# ANNEX B

Dose-response relationships for radiation-induced cancer

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Introduction

1. It has long been recognized by UNSCEAR that radiation-induced malignant diseases are the most important late somatic effect in human populations exposed at high doses for which direct observations are available [U6, U7, U9-U12, U24]. For evaluation of radiological risk or detriment [I2] this importance derives from the fact that these diseases are often lethal and they are the only statistically verifiable cause of radiation-induced life shortening at intermediate and low doses [B17, B18, J1, K39, K40, U24]. Radiation-induced cancer belongs to those radiobiological effects whose frequency of occurrence (but not severity) is believed—as a rule—to correlate with dose.4 The postulated probabilistic nature of the relationship between dose and frequency of malignancy5 has led to the acceptance of the term "stochastic" for effects of such type.

2. Assessment of risk, from environmental and occupational radiation sources in the dose region from fractions of mGy to a few tens of mGy, would be greatly facilitated by knowledge of the shapes of the dose-response relationships for radiation-induced cancers in humans. This knowledge is not available at present and is not likely to be obtained by direct observation. Two features of the dose-response relations are most important for evaluation of the risk at low doses: the possible presence of a threshold dose below which the effects would not occur, and the shape of the dose-response curve.

3. Lack of threshold for a given effect is usually assumed if the response for this effect, plotted against the independent variable (causal factor, or, specifically, dose), permits extrapolation by eye to the origin of the coordinate system. or when the calculated regression line intercepts the abscissa at values that are not significantly different from zero. Conversely, a threshold is usually assumed when the fitted regression function crosses the abscissa at a value significantly greater than zero. However, proving or disproving a threshold below the levels of direct observation may be impossible, due to statistical fluctuations of the spontaneous level and of the presumably induced response. Therefore, assumptions regarding a threshold are based essentially on theoretical considerations of the mechanisms of radiation interaction with the biological targets for initiation of neoplasia, supplemented by empirical observations to support the hypothesis. Although absence of the threshold is often assumed, this has not been proved for any form of radiation-induced malignancy [U6] and must be regarded as a working hypothesis.

4. In annex I of the 1977 UNSCEAR report [U6], the available data concerning experimental radiation carcinogenesis in numerous animal species were reviewed. The large variation of susceptibility to cancer induction in different tissues was emphasized. Physical and biological factors modifying the frequency of induction were discussed in great detail and interactions of other agents (e.g., viruses) with radiation were also reviewed. The extreme complication and unsatisfactory understanding of the pathogenesis of all forms of cancer, including those induced by radiation, were particularly stressed.

5. Various physical factors, such as dose, dose rate, and quality of radiation, were also considered, and general patterns could be recognized in cases where such factors were systematically studied in a given strain of animals, and for specific tumour types. Among the patterns identified, were a sparing effect of dose fractionation and protraction of low-LET radiation (x or gamma rays) upon the frequency of induced cancer and the absence, or even reversal, of such an effect for high-LET radiation (neutrons, alpha particles).

6. Current theories of cancer induction by radiation and some other agents (viruses, chemicals) were also briefly reviewed in the 1977 UNSCEAR report [U6]. None of these was able to accommodate all the known facts and to allow development of a theory or of...
comprehensive models of cancer induction by ionizing radiation. It was recognized, however, that known carcinogens have a common target within the susceptible cells, which is most likely the nuclear DNA or genome.

7. Since the 1977 report [U6], new information has been published on experimental induction of cancer by radiation. Some of it refers to observations at intermediate and low doses. Of particular importance is the information dealing with high-LET particles (mostly neutrons). This is reviewed here when it appears relevant to models of radiation-induced malignancies.

8. In annex I of the 1977 report [U6], the response relationships as a function of single acute doses for various forms of experimental radiation-induced cancer—both after whole-body and localized irradiation—were reviewed thoroughly. Tumours could be broadly subdivided into three categories:

(a) Those showing an increasing incidence with increasing dose up to a maximum, with a decline following that maximum (most forms):

(b) Those displaying a negative correlation between incidence and dose, as observed in tumours with an unusually high spontaneous frequency:

(c) Those showing no clear rise with increasing dose up to several Gy.

For occupational and environmental exposure of man, type (a) is the most relevant. Schematic examples of dose-response curves are shown in Figure I.

9. UNSCEAR [U6] also identified a large variability of the net incidence of various tumour types at intermediate to high doses between different species and, within species, between inbred strains. It was also found that in many cases a particular tumour could be induced by radiation in only one or two strains of a given species, an observation that must raise questions as to whether such tumours may represent adequate models of corresponding human diseases. Similar doubts would also apply to some observed forms of dose-response relationships. In some cases, dose-response relationships differ from species to species, although in many cases consistent patterns have been found. For these reasons, the increased incidence per unit dose of a given form of cancer cannot—as a rule—be extrapolated between species.

10. For dose-response relationships of category (a) some regularities were pointed out that appeared to conform to other radiobiological phenomena occurring in single cells (e.g., cell killing, induction of mutations and chromosome aberrations). These common features were as follows:

(a) The RBE values for densely-ionizing radiation relative to x and gamma rays are higher than 1 and decrease as doses increase:

(b) With acute doses of high-LET radiation the dose-response relationship is closer to linearity than for sparsely-ionizing radiation, for which upward concave curvilinearity is usually observed:

(c) The tumour yield often shows little dependence on dose protraction and fractionation for high-LET radiation, while for x and gamma rays the yield usually declines.

11. Since publication of the 1977 report [U6], additional information has appeared on tumour induction and life shortening in the low and intermediate dose region. It shows that after acute (high dose rate) high-LET exposure, in some cases at intermediate and in most cases at high doses, the incidence of tumours per unit dose decreases with increasing dose. For low-LET acute exposures, such a decline is usually observed only at high doses (above several Gy). These and other observations, together with some notable exceptions, will be discussed in detail in chapter IV.

![Figure I. Typical dose-response curves for radiation-induced tumours. Curve 1: Downwards concave; Curve 2: Linear; Curve 3: Upwards concave; Curve 4: Threshold type; Curve 5: A high spontaneous tumour rate declining with dose. The shaded area represents the spontaneous (control) incidence.](image-url)
12. In annex G of the 1977 report [U6], UNSCEAR presented a comprehensive review of epidemiological data on radiation-induced cancer in man. Absolute risk estimates of mortality per unit dose were examined in detail for malignant diseases of various organs, and confidence limits were attached to these estimates, derived in most cases from irradiation with doses at or above 1 Gy. Also, an approximate life-time risk of mortality from cancer at all sites per unit dose was estimated from the ratio of incidence of all non-leukaemic malignancies to the incidence of leukaemias in several groups (atomic bomb survivors, American radiologists, and patients treated for ankylosing spondylitis and metropatiha haemorrhagica). However, the previously available dosimetry at Hiroshima and Nagasaki has been questioned, and the new dosimetric system (DS86) is expected to yield improved risk coefficients for atomic bomb survivors, who are one of the most important sources of information.

13. The merit of such risk estimates lies in the fact that they are derived directly from human data and thus avoid interspecies extrapolations of doubtful validity. The precision of the 1977 risk estimates was the best possible under the conditions of exposure and follow-up then available, but many limitations of the estimates were discussed extensively.

