour yield declined with a half-time of about 1.8 hours. At the intermediate dose, an initial increase was followed by a decline, with a half-time of 3-4 hours; at the highest dose (23 Gy) the tumour yield increased, presumably as a result of the spacing effect on cell lethality. The maximum effect of dose fractionation (14.5 Gy, two fractions, 6.3 hours) gave a reduction in tumour yield by a factor of about 2.

193. In a more recent series of papers [O5, O6], skin tumour incidence has been measured in female ICR mice. The backs of the animals were repeatedly irradiated with beta particles from ^{90}Sr-^{90}Y (2.24 Gy min^{-1}, surface dose). For doses of 2.5-11.8 Gy per exposure, three times weekly throughout life, 100% incidence of tumours was observed. At doses of about 1.5 Gy per exposure, however, there was a marked delay in the appearance of tumours. In a further study [O7], groups of 30 or 31 mice were irradiated with ^{90}Sr-^{90}Y three times weekly throughout their life with doses of 0.75, 1.0, 1.5 and 8.0 Gy at each irradiation. The study demonstrated that tumours appeared later in the groups given 1.0 or 1.5 Gy per exposure than in the group given 8.0 Gy per exposure, although tumour incidence was 100% with these two doses. At 0.75 Gy per exposure, no tumours appeared within 790 days, although an osteosarcoma and one squamous cell carcinoma did finally appear. There was no effect on the life-span of the animals. In a further group of 50 mice given 0.5 Gy per exposure no tumours were obtained [O8, T11]. This observation of an "apparent" threshold in response may be accounted for by the small number of animals involved, but it is more likely arises as a result of the characteristics of this particular tumour, in which induction appears to be more dependent on dose per fraction than on total dose; single doses of up to 30 Gy alone do not induce tumours. At the higher doses and dose rates tumour development is likely to be influenced by radiation effects on the tissue surrounding initiated cells.

194. *Summary.* A series of studies in rodents has shown that when irradiation of the skin is fractionated the incidence of skin tumours is less than with acute exposure. In the majority of studies, however, the total doses have been large, and dose fractionation has been seen to have an effect only for doses per fraction of less than about 5-6 Gy. In general, the effect of dose fractionation under these conditions has been to reduce tumour yield by a factor of about 2. In one study, in which female ICR mice were repeatedly irradiated with beta particles from ^{90}Sr-^{90}Y, an apparent threshold for tumour induction was obtained at a dose per fraction of about 0.5 Gy. This may have been the result of delayed tumour appearance or the influence of radiation damage to surrounding tissues at the high doses and dose rates used.

(k) Tumour induction in rats

195. In male Sprague-Dawley rats (3 months old) exposed to ^{60}Co gamma rays to give 2.83 Gy (304 rats, 2.2 mGy min^{-1}), 1 Gy (505 rats, 1.3 mGy min^{-1}) and 3 Gy (120 rats, 1.3 mGy min^{-1}), an effect of dose rate on overall tumour induction was observed, although this varied with the tumour type [M43]. At lower dose rates the incidence of radiation-induced carcinomas (excluding thyroid, pituitary and adrenals) was lower than at high dose rates for a total dose of about 3 Gy. The frequencies of carcinoma were 6.8% ± 1.9%, 10.1% ± 3.8% and 25.8% ± 8.2% in controls and animals exposed at 1.34 mGy h^{-1} (total dose: 2.8 Gy) and 78 mGy h^{-1} (3 Gy), respectively, implying a reduction in excess cancers by a factor of about 6 at the lower dose rate. Although the incidence of most carcinomas showed an increase with increasing dose rate, the effect was most significant for the digestive and urinary systems.

196. For a group of sarcomas that are poorly inducible in the rat (nervous system, leukaemia, lymphosarcoma, bone and mesothelioma) but frequent in man, no dependence on dose and dose rate was observed. For a second group of sarcomas (angiosarcomas and fibrosarcomas of internal organs and of soft tissue), which are infrequent in man but common in the rat, the incidence did increase with dose (from 1 to 3 Gy), although again it was independent of dose rate.

197. *Summary.* The results of this study in male Sprague-Dawley rats indicate that, as with the studies in mice, the effect of dose rate on tumour induction varies between different tissues. For dose rates varying by a factor of about 60 and for a total dose of about 3 Gy, DDREFs in the range from about 1 to 6 have been obtained for tumours in various tissues. Taken

together, the cancer rate was reduced overall by a factor of about 3.

(l) Uncertainties in the calculation of DDREF

198. A particular problem in estimating dose-rate effectiveness factors from the ratio of cancer yields following exposure to acute (high-dose-rate) and chronic (low-dose-rate) irradiations is that very often the standard errors attached to the linear fits to the data are relatively large (Table 9). Formulae have therefore been developed that allow an estimate to be made of uncertainties in the calculation of the DDREF. If α_H and α_L represent the yield coefficients for high and low dose rates, respectively, then the DDREF may be estimated by:

$$\text{DDREF} = \alpha_H/\alpha_L\,[1 + (\sigma_L^2/\alpha_L^2)] \qquad (16)$$

where σ_L is the standard error on α_L. The correction term exists to represent the skewed distribution of the ratio when α_H and α_L are both normally distributed [E8]. The correction is of little importance unless σ_L/α_L exceeds about 0.3. Examples of calculations of DDREF that allow for this correction are shown in Table 10. These values may be compared with the point estimates of DDREF for the same studies given in Table 9. In all cases the values of DDREF that allow for this correction are larger than the values of those that do not, although by very variable amounts; the effect on the estimate of DDREF increases substantially when the standard error σ_L exceeds α_L. The central estimates of DDREF given in Table 8 for the range of tumour types described in this Chapter do not include this correction.

(m) High-LET radiation

199. The implication of the results described earlier, i.e. that at low doses of high-LET radiation the effects of fractionated or protracted exposures on life shortening are very similar to the effect of acute exposures (Section II.A.1.c), would suggest a similar lack of effect on tumour induction in individual tissues. The effects of neutron and alpha-particle irradiation are described separately.

200. *Neutron irradiation.* The effects of protracted and fractionated neutron irradiation on the induction of tumours differ in different tissues and have recently been reviewed by Fry [F10]. Grahn et al. [G12] have examined the main categories of cancers found in B6CF1 mice, namely, those of lymphoid and epithelial tissues. The results obtained so far indicate that mortality from lymphoma and leukaemia is greater after fractionated exposures than single exposures, whereas mortality from epithelial tumours is less after the fractionated than single exposures. Since no dose-fractionation effect is seen on the overall life-span in the dose range up to 0.2 Gy, it must be assumed that the two effects cancel each other out.

201. There is little further information to suggest that dose rate influences leukaemogenesis in experimental animals. Upton et al. [U21] did not report any difference between the effects of protracted neutron exposures at low dose rates on the induction of myeloid leukaemia in RFM mice and the effect after single doses. Huiskamp et al. [H28, H29] reported no effect of dose rate on either the induction of acute myeloid leukaemia (AML) or survival in male CBA/H mice exposed bilaterally to fast fission neutrons (mean energy 1 MeV) at 2, 10 and 100 mGy min^{-1} to give a total dose of 0.4 Gy. No AML was observed in the sham-irradiated controls. The observed AML frequencies in the irradiated groups were 11.4%, 12.3% and 9.8%, respectively, indicating that the incidence of AML was not influenced by a fifty-fold change in dose rate. Besides AML, lymphosarcomas were observed in all experimental groups with a suggestion of a slightly higher frequency in the high-dose-rate group, although numbers were small and with no clear trend with increasing dose rate. Although survival was significantly reduced in the exposed animals, it was independent of dose rate.

202. Ullrich [U16] examined the effect of dose rate or dose fractionation on tumour induction in BALB/c mice exposed to total doses of 0.025-0.5 Gy from ^{252}Cf neutrons. The animals were exposed at high dose rate (0.1 Gy in 20 hours daily), or in two equal fractions separated by 24 hours or 30 days, or at low dose rate (0.01 Gy in 20 hours daily). The effect of dose fractionation and dose rate on the tumorigenic response depended very much on the tissue. For ovarian tumours, the response to fractionated exposures was similar to that obtained for a single acute exposure; however, at the low dose rate the response was reduced. This would be consistent with the need for tissue damage and hormonal imbalance for ovarian tumours to manifest themselves. For lung tumour induction splitting the dose into two equal fractions separated by 24 hours had no effect on the response, although separating the fractions by 30 days gave a higher incidence of lung tumours at a total dose of 0.5 Gy (there was no difference at doses up to 0.2 Gy). These results suggest that the number of cells at risk may have increased in the 30-day interval between fractions, possibly as a response to cell killing by the first fraction. This might also explain why the increased effect occurred only when the initial dose was 0.25 Gy or above. For mammary tumours the response to dose fractionation was similar to that for lung tumours. For both lung and mammary tumours the tumour incidence was greater at low dose rates

than with acute exposures, and the increase was most marked at intermediate doses of about 0.1-0.2 Gy. In another study, the induction of Harderian tumours was little different in single exposures and in fractions of 25 mGy up to a total dose of 0.4 Gy [F9]. It has also been reported that, relative to acute exposures, protraction of neutron irradiation advances the time of appearance of mammary tumours in rats [U3].