14. Uncertainties regarding the shape of the dose-response relationships for radiation-induced malignancies, and the related risk estimates in man, derive mostly from the following conditions:

(a) The short duration of follow-up of irradiated populations compared to the length of latent period of most tumours. Whereas 20 to 30 years may be sufficient for manifestation of leukaemias and bone sarcomas after short-term irradiation, it is not so for other cancers. At present, the time distribution of their latent periods is not known in detail, and for some cancers it depends upon dose (or dose rate for chronic exposure), and also on age at irradiation. In addition, several, if not all, radiation-induced tumours tend to have an age distribution similar to that of their spontaneous counterparts. This means that greater absolute incidence per unit dose will be observed in cohorts of older than of younger ages. On the other hand, the expression of induced cancers will be truncated in cohorts irradiated at older ages, owing to their limited survival. Thus, the shape of the dose-response curve may depend upon the length of follow-up and on the age structure of the irradiated population. In most studies, the mean follow-up period is substantially shorter than the time needed for full tumour expression. In addition, a negative correlation between dose and latent period could, if present, affect the shape of the dose-response relationship, because it could not permit comparable tumour expression after high and low dose.

(b) The sex and age composition of the population under study. Since for certain tumours the age at irradiation and sex have a pronounced effect upon the risk of later development of the malignancy, a given dose-response relationship may not be representative of populations of different composition.

(c) There is a pronounced geographical, socioeconomic and ethnic variation of the spontaneous incidence of cancers in most organs. (For a review of this point see [D17, W9].) This suggests that epidemiological observations on radiation-induced cancer cannot be applied indiscriminately to populations of different ethnic, socioeconomic or geographic characteristics. On the other hand, such differences are not necessarily reflected in the value of the absolute risk or the shape of the dose-response relationship. For instance, in spite of large differences in the age-specific incidence, the age-corrected excess risk of breast cancer and dose-response curves in Japanese atomic bomb survivors are very similar to those in women irradiated in the United States for medical reasons.

(d) Deficiencies of tumour ascertainment in retrospective studies from available records of incidence or specific mortality.

(e) Difficulties in the selection of suitable comparison groups for the calculation of the expected (control) incidence or mortality due to a given tumour.

(f) Presence of confounding variables and modifying factors (promoters, inhibitors) that, if correlated with dose, or per se, could modify the shape of the dose-response relation.

(g) Questionable accuracy of dosimetric estimates, particularly when these involve retrospective reconstructions of complex situations. Numerous examples of such uncertainties are given in chapter V. In this category belongs also the very narrow range of doses to which a population may have been exposed, as well as the non-uniformity of dose distribution in the target organs. The latter condition may distort the relationship when the mean organ dose is used as the independent variable and induction is not a first-order process.

(h) Statistical uncertainties in the estimates of incidence or mortality at given dose levels.

15. Because of such limitations, in view of the presence of numerous and often unknown biological variables affecting cancer incidence, and of the lack of understanding of the pathogenesis of cancer, UNSCEAR cautioned against the indiscriminate use of risk estimates under conditions other than those for which they had been derived. For example, direct application of the risk coefficients to doses in the range from 1 mGy to 0.1 Gy involves a procedure of linear extrapolation, i.e., an assumption that the incidence per unit dose does not vary with dose. Such a procedure could, however, lead to over- or under-estimates of risk, depending on the actual shape of the dose-response curve. It was generally concluded, in the 1977 report, that the real risk per unit dose of low-LET radiation at low doses and/or dose rates would be unlikely to be higher, but could be substantially lower, than the values derived for the range of a few tens of mGy. The derivation was based essentially on
observations made above 1 Gy, but some reduction of the effect at low doses was already assumed (e.g., for leukaemia by a factor of about 2).

16. The wide confidence limits on the data from man allow various mathematical functions to be fitted to the same epidemiological series [B17, B20, B24, C29, M31, R21, S50]. Consequently, the probability of being able to discriminate between the statistical goodness of fit of various alternatives, or to reject some of them, is too low. In addition, the extrapolation of a relationship beyond the region of direct observation is always questionable when the underlying mechanism is not well understood.

17. In view of all these difficulties UNSCEAR has followed another approach in preparing the present annex. It has reviewed evidence at the subcellular and cellular level, from which inferences could be made as to the possible nature of the dose-response for cancer initiation by radiation. It has also examined how initiation of cancerous clones, and their progression to clinical tumours, may affect the shape of the dose-response relationship. Finally, it has reviewed published models of cancer induction and tested them for compatibility with epidemiological and experimental findings. It is hoped that this complex exercise may help to establish, with some confidence, the shape of dose-response relationships, and thereby limit the uncertainty in the extrapolation of the risk to low doses.

18. Thus, the objectives of this annex may be summarized as follows:

(a) To review the critical assumptions involved in the formulation of models linking radiation-induced cancer to dose;

(b) To review and discuss dose-response relationships for effects at the cellular level that could basically be linked with malignant transformation;

(c) To discuss models of cancer induction by radiation from the standpoint of resulting dose-response relationships for tumours of some organs and tissues;

(d) To review the effects of the mode of dose delivery (dose rate, fractionation) and quality of radiation upon the dose-response relationships;

(e) To identify possible general trends, and interspecies similarities, brought about by changes in the above variables upon the dose-response relationships for various types of cancer.

UNSCEAR wishes to stress that in pursuing this exercise it does not intend to give more weight or credit to one or another model of tumour induction, nor to depart from previously established policies in risk estimation adopted within the Committee. This review is meant to be a purely scientific analysis of data aiming at an assessment of systematic errors in the risk estimates derived from existing epidemiological evidence when one or another model is assumed in interpreting such evidence.

1. DOSE-RESPONSE RELATIONSHIPS FOR RADIATION-INDUCED CANCER

19. Radiation-induced cancer, as a stochastic phenomenon, can be analysed in terms of probabilistic concepts such as the distribution function of the time from irradiation to the occurrence of cancer. Depending on the data, the event may be assumed to occur at the time of diagnosis for readily apparent tumours, or at the time of death for rapidly lethal ones. The definitions and procedures of estimation are similar, but the two cases should not be confused because this could lead to serious errors, particularly with cancers for which effective therapy is available.

20. In experimental work, the populations under study consist generally of inbred animals standardized for species, strain, sex and age. They are irradiated under controlled conditions and followed for a specified time or up to death. Appropriately matched control groups are followed concurrently under similar conditions. The time of death and, at least in some experiments, the cause of death, can be ascertained for each animal. Such data may undergo sophisticated statistical treatments.

21. Easily diagnosed or rapidly lethal tumours are readily discovered. For such "manifest" neoplasms, established mathematical procedures can be applied to correct for competing risks, e.g., intercurrent mortality not related to the tumour incidence. Under these conditions, the time to the expression of the tumour is known only for some of the individuals in the collective, while others die or disappear from observation due to unrelated causes before a tumour is observed. For these latter individuals one knows only that the hypothetical time to the tumour would have been longer than the observation time, i.e., it would lie to the right on the time scale. Hence one speaks of "right censored" data.

22. If tumours are "occult", in the sense that they are discovered only incidentally in animals killed or dying for other reasons, one speaks of "double censored" data. In this case, one knows either that the time to the expression of the tumour is shorter than the observed time of death or that the hypothetical time would be longer than the time of death, according to whether the dead animals carry a tumour, or not. Under these conditions the expression "double censored data" is used (meaning that the data are both "left and right censored") and the methods for a competing-risk-corrected analysis are more complex (see paragraph 31). There are special difficulties for partly lethal tumours, but a four-point grading of the tumours is usually practicable and sufficient for the analysis; it ranges from "definitely incidental" to "definitively manifest (e.g., lethal)" [P18, P19].