203. Di Majo et al. [D3] have reported dose-response relationships for liver tumour induction in $BC3F_1$ male mice exposed to fission neutrons. Three-month-old mice given doses from 0.17 to 2.14 Gy (0.05-0.25 Gy min^{-1}) showed an increased tumour incidence in all the irradiated groups [C29, D3]. A linear model gave a best fit to the dose-response data [I(D) = 11.3 + 34.6D], implying that no dose-rate effect would be expected. Because of the different shapes of the dose-response curves for x rays and neutrons (Figure XII), the RBE depended on the dose at which it was calculated, with a value of 13 estimated at 0.17 Gy.

204. *Summary.* These results indicate that there are differences between tissues in the tumorigenic response following fractionation and changes in dose rate for neutron irradiation. These differences may relate to the different mechanisms of tumorigenesis involved in the different tissues. Taken together, however, any effects of dose rate and dose fractionation on tumour induction in the various animal experiments that have been reported are small.

205. *Alpha particle irradiation.* Information is also available on tumour induction in experimental animals following intake of alpha-emitting radionuclides. The interpretation of such data is, however, considerably more difficult than is the case for neutron exposure. The spatial and temporal distribution of dose throughout a tissue depends on the age of the animal and the pattern of intake, as well as on the radionuclide itself and the chemical form in which it enters the body. Thus, even for the same radionuclide given in the same chemical form, the distribution of dose may be very different after acute and protracted exposures, and this is likely to affect the tumour yield. This is particularly the case for alpha emitters deposited in the skeleton, where rates of bone turnover that vary both with age and site in the skeleton result in a very heterogeneous deposition of the radionuclide, which in turn can result in quite different distributions and hence dose with various patterns of intake. Local deposition of radionuclides can also produce hot spots that could lead to local cell killing, thus reducing the tumour yield [P11]. Much of the published data has recently been summarized [S28].

206. Two lifetime studies with adult beagle dogs injected with ^{226}Ra to give a wide range of radiation doses, either as a single injection (0.22-370 kBq kg^{-1}) or fractionated (to give total injected activities of 0.88-370 kBq kg^{-1}) have been reported. The data suggest that for both methods of administration, ^{226}Ra was equally effective in inducing skeletal osteosarcomas. However, ^{226}Ra was more *effective* at inducing osteosarcoma (per unit dose) at low total doses than at high total doses, where cell killing and wasted radiation may be significant [G13, T19].

207. Fabrikant et al. [F11] have compared osteosarcoma induction by alpha-radiation in young male rats given ^{239}Pu (110 kBq kg^{-1}) by intravenous injection, either as a single dose or fractionated over months (37 kBq kg^{-1}, then 19 kBq kg^{-1} at 2, 4, 6 and 8 weeks). Although the number of animals in each group was small (25), the tumour incidence in the two groups (52% and 56%, respectively) did not differ significantly. There was a tendency for tumours in animals given fractionated injections to occur earlier. It was notable that in animals given single injections of ^{241}Am at a similar dose the incidence of bone tumours was about one fourth that in the animals given ^{239}Pu. This is likely to be due to differences in the distribution of the two radionuclides in the skeleton.

208. The effects of dose protraction on osteosarcoma induction have also been examined in female NMRI mice given either single or repeated injections of ^{224}Ra (half-life: 3.5 days) [M40]. One group received a single injection (18.5 kBq kg^{-1}, corresponding to a mean skeletal dose of 0.15 Gy) and the another group received a similar amount in 72 fractions given twice weekly over 36 weeks. In the group given fractionated administration, lymphomas appeared early (13.5%, 42/299 mice; controls 1%, 1/98 mice); osteosarcomas occurred during the second half of life of the animals (7.1%, 21/299; controls 3%, 3/98). In contrast, the group given a single injection did not develop early lymphomas and showed a later occurrence of osteosarcoma with an incidence of 5.8% (17/295). Although the incidence of osteosarcoma was similar up to 800 days in the two experimental groups, after that, it was different: no additional cases of osteosarcoma were observed in the single-injection group, but one third of all osteosarcomas occurred after 800 days in the fractionated group. Because of the very short half-life of ^{224}Ra administered in the study, much of the dose is delivered while the radium is on bone surfaces shortly after administration, and thus local doses will have been significantly higher than the average bone dose calculated (0.15 Gy). In contrast, the dose received following protracted administration would have been more uniformly spread over the skeletal tissues, and this might well have accounted for the observed differences in tumour response.

209. Information is available on lung tumour induction in rodents exposed to alpha emitters. Sanders et al. [S29] compared lung tumour rates in rats exposed by inhalation to aerosols of $^{239}PuO_2$ and $^{244}CmO_2$. The dose distribution throughout the lung was similar for the two radionuclides, although $^{244}CmO_2$ is more soluble in the lung than $^{239}PuO_2$ and, as a consequence, is cleared more rapidly. Despite this, the dose response for lung tumour induction following inhalation of soluble $^{244}CmO_2$ was similar to that for insoluble $^{239}PuO_2$ up to average radiation doses to the lung of a few gray. At greater radiation doses, rats exposed to $^{244}CmO_2$ died earlier from radiation pneumonitis than those exposed to $^{239}PuO_2$, reflecting the differences in dose rate and distribution of activity throughout the lung tissue. The effect of cell killing on tumour induction became apparent at doses of about 2 Gy for $^{244}CmO_2$ and about 30 Gy for $^{239}PuO_2$. Thus, at high total doses, exposure from ^{239}Pu appeared more effective for tumour induction than exposures from ^{244}Cm. Sanders et al. have also shown [S30] that further protraction of the dose from ^{239}Pu by fractionated exposure does not increase the lung tumour incidence in rats, indicating that lung-tumour promotion is not so much a function of the temporal dose-distribution pattern as of the spatial dose-distribution pattern.

210. In further studies on tumour induction in rats exposed to $^{239}PuO_2$, groups of animals were exposed to various levels of activity giving average lung doses between 0.01 Gy and 62 Gy (based on initial lung deposits measured with a ^{169}Yb marker and knowledge of the retention function for plutonium in the lung). This was a large study involving 1,052 female, SPF, Wistar, sham-exposed rats and 2,105 rats exposed in groups to give different initial lung deposits. The dose from inhaled ^{239}Pu is accumulated over an extended time because of the insolubility of the particles and the long retention time in the lung [S43, S20]. Of the 97 primary lung tumours found in this study (93% malignant and 80% carcinomas) 1 was in controls and 96 in exposed rats. Survival was significantly reduced only in rats with lung doses >30 Gy. Of the malignant lung tumours 49 were squamous carcinoma and 22 adenocarcinoma with the remainder consisting of haemongiosarcoma (9), adenosquamous carcinoma (7), and fibrosarcoma (3). No squamous cell carcinomas were found at average lung doses less than 1.5 Gy, and for adenocarcinoma the threshold dose was 3.1 Gy. The other tumour types were seen only at higher lung doses. In this study the predominant tumour type was therefore squamous carcinoma, which is known to develop in the rat lung following the development of squamous metaplasia [S44], which occurs mainly in regions of high deposition of ^{239}Pu, where the local dose would be substantially in excess of the average lung dose. For this tumour type a threshold for the response would therefore be expected, although this would not necessarily be the case for other tumour types or for tumours occurring in man. It was concluded that, at least in the Wistar rat, average lung doses in excess of 1 Gy (20 Sv assuming a radiation weighting factor, w_R, of 20) are needed to give a significant increase in lung tumours.

211. The induction of lung cancer after single or protracted irradiation with alpha particles was also examined by Lundgren et al. [L19] in mice exposed to $^{239}PuO_2$. After single or repeated inhalation exposures giving average lung doses of 2.8 and 2.7 Gy, respectively, lung tumour incidence was about 2.7 times higher after repeated exposures, although the difference between the groups was not significant ($0.05 < p < 0.10$). In contrast, a significant difference was obtained in mice receiving pulmonary doses of 14 Gy in a single exposure or 19 Gy in repeated exposures, the percentage with pulmonary tumours being about 3.5 times greater among the repeatedly exposed mice ($0.01 > p > 0.025$). It seems possible, however, that, as with the study by Sanders et al. [S29], the differences in effect could be attributed to higher dose rates from the single exposures resulting in more cell killing.