23. In epidemiological work on human populations, the situation is quite different. The series are, in most cases, retrospective: the final data on morbidity and mortality are frequently incomplete: and the composition of the group is often heterogeneous with respect
to sex, age, socio-economic status, health conditions and exposure to carcinogenic or promoting agents other than radiation. Also, the control population is seldom fully adequate; follow-up to extinction is rarely achieved owing to the long life span of man; and dosimetry is frequently uncertain. The statistical treatment of such data must obviously follow methods different from those applying to prospective experiments.

A. THE INDEPENDENT VARIABLE

24. Dose-response curves are functional relationships between an independent variable, the radiation dose in a given organ or tissue, and a dependent variable represented by a suitable measure of the response. The specific energy, $z$, absorbed in a cell or in its critical structures, is a random variable. The mean value of $z$, i.e., the absorbed dose, $D$, is commonly used as the quantity of reference, but at equal values of absorbed dose the distribution of the values of specific energy can vary greatly, depending on the tissue volume for which the specific energy is determined and the value of the absorbed dose, as well as the radiation quality (see III.B.2). Furthermore, the same dose may be delivered at different dose rates. In the present context, the following terminology will be adopted for sparsely-ionizing radiation: low doses, $< 0.2$ Gy; intermediate doses, $0.2-2.0$ Gy; and high doses, $> 2.0$ Gy. For densely-ionizing radiation (e.g., fast neutrons) doses $< 0.05$ and $> 0.5$ Gy will be referred to as low or high, respectively, with intermediate doses falling between the figures quoted. Low dose rates for all radiations are $< 0.05$ mGy min$^{-1}$; high dose rates are $> 0.05$ Gy min$^{-1}$; and intermediate dose rates fall between these limits. Other quantities will at times be used as the independent variable, such as the injected activity of a specified radionuclide, or the time-integrated concentration of alpha-energy of short-lived radon daughters ultimately to be released in air. With some oscillations, such quantities are proportional to dose.

B. THE DEPENDENT VARIABLES IN EXPERIMENTAL WORK

25. In experimental work on radiation carcinogenesis, various expressions of the response may be adopted (see annex 1 in [U6]). The simplest, and most commonly used, is the fraction of animals incurring a tumour after irradiation with a given dose (crude incidence). It has been stressed repeatedly [F1, G17-G19, H15, M32, R9, S37, U2-U5, U20-U22] that such way of expressing the response leads to erroneous results. The reason is the interference of competing risks and of the different duration of life between animals receiving different doses. Actually, animals receiving the highest doses tend to die earlier and thus have less chance of expressing the tumours that may be induced.

26. Corrections for differences in the distribution of survival times between control and irradiated animals may be made by approximate methods, as, for example, in studies by Ulrich and Storer [U2-U5, U20, U21, U23-U26]. In this approach, the data are truncated at the time when the group is extinguished through natural death and the observed incidence in the treated group is corrected by a factor equal to the ratio of the mean lifetime for the control and the irradiated groups. This approach can provide approximate age corrections, but it may be misleading when the frequency of tumour appearance varies considerably with time after exposure.

27. Rigorous corrections for age and intercurrent mortality may be made by following the response of irradiated and control individuals throughout their life after irradiation or during a pre-selected post-irradiation period, with appropriate methods of investigation, including careful post-mortem pathology. The relevant parameter is then the age- or time-dependent rate of tumour appearance [C18, C19, H15, K8, S37] or a related cumulative quantity that can be more readily determined in the experiment. The basic quantities in this approach and their competing-risk-corrected estimates for manifests tumours are:

(a) The tumour rate, $r(t)$, as a function of age or time $t$ after irradiation. It is the probability at time $t$ per individual to develop a tumour per unit time. This quantity, $r(t)$, is to be interpreted as a mean value for the population under study. Since, for tumours diagnosed during the lifetime, the actual time of origin of the tumour is unknown, the time of its first observation is generally used: the time of death is used for rapidly developing, lethal tumours. In experimental work one derives $r(t)$ as an average value in a group of animals at time $t$. If $N$ animals are observed (i.e., are at risk) over the interval $t - dt/2$ to $t + dt/2$, and $n$ tumours appear within the interval, the estimate of the tumour rate is $R(t) = \frac{n}{N dt}$. For incidentally observed tumours a direct estimate of the tumour rate is impossible: the tumour prevalence can, however, be estimated (see paragraph 31):

(b) The cumulative tumour rate, $R(t)$. Estimates of this quantity are less affected by statistical fluctuations and are therefore more readily derived. The quantity is defined as the integral of the tumour rate from the time of exposure ($t = 0$) up to time $t$:

$$R(t) = \int_0^t r(t') \, dt' \quad (1.1)$$

$R(t)$ is the number of tumours per animal up to time $t$ under the hypothetical condition that one could keep the number of animals at risk constant in spite of intercurrent mortality and the occurrence of tumours. $R(t)$ exceeds, therefore, not only the crude incidence, but also the incidence $I(t)$, corrected for competing risks (see paragraphs 29 and 30). A competing-risk-corrected estimate of the integral tumour rate is $[A1, N3, S37]$

$$\hat{R}(t) = \sum_i (n_i/N_i \, dt) \, dt = \sum_i n_i/N_i \quad (1.2)$$

for all $i$ with $i \, dt < t$, where $n_i$ is the number of tumours appearing within the time interval $(i-1) \, dt$ to $i \, dt$, and $N_i$ is the actual number of
individuals still at risk at this time, i.e., individuals without a tumour. The standard error for equation (1.2) can be obtained by the relationship [S37]

\[ \sigma_{\text{R}} = \sqrt{\frac{\Sigma n_i}{N_i^2}} \]  

(1.3)

28. If multiple non-lethal tumours occur, estimates of the tumour rate or the integral tumour rate can be based also on all observed tumours [S37]. In this case all animals still at risk are included in N_i (equation 1.2), regardless of whether these animals had developed a tumour or not. With this modification, similar estimates are obtained, provided that the animals without previous tumour had experienced the same tumour rate as the animals that had already incurred a tumour. This is so because both the numerator and the denominator in equation (1.2) are increased. If, on the other hand, there are inherent variations of the tumour rate within a population, or if the occurrence of a tumour increases the probability of subsequent tumours, the tumour rate estimated from all tumours will be larger than the rate estimated from first tumours only. It is mandatory, therefore, to specify whether the estimates of the integral tumour rate are based on the first or on all observed tumours. For partly lethal or rapidly developing lethal tumours the estimate can be based only on first tumours.

29. A frequently used quantity, related to the cumulative tumour rate, is the actuarial incidence, or incidence corrected for competing risks. I(t). It is the probability of an animal at risk up to time t to have incurred a tumour. In the absence of competing risks, the actuarial incidence equals the crude incidence (see paragraph 25). In the presence of competing risks, and for manifest tumours, a quantity can be obtained in terms of the product limit estimate [K1]:

\[ \hat{I}(t) = 1 - \prod_{i=1}^{N_1} \left( 1 - \frac{n_i}{N_i} \right) \quad t_i \leq t \]  

(1.4)

where the product extends over a number of time intervals (i) up to time t; n_i is the number of animals with tumours appearing within the time interval t_i = t_i - t_i; and N_i is the number of animals without tumours still at risk at time t_i. The standard error of the product limit estimate is expressed by the so-called Greenwood formula:

\[ \hat{\delta}_{\text{R}} = \sqrt{\frac{[1 - \hat{I}(t)]}{\prod_{i=1}^{N_1} n_i N_i^2}} \quad t_i \leq t \]  

(1.5)

When N_i is very small the log-rank test is preferable (see paragraph 33).

30. If few individuals incur the tumour, the actuarial incidence and the integral tumour rate, based on first tumours only, are nearly equal. At high frequencies, the actuarial incidence can approach 1, and the integral tumour rate may exceed 1. The sum limit estimate (equation 1.2) is largely equivalent to the product limit estimate (equation 1.4), i.e., the integral tumour rate can also be obtained from the product limit estimate by the relationship:

\[ R(t) = -\ln \left[ 1 - \hat{I}(t) \right] \]  

(1.6)

Similarly, the actuarial incidence can be obtained from the sum limit estimate by the relationship:

\[ \hat{I}(t) = 1 - \exp \left[ -R(t) \right] \]  

(1.7)

31. For occult tumours (see paragraph 22), which frequently occur in short-lived animals, the actuarial incidence (which is then usually called prevalence) or the integral tumour rate are more difficult to estimate. Theoretical analyses have shown that a combination of serial killing and survival data is required for such estimates in the case of tumours with unknown degree of lethality or life shortening [C36, M22, N4, R8]. If occult tumours are definitely non-lethal, estimates can be obtained by serial sacrifices at specified times after irradiation. However, this approach requires large numbers of animals. As Heei and Walburg have pointed out [H29], the method of isotonic regression (see also [B84, K33]) may be used to estimate the competing-risk-corrected incidence from survival experiments. This provides a maximum likelihood solution with the constraint of monotonicity of the estimate. The algorithm for isotonic regression is straightforward and has been utilized for the analysis of radiation carcinogenesis [C36]. At present, however, there are no methods to derive standard errors.

32. In most experimental studies in which tumours are seen in various organs of the same animals, it is usually assumed that such tumours occur independently of each other. However, Storer has shown [S33] that this is not necessarily so. In irradiated female BALB/c mice, 21 out of the 66 pairs of tumours tested showed significant positive or negative correlations. Some of the negative associations were due to rapid lethality caused by one of the tumours, and this could be corrected for by appropriate methods allowing for intercurrent mortality. Of the remaining 13 significant correlations, 6 involved tumours known to be endocrine-related, and 7 applied to tumours of other organs. Alterations in host factors were believed to be responsible for the observed associations. These possible complications should be borne in mind in the analysis of dose-response relationships on the assumption of random, i.e., independent, tumour occurrence.

33. The logrank test, the Breslow test, or the wider class of non-parametric generalized rank-sum tests are suitable for the comparison of tumour rates in two or more groups in the case of manifest tumours [K37]. Analogous tests do not exist for double censored data from survival experiments, i.e., for tumours found incidentally. With such data, one must use tests based on the assumption of the equality of competing risks in the two groups under comparison, or one requires knowledge of the degree of difference of competing risks. For experiments with serial killing, suitable standard tests exist.

34. The quantities discussed in paragraphs 28-29 are not based on specific models. In experiments where various groups, exposed to different doses, are compared, estimates may be used that are also non-parametric but are based on models. Most frequently, the proportional hazards model is used. This model is based on the assumption that the tumour rate or the integral tumour rate in non-irradiated animals is increased by a dose-dependent factor:

\[ r(t, D) = \lambda(D) r_0(t) \quad \text{or} \quad R(t, D) = \lambda(D) R_0(t) \]  

(1.8)
where \( r_0(t) \) and \( R_0(t) \) are the tumour rates and integral tumour rates for the non-irradiated animals, and \( r(t,D) \) and \( R(t,D) \) are the tumour rates for the individuals exposed to dose \( D \). By equations (1.1) and (1.6) one could express this model in terms of the actuarial incidence, \( I(t) \). However, such expression would be more complicated. The reason is that tumour rates and cumulative tumour rates from independent causes are additive. While the incidence is additive only when its value is small. For manifest tumours, there is a relatively straightforward algorithm for calculation of the proportional hazard coefficients, \( \lambda(D) \), employing the method of partial likelihood [K37]. For incidentally observed tumours, one must make use of more complex methods. requiring computer algorithms for non-linear optimization, with the constraint of monotonicity [C36, K38]. The analysis can also be based on the model of accelerated failure times by use of the non-linear optimization methods. This model assumes that the competing-risk-corrected incidence, \( I(t,D) \), rises earlier in a way that can be described by a dose-dependent acceleration of the incidence \( I_0(t) \) for the control groups:

\[
I(t,D) = I_0[a(D)t] \quad \text{and} \quad R(t,D) = R_0[a(D)t]
\]

(1.9)

A similar model is that of time shift [C36], which assumes that the tumour rates, the integral tumour rates, or the incidence may attain the same values at earlier times, in a manner that can be described by a forward shift in time:

\[
I(t,D) = I_0[t+\tau(D)] \quad \text{and} \quad R(t,d) = R_0[t+\tau(D)]
\]

(1.10)

With both the accelerated time or the time shift models, algorithms for non-linear optimization are required for either manifest or incidentally found tumours.

35. As a further step towards the derivation of coherent time and dose dependencies, parametric models have been used. Particularly important among these is the so-called Weibull model [K37], which postulates tumour rates, and integral tumour rates, increasing as a power of time:

\[
r(t) = c t^p \quad \text{and} \quad R(t) = c t^{p+1}/(p+1)
\]

(1.11)

The coefficient \( c \) is assumed to depend on dose, while the exponent \( p \) may or may not be treated as a parameter that varies with dose. The Weibull model is a special case both of the proportional hazards model and of the accelerated time model. Another frequently used model envisages a log-normal distribution of the times to the tumour, i.e., of a competing-risk-corrected incidence that depends on time as a log-normal sum distribution. Various other models, for example the logistic model, have also been utilized.

36. The preceding paragraphs refer only to acute irradiation. In case of continuous or fractionated long-term exposure, additional complexities are introduced. Under such conditions, the dose increases with time and it may be difficult to identify the relevant value of the accumulated dose. The process of cancer induction is followed by a period of growth until the tumour becomes observable. The dose absorbed during this period is not relevant to the appearance of the tumour. Corrections may therefore be applied by subtracting from the total dose the portion received after the presumed onset of neoplastic growth.

C. THE DEPENDENT VARIABLES IN EPIDEMIOLOGICAL STUDIES

37. Whereas experimental studies use inbred animals that are uniform as regards sex, age at exposure and other conditions, no comparable uniformity is ever encountered in epidemiological human studies. Moreover, various human populations are often subject to a spectrum of influences, of which only some are known or accounted for. In an ideal case, a multivariate analysis should be used to assess the relative importance of factors other than radiation. As this is seldom possible, less rigorous analyses must frequently be accepted which, in addition to the basic quantities previously discussed, use other, somewhat crude ones. In epidemiological investigations, the follow-up may start shortly after irradiation (prospective studies) or at later times when some or all of the expected tumours may have occurred (retrospective studies). Reliable data collection is more easily achieved in the first case, but the majority of epidemiological studies are based on retrospective analyses.