212. Some information on the effect of dose rate on the induction of lung tumours has also been obtained following intratracheal instillation of ^{210}Po in saline [L18]. Protraction of the dose over 120 days was more carcinogenic at lower total doses (0.24 Gy) but less carcinogenic at higher doses (2.4 Gy), in comparison with an exposure limited to a 10-day period. However, the development of tumours was also markedly enhanced by the weekly instillation of saline alone, emphasizing the importance of other factors in the expression of radiation-induced cancer.

213. A number of studies have been reported on the exposure of animals, particularly rodents, to varying concentrations of radon and its decay products. Studies at the Pacific Northwest Laboratory in the United States, which have examined the effects of exposure to radon under a range of exposure conditions in experimental animals, have recently been reviewed [C2, C3]. The predominant effect of the inhalation of radon is tumour induction in the respiratory tract. The main tumours arising are adenocarcinomas, bronchiolar carcinomas, adenocarcinomas, epidermoid carcinomas, adenosquamous carcinomas and sarcomas. Acute effects, although species-dependent, do not appear to have occurred at exposure levels of less than 1,000 WLM (3.5 J h m^{-3}). Excess respiratory tract tumours were, however, produced in rats at exposures well below 100 WLM. The results of a series of studies in rats exposed at 5, 50 and 500 WLM per week to give

a range of cumulative exposures are given in Figure XVI. With a few exceptions, the incidence of adenomas and sarcomas was well below 10%. A decrease in exposure rate, at a given exposure level, increased the overall incidence of lung tumours at all but the lowest exposure level (320 WLM). This increase was specifically the result of an increasing incidence of epidermoid carcinomas, most of which (>70%) are fatal. In rats, most (~80%) of the tumours are considered to originate peripherally and to occur at the bronchiolar-alveolar junction. The remaining 20% are considered to be centrally located in association with the bronchi. It should be noted that these are interim results. The shape of the dose-response curve remains uncertain. Most of the exposures below 100 WLM are not yet complete or analysed. At the lowest exposure rate (5 WLM per week), the data suggest that the exposure-rate effect (but not the risk) tapers off, and the risk might still best be described by a linear model, at least at low doses (Figure XVI) [C3].

214. A series of studies has also been conducted by COGEMA in France on the effects of radon exposure [G24, M24]. 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}). Theses 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 apparent reductions in tumour induction at high doses may chiefly have been the result of acute damage. Later experiments have, 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) [M24]. 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. It is significant, however, that no increase in risk was observed with a decrease in exposure rate.

215. The two-mutation (recessive oncogenesis) model of Moolgavkar and Knudson [M38] has been used to model lung tumour induction in rats exposed to radon. This model postulates transitions from a normal cell to an intermediate cell to a malignant cell with quantifiable transition rates and takes account of the growth characteristics of the normal cell and intermediate cell populations. The model describes well the rat lung cancer data following exposure to radon [M6]. The findings suggest that the first mutation rate is very strongly dependent on the rate of exposure to radon progeny and the second mutation rate much less so, suggesting that the nature of the two mutational events is different. The model predicts that (a) in rats radon doubles the background rate of the first mutation at an exposure rate of approximately 0.005 J h m^{-3} wk^{-1} (1.35 WLM wk^{-1}), an exposure rate in the range of exposures to miners; (b) radon doubles the background rate of the second mutation at an exposure rate of about 1.4 J h m^{-3} (400 WLM wk^{-1}); consequently, the hypothesis that radon has *no* effect on the second mutation rate cannot be rejected; and (c) the net rate of intermediate cell growth is doubled at about 0.12 J h m^{-3} wk^{-1} (35 WLM wk^{-1}). The model also predicts a drop in hazard after radon exposures cease, paralleling the exposure-rate effect noted previously, and that fractionation of exposure is more efficient in producing tumours, although further fractionation leads to a decreased efficiency of tumour production.

216. *Summary.* It is clearly difficult to generalize from these results on the effects of neutrons and alpha-emitting radionuclides on tumour induction in experimental animals. Despite this, there is little evidence to suggest that, in the absence of cell killing, there is an appreciable enhancement of tumour induction when the dose from alpha-irradiation is protracted or fractionated rather than administered in a single exposure. For the present, the data seem to be reasonably consistent with the assumption of a linear dose-response relationship, at least at low doses.