38. It is not the objective of this annex to deal in detail with all factors and variables affecting the accuracy of risk assessment of radiogenic cancers. However, for the understanding of the following text it is necessary to discuss briefly the expression of the response in absolute and relative terms. The question of risk projection beyond the period of direct observation is intimately linked to the use of so-called absolute and relative risk projection models. These will not be discussed in detail in this annex.

39. As mentioned above, the occurrence of radiation-induced neoplasms may depend on time after irradiation and on the absorbed dose in a variety of ways. In epidemiological investigations it is possible, ideally, to envisage two different situations frequently referred to as the absolute or the relative risk model.

(a) The radiation-induced excess tumour rate—or the incidence rate, as more frequently determined in epidemiological studies—after a latent period increases independently of the spontaneous incidence but as a function of absorbed dose, i.e., the spontaneous and induced rates are additive. Panel (a) of Figure II illustrates this case schematically;

(b) The excess tumour rate, or the net incidence rate, is proportional to the spontaneous age-specific incidence rate, i.e., the dose results in a multiplicative effect of the spontaneous tumour rate over the life span. This relative risk model, corresponding to the proportional hazards model described in paragraph 34, is shown in panel (b) of Figure II [C29].
40. For most human tumours, the spontaneous tumour rate is a steep function of age, but quantitatively this dependence varies. For radiation-induced tumours with relatively short latent periods and full expression within a short interval (leukaemia and bone sarcoma) one may distinguish between absolute or relative risk models. A simple absolute model will apply when irradiation at any age is followed by a temporary increase of the tumour rate that does not depend on the magnitude of the spontaneous age-specific rate. A relative model will be preferable when the temporary increase of the tumour rate becomes larger with increasing age at exposure. The latter case is illustrated in panels (c) and (d) of Figure II.

41. The relative risk projection model implies that the absolute (attributable) risk increases with age; on the other hand, the absolute risk model implies that the excess risk when related to the spontaneous incidence rate, decreases with age. Actually, epidemiological data often show an intermediate pattern, and a decision as to which model is most appropriate for risk estimation is not always possible [C29, T12]. However, recent evaluations of cancer incidences in atomic bomb survivors lend rather strong support to the relative model. This is so because, for the same age cohort, excess deaths from cancer other than leukaemia increase with age at death in proportion to the agespecific death rate from these cancers in the population of Japan [K39, S59]. A similar conclusion was reached when the time pattern of appearance of second cancers, presumably radiation-induced, was studied in women treated by radiation for carcinoma of the cervix [B93]. Constancy of the relative risk of lung cancer with time after irradiation was also noted in Swedish iron-ore miners [R41] and in United States of America and Czechoslovak uranium miners [15]. The two projection models have been used for prediction of the numbers of various tumour types in a population having an age distribution similar to the one in the United States, assuming a series of dose-response relationships and applying the appropriate corrections for intercurrent mortality by the life-table technique [C29]. When considering the risk of radiation-induced cancer, whatever the model applied, it is being currently assumed that the distribution of the sensitivity in human population is unimodal, although the character of the distribution is virtually unknown. The question of the possible exceptionally elevated susceptibility of some individuals is discussed in paragraphs 93-97.
1. The risk expressed in absolute terms

42. The tumour response of an irradiated population may be characterized by the time average net incidence rate, $\hat{I}_{TA}$, which is defined as the number of additional tumours diagnosed per person-year (PY) at risk. This quantity should reflect a net increase above the spontaneous incidence rate in suitably matched controls. It is calculated from the formula

$$\hat{I}_{TA} = \frac{X}{P} - \frac{J}{Q} \quad (1.12)$$

where $X$ is the number of persons with a diagnosed tumour in the exposed group; $P$ is the number of person-years in this same group, obtained by summing the number of years at risk for all individuals; $J$ is the number of persons with the same type of tumour in a control group that is matched or corrected for sex, age and calendar years; and $Q$ is the number of person-years in the control group.

43. The period at risk for an irradiated subject is the time, usually in years, from irradiation to cancer diagnosis, or death, or to loss from observation, or termination of the survey. For cancers other than leukaemia and bone sarcoma, an assumed minimum latency is usually subtracted from the time at risk. The value of $\hat{I}_{TA}$ is given as "cancer cases/person-year at risk", or more generally as the annual probability of occurrence of a specified cancer in a given population. The dose, $D$, will commonly be an average of the different doses that the members of the population have received.

44. If a suitable control group is not available, age- and sex-specific incidence rates of tumours in the general population may be used. Under these conditions, however, tumour ascertainment in the groups may not be fully comparable, and possible selection by conditions that prompted irradiation, or by other covariates, may result in gross errors.

45. In order to obtain the time average net incidence, $I_{TA}$, the value obtained from equation (1.12) is multiplied by the average time at risk, i.e., by the average period of observation or, for predictions beyond follow-up, by an assumed time for full expression of the malignancy. For leukaemia and bone sarcoma, this time is known not to exceed significantly 30 years (see Chapter V). For other radiation-induced tumours, the period of expression is unknown. In order to derive meaningful projections, a correction for intercurrent mortality becomes necessary, i.e., average life expectancies have to be used. When the distribution of the population by age and sex is known, life tables can be used to this purpose. As in $[C29]$, as the quantities $\hat{I}_{TA}$ and $I_{TA}$ can be affected by significant errors, due to several circumstances, they must be used with caution. First, as the latent period is not known precisely, the correction for it can only be, at best, an approximation. Secondly (and apart from the above correction) it is unrealistic to assume constancy of the incidence rate over time; if the observation periods for two collectives exposed to different doses do not coincide, then the observations cannot be strictly comparable.

46. Annual risk coefficients for radiation-induced cancer can be expressed in terms of the time average net incidence rate per unit dose, $F_{TA}$, that is, the probability per unit time per unit dose per person that a tumour can be seen in the observation period. This risk may depend on sex, age at irradiation, genetic disposition, the organ exposed, and a variety of other factors. The quantity is obtained by dividing $I_{TA}$ by the mean absorbed dose received in the exposed group. Numerical values are commonly given in "cases $10^{-6}$ a$^{-1}$ Gy$^{-1}$".

47. In order to obtain the life-time risk coefficient (net incidence per unit dose), $F_{TA}$, the quantity $F_{TA}$ is multiplied by the time at risk, i.e., by an assumed average time of full expression of the malignancy. The considerations in paragraph 45 that refer to the quantity $I_{TA}$ apply also to $F_{TA}$.

48. When the risk coefficients $F_{TA}$ and $F_{TA}$ are calculated for groups exposed to different average doses, changes of these quantities with increasing dose may provide approximate information about the shape of the dose-response curve. Thus, constancy of the coefficient indicates proportionality between response and dose within the range of doses studied; a rise or a decrease with increasing dose may result from an upward or downward concavity of the dose-response curve. However, if the radiation dose is correlated with a variable that affects the response (e.g., age) spurious effects may be seen and normalization procedures are called for. Furthermore, if the dose-incidence relationship is not linear, expressing the observations in terms of a probability per unit dose distorts the data and introduces additional inaccuracies if individual doses within the exposed population deviate substantially from the average dose to the population. For instance, if the dose relationship contains a dose-squared component, the contribution to the response by individuals with high doses is greater than if linearity applies.

2. The risk expressed in relative terms

49. When the risk is expressed in relative terms, the response is related to the risk of spontaneous cancers (incidence or mortality) in an unirradiated control population. The response variables used are the standardized mortality ratio (SMR), defined as the ratio of mortalities in the exposed group to the mortality in a control group multiplied by 100; and/or the relative risk (RR), which is the ratio of risks observed over expected (each expressed, for instance, per $10^{-6}$ person and over a given follow-up time). The latter quantity may be applied both to the incidence and mortality indices. The proportional hazard model specifies that if $i$ is the incidence rate of a disease, or the mortality rate for a specific cause of death among subjects in dose group $i$, it can be expressed as $i = \lambda_0 (RR_i)$, where $\lambda_0$ is the background or spontaneous rate of mortality or incidence (i.e., the rate experienced by subjects in the absence of exposure to radiation) while $RR_i$ is the relative risk associated with dose group $i$. The starting point for investigation of a radiation dose-response is the function...
\[ RR_i = 1 + yD_i \], where \( y \) represents the excess relative risk coefficient \((RR - 1)\) per unit dose. Variation of \( y \) with dose provides information as to the form of the RR-dose relationship.

50. Various statistical methods, including multiple regression analysis, have been used to obtain dose-response relationships, and are presented and discussed in detail in numerous publications, e.g., [G36, J8, K42, S60, W17]. Careful corrections for sex, age at irradiation, attained age at observation and other confounding variables (e.g., ethnicity, exposure to other carcinogens and promoters) are necessary when the shape of the dose-response curve is the object of the study.

D. TEMPORAL RELATIONSHIPS

51. As pointed out in preceding paragraphs, the tumour rate, the cumulative tumour rate and the actuarial incidence depend on time after irradiation and on other factors, such as age at exposure or absorbed dose. It has already been mentioned that the absorbed dose may change the time dependence in a variety of ways. It is necessary, therefore, to consider temporal relations in somewhat more detail.

1. Latent period

52. The latent period is the time between irradiation (e.g., single acute exposure) and manifestation of a tumour. It may be divided conceptually into true latency (the time required from initiation to the beginning of unrestrained growth) and tumour growth (time until the neoplasm can be diagnosed). Reported data are usually latent periods, i.e., the sum of the above times. The length of true latency may be obtained by subtracting an estimated time of tumour growth from the observed latent periods. There are considerable differences between the latent periods of various tumours. Two malignancies in man, leukaemia and bone sarcoma, appear to have relatively short latencies and show an upper limit of the latent periods, so that their full distribution in a population having a normal age structure can be observed. For other tumours the distribution of the latent periods is unknown, because it extends to very long times; the distributions are then usually truncated by the length of the observation period. Median times recorded so far are of the order of 20 to 30 years, but they should increase with extension of the follow-up of the respective groups.

53. If the follow-up is shorter than the minimum latent period, radiation-induced tumours cannot be expected. For more extended studies an inferred minimum latent period is frequently subtracted from the total time at risk, as mentioned before. The minimum latent period is often identified with the time after irradiation at which the incidence rate becomes statistically significant at a given level of confidence above the control values. Figure III shows, however, that, for statistical reasons, the minimum latent period so estimated must vary with dose even...

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Figure III. Presumed effect of the magnitude of dose upon estimate of minimum latent period. Two doses \( D_1 < D_2 \) produce different absolute effects, which have similar time distributions of the latent periods. Shaded area: spontaneous (control) incidence rate; Broken line: upper confidence limit of the spontaneous incidence rate.
under the assumption of the proportional hazard model, i.e., when the tumour rate is increased by a constant factor without change of the underlying temporal dependence. Similarly, shorter minimum latent periods are estimated if the number of individuals in the observed population is larger, because predetermined levels of statistical significance are reached earlier in a larger sample. Other statistical procedures have been developed to analyse the dependence of the latent period on dose [L32].

54. For some cancers, the minimum observable latent periods may change with age at irradiation, as suggested, for example, by studies of human breast carcinoma [B26, M17, S16, T12, T23] and lung cancer incidence in Japanese atomic bomb survivors [K39]. These radiation-induced tumours appear with high frequency at ages when the spontaneous incidence is also relatively high. It follows, therefore, that latency is longer for cohorts irradiated in childhood and adolescence than for individuals exposed in their twenties or thirties. If so, the follow-up to include a complete (i.e., life-time) expression of the effect must vary with age at exposure for different cohorts. It is not known whether this observation can be applied in a general sense to all forms of radiation-induced tumours with long latent periods.

55. In cohorts of advanced age, the life expectancy may be shorter than the average latent periods, and only a fraction of induced neoplasms will be seen. The younger the age at irradiation, the fuller the expression of induced tumours. In the common case of neoplasms with a distribution of latent periods extending significantly beyond the follow-up, the dose-response relationship cannot be based on complete data. The estimate of $L_{TA}$ will then change with the duration of the observation period; however, for those cancers for which the relative risk model appears adequate, the relative risk should be a less susceptible quantity in this respect. Analogous changes of the dose-response relationships have been shown in experimental work [S37]. Whenever the distribution of latent periods varies with the absorbed dose, the form of the derived dose-response relationships will depend critically on the length of the observation period. At low doses, the minimum latent periods may exceed the average life span of the individuals and it has been postulated that so-called practical thresholds may result [E3, M8, M10, R28]. This inverse relationship between latency and dose is most common in studies of continuous long-term irradiation. It was first described for bone sarcoma induced by long-lived isotopes [B48, E3, E4, F8, M4, R18-R20]. The present evidence does not substantiate the existence of such relationships for other tumours commonly induced by radiation in man, for which sufficiently detailed data are available, namely cancer of the breast [K39, L22], of the thyroid [S38], and of the lung and stomach [K39, L32]; an inverse relationship between absorbed dose and latent period may exist for leukaemia [L24], but has not been definitely proved.

56. All the above factors must be borne in mind in any discussion of the accuracy and reproducibility of dose-response relationships. Except for tumours with short latencies, such as leukaemia and osteosarcoma, there is generally a lack of data extending to follow-up periods long enough for the dose-response relationship to be assessed with reasonable completeness.

2. Relationships between dose and tumour-free lifetime lost as a result of induced cancers

57. As mentioned above, various malignancies may be characterized by different latent periods. For some malignancies and modes of irradiation the latent periods (mean, median) may vary with dose, the mean values normally increasing with decreasing dose. A quantity that may provide a first approximation to a common denominator for assessment of the harm per induced neoplasm is the tumour-free lifetime lost [I6]. The relationship between this quantity and dose is therefore of interest. Very few papers provide information on the subject, but this will be reviewed. when it is available, in relation to different types of tumour.

E. SUMMARY AND CONCLUSIONS

58. Cancer, as a stochastic radiation effect, can be studied for its dependence on dose and time after irradiation. Different degrees of precision are attainable in experimental and epidemiological studies of radiation-induced tumours. The methods for the estimation of dose-response relationships reflect these differences.

59. The absorbed dose in the relevant tissue is the independent variable for dose-response studies. The magnitude of the dose and its temporal pattern of delivery (dose rate, fractionation) may vary greatly. In the present context, low doses are taken to be those up to 0.2 Gy of low-LET radiation, while those in the interval 2.0–3.5 Gy are regarded as high doses, with the intermediate doses lying between these ranges. Low and high dose rates are taken to be below 0.05 mGy min$^{-1}$ and above 0.05 Gy min$^{-1}$, respectively. Intermediate rates are those in between these values. For high-LET radiation low and high doses are assumed to range up to 0.05 Gy and down to 0.5 Gy, respectively. Dose rates may be classified in the same way as for low-LET radiation. In certain studies, more readily measurable quantities that are proportional to absorbed dose—at least over some range—may be used as the independent variable.