(n) Summary

217. A number of studies have been published that permit the effect of dose and dose rate on tumour induction in experimental animals exposed to low-LET radiation to be examined. The majority of the data are for external radiation exposure but some information is also available for incorporated radionuclides. The data that have been reported by various authors cover a wide range of dose-response relationships and in general show an increasing risk with increasing dose and dose rate at low to intermediate doses. Although the results from a number of studies can be fitted by linear-quadratic functions, this is by no means universal, and many other dose-response relationships have been obtained. The assessment of the extent to which changing the dose rate increases the effectiveness of the radiation depends, therefore, on the dose range over which the dose and dose-rate effectiveness

factor (DDREF) is calculated. The majority of studies also show that at high doses and high dose rates, cell killing becomes significant and reduces tumour yield. In these circumstances the risk of tumour induction at low doses may be underestimated by fitting a linear function to the data obtained in this region. Estimates of values of DDREF from the different studies that have been reviewed have therefore been made in the dose range in which no cell killing is apparent. The results of these analyses are given in Table 8.

218. A wide range of DDREFs for tumour induction in a variety of different tissues has been found, with most studies being carried out in the mouse. It must be stressed that some of the tumour types for which information is available are not found in man (Harderian gland) and others (ovarian tumour, thymic lymphoma) appear to involve substantial cell killing and/or changes in hormonal status. For other tumours there is a human counterpart (tumours of the lung, breast, pituitary and thyroid), although the tumours involved may not be strictly comparable to the human disease. In practice, the DDREFs found in these two groups are little different, falling in the range from about 1 to 10 or more for dose rates that vary by a factor of 100 or more, and there was no clear trend with tissue type. The data reported on myeloid leukaemia induction in different species and sexes also give DDREFs in the range from 2.2 to >10. The one reasonably consistent finding is that DDREFs for tumour induction in mammary tissue tend to be lower than for tumours in other tissues, although even here one author [U26] has reported a substantial effect of dose fractionation and, hence, a relatively high value of the DDREF (~10) for mice.

219. The main conclusion to be drawn from the results of both the studies on radiation-induced life shortening (Section II.A.1) and those on the induction of specific tumour types is that the tumour response to low-LET radiation is dependent on the dose rate. While the absolute value of the DDREF varies with the conditions of exposure, the animal strain, tissue/tumour type and the dose range over which it is calculated, there is in general a consistent finding that tumour yield decreases with a substantial reduction in the dose rate. There is also some evidence that if the dose rate is sufficiently protracted initiated tumours are unlikely to be fatal in the life-span of the animal. These results may be expected to apply to human tumours as well as to those in experimental animals.

220. A number of the animal studies also indicate that a dose-rate effect cannot necessarily be inferred from exposures at high dose rates alone, as the dose-response data for tumour induction can be adequately fitted by a linear function. This implies the absence of a visible quadratic (i.e. multi-track) function in the dose response, which, according to conventional interpretations, would appear to be a necessary prerequisite for an effect of dose rate on tumour yield. It is thus clear that where information is available only for exposures at high dose rates, as is normally the case for human exposure on which risk estimates are based [C1, I2, U1], any attempt to assess the effect at low doses and low dose rates, and hence a value of the DDREF, by simply attempting to fit a linear-quadratic or similar function to the dose response is unlikely to succeed fully. The limiting factor is the amount of information available at low doses from which the initial linear term (α_L of Figure I) can be accurately determined. In planning future animal studies it should be noted that most information is likely to come from studies on animals exposed at different dose rates rather than from studies that attempt to obtain information on the risks at very low doses. It is to be hoped that more work will be carried out to supplement the very limited information presently available.

221. From the limited and somewhat disparate data on high-LET radiation it is difficult to generalize. There is, however, little experimental data to suggest that, in the absence of cell killing, there is a need to apply a DDREF to tumour incidence data obtained at high dose and dose-rate exposures to calculate risks at low doses and dose rates. Similarly, there is little evidence to suggest that there is an appreciable enhancement of tumour yield when the dose from high-LET radiation is protracted or fractionated. Some data suggest that if radiation exposure is protracted this results in a delay in the appearance of tumours, and in practice they may not arise in the life-span of the animals.

B. *IN VITRO* CELL TRANSFORMATION

222. As has been indicated, oncogenesis is a complex, multi-stage process that is modified by both environmental and physiological factors. *In vitro* cell transformation systems, which have developed rapidly in recent years, have been used to study part of this process in single cells free from host-mediated influences, such as hormonal and immunological factors, and from environmental agents. Even here, however, cell-cell interaction cannot be discounted. Such systems have the advantage that they allow the relative importance of cell killing and transformation to be measured in the same target population of cells. They can also be carried out in a much shorter period of time than animal studies designed to examine tumour induction, and they can be more readily analysed, not having the problem of competing health risks, which is inherent in animal studies.

223. Transformation describes the cellular changes associated with loss of normal control, particularly of

cell division, which results in the development of a neoplastic phenotype. Although exact definitions depend on the experimental conditions, enhanced growth rate, lack of contact inhibition and indefinite growth potential, anchorage-independent growth and the ability to form malignant tumours when transplanted into a suitable host are the main features of the transformation systems currently in use [O1]. Whereas *in vivo* models involve the whole process of carcinogenesis, *in vitro* cell transformation considers events at the level of the initial target cells. However, cell transformation is in itself a complex, multi-stage process by which a cell acquires progressively the phenotypic characteristics of a tumour cell. In practice progression to complete transformation may not occur [L7, L11].

224. The two most common cell lines used in cell transformation assays are the NIH BALB/c3T3 and the C3H10T½ mouse-embryo-derived fibroblast line. There are inevitably disadvantages associated with the use of such cell transformation systems as a model for carcinogenesis in man. The lack of close intercellular contact and the necessity for an artificial growth medium can alter the reactions of cells. Cell handling techniques, such as the degree of trypinization and changes in culture medium, may substantially alter the results obtained [E6, T13]. In particular, in the cultivation of mammalian cells, the properties of serum, constituting 10% of the growth medium of C3H10T½ cells, can be very variable. Thus Hsiao et al. [H27] found large differences in the ability of serum to support the expression of transformed phenotype of C3H10T½ and rat embryo cells. A particular problem with current work on cell transformation is that it is largely based on fibroblasts of rodent origin, whereas tumours of epithelial origin are the main radiation-induced cancers in man. Reliance on data from experimental models that utilize cultured rodent cells for extrapolation to man, without experimental support can, and has, led to serious errors of interpretation [S21]. Thus, a correlation between anchorage-independent growth and the tumorigenic phenotype has been established in rodent cells [F5, O1, S22], which has allowed for the selection of neoplastically transformed cells by growth in soft agar. This does not apply, however, to cultured human cells, as normal human diploid fibroblasts are capable of anchorage-independent growth when cultured in the presence of high concentrations of bovine serum. More relevant cell lines based ideally on human epithelial cell systems are needed for studying mechanisms of tumorigenesis. There are indications that such models can be developed; a recent paper has described transformation in a human colonic epithelial cell line [W11]. A number of studies have also reported neoplastic transformation of human fibroblasts by ionizing radiation and other carcinogens using anchorage-independent growth as an assay (see, e.g. [M26, S23]).

225. Rodent cells seem to have a much greater ability than human cells to undergo the immortalization stage of transformation *in vitro*, either spontaneously or as a result of treatment with a whole range of carcinogens. This may reflect a fundamental difference in their response and must be taken into account in any interpretation of radiation-induced transformation studies employing the currently available rodent cell lines [L9, L11]. Complete transformation of normal human diploid fibroblasts by physical or chemical agents has rarely been achieved [M3, N5]. Immortalization leading to tumorigenicity is a rare event in human diploid cells, whereas certain characteristics of morphological transformation, such as anchorage-independent growth, may be induced quite easily [L10, L11].

226. Nevertheless, there are several general characteristics of cell transformation *in vitro* that support its relevance as a model system for studying the early stages of radiation-induced carcinogenesis *in vivo* and the effects of dose rate. These have been summarized by Little [L11] as follows:

(a) the commonly used cell transformation systems provide quantitative information on the conversion of non-tumorigenic to tumorigenic cells;
(b) there is a high correlation between the carcinogenicity of many chemicals tested in both animals and cell transformation systems. Similar correlations hold for a number of inhibitors and promoters of carcinogenesis that have been tested both *in vitro* and *in vivo*;
(c) cell transformation responds to initiation and promotion similarly to two-stage carcinogenesis in tissues of experimental animals;
(d) transfection assays have shown that cells transformed *in vitro* have activated oncogenes that can be isolated and will transform recipient cells. The DNA of parental, non-transformed cells is inactive in such transfection assays. These findings are analogous to those with human tumours and normal cells in the same DNA transfection assay.