60. In experiments on radiation carcinogenesis, various measures of the response may be used. The crude incidence, uncorrected for mortality, is not infrequently applied, but it is unsatisfactory because it neglects differences in life span after different exposures; accordingly, it may be strongly affected by intercurrent mortality due to causes other than cancer. Competing risk corrected quantities such as the tumour rate, $r(t)$, the cumulative tumour rate, $R(t)$, or the actuarial incidence or prevalence, $I(t,D)$, can be used for more meaningful expression of the response. These quantities are always dependent on the post-irradiation time, and must be reported accordingly. They are obtained from the observed time sequence of
tumour appearance and from the variable number of individuals at risk during the observation period. They may be calculated by appropriate algorithms that are different for manifest tumours, i.e., rapidly lethal or readily discovered tumours, and for incidentally observed tumours, i.e., tumours that do not contribute to lethality and that are found only at necropsy. Only a few experiments have so far been analysed according to the requirements of the competing risk theory, and this circumstance has often reduced the potentially useful information from numerous studies.

61. In epidemiological surveys, the response is, in most cases, assessed retrospectively, but less accuracy can be achieved than in well-designed controlled experiments. The genetic composition, the distributions of ages and sex, the forces of selection, and the follow-up periods are often different in irradiated and control groups and require appropriate adjustments. Furthermore, the doses to individual members of a cohort are usually subject to substantial variation, making it necessary to group exposed individuals over broad dose intervals. Also, the less rigorous quantities used in epidemiological studies, $T_A$, $F_A$, and their rates, are complex functions of time post-irradiation. The scoring of radiation-induced tumours depends therefore, in a complex way, on the age at irradiation, duration and completeness of follow-up, latent period, and life expectancy of the population. The duration of follow-up may especially influence the magnitude of the response and the shape of the derived dose-response relationship in terms of the absolute (attributable) risk. If the tumours of interest show an increment of the relative risk per unit dose that is not strongly dependent on age at irradiation, the shape of the observed dose-response relationship is less susceptible to duration of the follow-up. The minimum latent period has been exceeded, the number of tumours is sufficient for verification of the postulated models, and other relevant co-variables have been corrected for.

62. Human tumours with relatively short latent periods, e.g., leukaemia and bone sarcoma, are not expected to appear after about 30 years. For these tumours, the dose-response relationships in young cohorts can be based on complete manifestation of the effect. For other tumours, of longer and unknown latency, dose-response relationships must be based on the incomplete information resulting from truncation of the response by the short follow-up. A possible dependence of latent periods on dose is of critical importance in determining the shape of the dose-response curve. When mean latent periods decrease with increasing dose, the shape of the relation depends on the observation time and, in some cases, practical thresholds may apply. However, except for $^{226}$Ra-induced bone sarcoma, little information of this nature is available from data on man.

63. Projection of tumour incidence or incidence rate beyond the period of follow-up for tumours with long latent periods may be achieved by two alternative models: the absolute and the relative risk projection model. In its simple version, the first is based on the assumption that the rates of spontaneous and of radiation-induced tumours are independent, so that the rate of radiation-induced tumours does not increase proportionally to the age-dependent rate of spontaneous incidence. The second model in its simplest form assumes that irradiation has a multiplicative effect on the spontaneous tumour rate and that the rate of induced tumours is a dose-related multiple of the spontaneous rate. Recent data from several epidemiological studies have shown that, in many cases, the relative risk projection model provides a better fit to the data than the simple absolute risk model. For numerous tumours, however, no firm conclusions can be drawn as yet.

II. ASSUMPTIONS AND LIMITATIONS OF EXISTING MODELS

A. BASIC PHENOMENA AND INFLUENCE OF MODIFYING FACTORS

64. Common features of malignant cells are the unrestrained growth, the ability to infiltrate neighbouring tissues, and often the capacity to give rise to metastases. In a general sense, cancerous cells transmit their malignant characteristics from one generation of cells to another, and therefore somatic mutation could be one of the mechanisms involved as an underlying factor [B71, C32, F14, K20, K29, M50, R31, S43]. On the other hand, epigenetic factors such as dis-differentiation, expression of previously suppressed genes, activation of oncogenes (see annex A to this report) or others [B71, K26, S43, R31, S55] may be envisaged, alternatively or in parallel. Continuous growth through cellular divisions might result either from inactivation of operating or regulatory genes, or from the inability of malignant cells to receive or recognize division-restraining signals, whatever their nature might be.

65. Owing to poor understanding of the origin of cancer, quantitative predictions regarding the effects of ionizing radiation (dependence on dose, dose rate, quality of radiation) cannot be developed from basic principles. Formulation of simplified hypotheses under the form of models is at present a practical step towards understanding basic issues. To this end, detailed knowledge of mechanisms of cancer initiation and promotion would be valuable, but even this cannot be secured at present in a comprehensive form. Qualitative models of cancer induction have been reviewed recently by Whitmore [W8] and Parfenov [P2].

66. Before reviewing selected models, it is necessary to discuss the concept of multi-stage development of cancer. Since the classical studies of Berenblum et al. on chemically induced skin cancer in mice (discussed recently with regard to in vitro oncogenic transformation by Borek [B76]) this concept has gained wide acceptance. The subject has been repeatedly reviewed [B71, C32, D18, F13, F16, F20, M50, R31, R42, S43]. It is commonly assumed that cancer induction is started by a phenomenon of initiation, occurring most likely in one cell, although participation or modifying influences from neighbouring cells cannot be excluded. Further development into a cancerous clone probably
requires several stages, covered under the concepts of promotion and progression, which result eventually in an overt malignancy. These concepts have been reviewed by the Committee in annex I of the 1977 report [U6].

67. Cellular phenomena that take place during development of a cancerous clone are not well understood. Direct involvement of DNA in oncogenic cell transformation has been demonstrated [B14, L33, L34]. Lesions affecting its structure and function must therefore be relevant. Somatic mutation(s)—such as changes of the DNA base sequence: transposition, deletion, translocation and transfection (from one cell to another) of genetic material: enhanced instability of DNA, leading to facilitation of non-repairable DNA damage; induction of recombination between nuclear and mitochondial DNA; disturbances of DNA methylation: activation of oncogenes—may be involved. But some of these mechanisms are largely hypothetical [B71, B83, F21, G33, R31, S43, S45, S55, V2, V3]. Initiation is followed by a long latent period, during which secondary influences (systemic: exogenous) may influence the final outcome of the process. During this period, the action of secondary factors may accelerate and stimulate (promote) or inhibit, or even reverse the process (anti-promoters, suppressors of malignant transformation). Arguments have recently been presented which show rather convincingly that not all initiated cells, or their clones, will eventually give rise to diagnosable tumours [F22].