227. Cell transformation systems currently in use divide into two main categories. The first one is short-term explants of cells derived from rodent or human embryos, e.g. Syrian hamster embryo cells. These have the advantage that they are normal cells with a normal karyotype allowing parallel cytogenetic experiments. As such, they have a limited life-span in culture. Immortal transformants are identified by their altered morphology as survivors against a background of senescing normal cells. Spontaneous transformation of these cells occurs at a low frequency (of about 10^{-6} per cell), and in these assays cell survival and transformation frequency are measured in the same cell

culture after about 10 days, the transformed cells being recognized by their distinctive clonal morphology. Being derived from the whole embryo, however, these cultures contain a mixture of cell types, with the possibility that there could be a subpopulation of sensitive target cells.

228. The second category of cell transformation systems is established cell lines that have undergone a growth "crisis" *in vitro*, resulting in the evolution of a derivative cell population capable of indefinite (immortalized) growth. This category includes the NIH BALB/c3T3 and C3H10T½ mouse-embryo-derived fibroblast lines. These cells are highly abnormal and contain a variety of chromosomal rearrangements. If not treated meticulously, the spontaneous level of transformation may increase dramatically. Cell survival and transformation are measured in separate cultures. Survival is normally measured after two weeks and transformation after six weeks, during which time the cultures have reached confluence, and foci of transformed cells that have lost the property of contact inhibition can be seen as distinct colonies that pile up on top of the layer of untransformed fibroblasts. This delay allows for the expression of lethal mutations in the cell population, and these may have a significant effect on the calculation of induced transformation frequencies [A2, E3, M7, S2]. Extrapolation to effects in human epithelial cells *in vivo* from results in these cell systems must be made with caution.

229. Transformed cells are found by scoring characteristic colonies identified in a culture, thus giving a direct measure of the transformation frequency per surviving cell. Many experiments are reported in this way, but information can also be obtained on cell survival as a function of dose, and on the plating effi-ciency. The transformation yield per initial cell at risk can then be determined by correcting for the surviving fraction in the culture and the plating efficiency. This second measure of transformation frequency commonly cannot be calculated from published values of the frequency per transformation of survivors, which may make it difficult to define accurately dose-response relationships.

230. There are a number of other transformation systems in use or under development that attempt to measure more relevant end-points, e.g. thyroid and mammary cell systems in the rat, in which survival and oncogenicity can be measured by transplantation into a fat pad of the animal [C7] and epithelial tissues grown in culture [M31]. As a general rule, the greater the relevance of the measured end-point, the poorer at present is the degree of quantification. Considerable effort continues to be expended in the search for a reliable, relevant and quantifiable human epithelial cell system that would be more representative of the majority of human tumours [C6] and more relevant to determining dose- and dose-rate-related effects.

231. Despite these limitations, transformation assays can provide practical guidance in understanding a number of areas in radiation carcinogenesis:

(a) the shape of dose-response relationships;
(b) the effect of variations in the dose rates of irradiation;
(c) the relative effectiveness of radiation of different qualities;
(d) the modification of radiation effects by interaction with other agents.

With the transformation systems presently available, the experimental cell system chosen to examine a particular end-point should reflect its suitability for answering the question asked. For example, questions about the relative effectiveness of alpha particles compared to gamma rays or about the effect of dose rate require a more quantitative system, such as the Syrian hamster embryo cell or C3H10T½ cell systems, whereas studies of oncogenic mechanisms and the effect of suppressor genes need a more relevant human epithelial cell system [H3]. (This topic is covered in more detail in Annex E, "Mechanisms of radiation oncogenesis".)

1. Dose-response relationships

232. Dose-response relationships for cell transformation following exposure to low-LET radiation were comprehensively reviewed in the UNSCEAR 1986 Report [U2] and more recently by Barendsen [B9]. A knowledge of the factors influencing the response to radiation is important for understanding the influence of dose fractionation and dose rate on cell transformation, and they are briefly summarized here.

233. The pattern of response is very dependent on cell cycle kinetics. The most reliable experimental evidence shows that when measuring the transformation frequency per cell at risk following exposure to low-LET radiation, a linear or linear-quadratic relationship can be fitted to most available data at lower doses, but above about 4-5 Gy cell reproductive death starts to predominate over the observed frequency of transformation per plated cell [B9, H4]. Figure XVII shows the typical form of the dose-response relationship. Parameters were selected by Barendsen [B9] to illustrate the importance of the linear and quadratic terms in the induction of transformation and of cell reproductive death. When expressed as transformation frequency per surviving cell, a plateau in the yield at high doses may be observed with C3H10T½ cells [H4, T1].