68. Whatever the nature of initiation, be it radiation-induced somatic mutation in the classic sense (induction coefficient of $10^{-5}$ to $10^{-6}$ Gy$^{-1}$ of low-LET radiation per locus at risk), or any other phenomenon similar to in vitro cell transformation (induction coefficient of $10^{-4}$ to $10^{-6}$ Gy$^{-1}$), the yield of recovered malignant tumours per irradiated cell in vivo is lower by many orders of magnitude. The possibility of a symmetrical “tumour-specific” chromosomal translocation was advocated [F21] as a very rare event that would remove part of this difficulty. However, recent studies in vitro and in vivo (chapter IV) suggest that initiation may not be a rare event when expressed per cell at risk. Therefore, if it is assumed that these processes operate in vivo with comparable efficiency, even for a small fraction of the susceptible cells ($10^{-4}$ to $10^{-6}$), a significant number of cells would be transformed every day because of background irradiation alone [M31].

69. The theory of sequential development of cancer may help to explain the discrepancy between data in vitro and in vivo, and the rarity of radiation-induced cancer. If a series of consecutive rare events is required for the initiated cell to become malignant, the final probability of emergence of the malignant clone will be the product of the probabilities of each event. Assuming that only the first event (initiation) is radiation-induced, and that the subsequent ones are rare phenomena, perhaps independent of irradiation. Clinical cancer could be many orders of magnitude less likely than initiation itself. Yet, clinical cancer could show a relationship with dose similar to that of initiation, even though (owing to the multi-stage nature of the process and possible systemic interferences) less regularity would be expected than that observed for stochastic radiation-induced phenomena in single cells under controlled conditions. That this may actually be the case is suggested by the reproducibility of dose-response relationships for some cancers in animals of selected strains. Also, supporting this contention is the fact that the absolute risk coefficient for cancer of the same organs derived from epidemiological observations of different populations (see chapter V) are often similar. Although there are exceptions to this rule (see, for example, Figure XXVIII), this may imply that the modifying influence of secondary factors may not greatly change the final probability of induction and the reproducibility of the dose-response relationships. It has been postulated that for some forms of malignancy, such as leukaemias, radiation may be the causal factor, not for the first, but for the last event in a series of rare phenomena [L24, K39]. In any case, initiation is a necessary step, although only a small fraction of initiated cells may develop into a viable clone and into a clinical tumour.

70. The principal aim of a model is to predict the shape of the incidence curves as a function of dose, dose rate, quality of radiation and possibly other factors. Ideally, a model for radiation-induced cancer could provide some basis for the evaluation of risk at low doses [D9, F12, T15] if it could characterize the following processes:

(a) Probability of malignant transformation of a defined target cell. This will most likely vary with the organ, dose, quality of radiation, and temporal pattern of dose distribution. In this respect, the model should include theoretical concepts concerning the nature of the initiating event(s) and not be purely empirical. Mathematical formulations should be compatible with general knowledge of radiation and cancer biology;

(b) Probability of interaction at the systemic level between transformed cells or between developing clones;

(c) Probability of cell killing with dose, as non-viable cells cannot give rise to cancerous clones. This factor, as discussed in chapter III, depends certainly upon the tissues concerned, some of which are particularly susceptible, and upon dose, dose rate and quality of radiation and on other irradiation conditions (in vitro, in situ) [D7, G14, G16, H8, H10, M31, M52, R2, S26, S27, U6, U7, U17];

(d) Effect of numerous host factors. Discussed in annex I of the 1977 UNSCEAR report [U6]. Among promoting factors, cell division is perhaps a good example [B36, B85, M6, N6, P23]. Enhanced proliferation can be induced by various mechanisms, including radiation-induced cell death, a phenomenon which is likely to produce a threshold-type dose response. Other factors could be the differentiation of initiated cells, immunological surveillance that suppresses development of malignant clones with antigenic characteristics foreign to the host-system, and hormonal influences:
(e) Effect of exogenous promoting agents leading to more rapid appearance of tumours, to an increase in tumour yield, and perhaps also to changes in the shape of dose-response curves, as shown by several experiments [F21, L26] including those on in vitro oncogenic transformation [H22, L26].

71. Immunological surveillance may be impaired by numerous factors, including ionizing radiation. The degree of impairment would certainly depend on dose, on whether the whole body or only part of it is irradiated, and then on what part and what proportion. It may be postulated that a reduction of immunological capacity of the organism follows a threshold-type response to irradiation. The importance of the immunological suppression in the low and intermediate dose range remains to be established although recent evidence points to a limited role of the immune system. Studies of Mole et al. [M52] on induction of acute myelogenous leukaemia in CBA/H mice, suggest that, at least in this tumour-host system, impairment of immunological surveillance is not very important. Similar conclusions were also reached by Nolibé et al. in respect to lung cancers induced by 239PuO2 in rats [N9].

72. Hormonal dependence is well known for some cancers [U6]. By partly or totally inactivating endocrine organs and creating a hormonal imbalance, radiation may accelerate or impair malignant growth in other organs (e.g., breast, thyroid, ovary). Again, it is likely that this type of response would show pronounced dose thresholds. Hormonal influences could also be responsible for the association of various tumours, as observed in experimental animals [S33].

73. In summary, the action of modifying factors might change the resulting dose-response relationships. It is a basic weakness of the models discussed in chapter III that mathematical functions relating cancer incidence to dose reflect only initiation and sterilization of the initiated cells. This is because the precise effect of other modifying factors on tumour induction—as a function of dose—cannot yet be described with reasonable credibility, even if some attempts have been made [M4, P22]. Models, therefore, provide greatly simplified concepts based on plausible assumptions and hypotheses, which may help in understanding the role of dose, dose rate and radiation quality. The relative importance of the parameters reflecting these quantities in a model can be deduced by comparing data with predictions. However, some of the parameters are the compounded expression of several elementary phenomena that cannot be resolved at present.

74. From available models, risk coefficients for man in the range from about 1 mGy up to about 0.1 Gy can only be obtained by extrapolation from the recorded values (in most cases above 1 Gy) to zero dose. Effects of the host factors discussed above (cell renewal, immunological surveillance, hormonal balance) and exogenous modifying factors upon dose-response relationships are uncertain, and only approximate evaluations may be given. For the purposes of the present annex, the levels of acute doses of low-LET radiation can be broadly separated as follows:

(a) < 0.2 Gy. At this level, data for most tissues are not directly available, but the damage to tissue functions is considered negligible. Therefore, dose-response relationships could be very close to those postulated by models that disregard host factors;

(b) 0.2-2.0 Gy. Damage to tissues, cell proliferation and influence of other host factors would not be expected to play a dominant role. Distortions in the dose-response relations should be relatively minor. and would not grossly bias the extrapolations;

(c) 2.0-10 Gy. Damage to tissues, to immunological functions (for irradiation of the whole or most of the body) and to hormonal feed-back systems may play a significant role. Extrapolations to low doses must therefore become more complex, particularly from the upper end of this range. Great caution is needed to avoid extrapolating from data that are already strongly affected by killing of initiated cells, as this might result in a substantial under-estimate of the risk at low doses;

(d) > 10 Gy. Severe damage to tissues and organs will dominate the response. Data in this dose region are most difficult to evaluate and so complex that extrapolation to low doses could be meaningless. Induced cell repopulation may also become a complicating factor for irradiation times longer than a few days.

A corresponding approximate classification of fast neutron doses would include the ranges: (a) 0.05 Gy; (b) 0.05-0.2 Gy; (c) 0.2-2.0 Gy; and (d) > 2.0 Gy. It should be noted that the relation between these ranges of low- and high-LET radiation may not be interpreted in terms of RBE.

B. THE THRESHOLD DOSE FOR CANCER INDUCTION

75. It has already been mentioned that for many types of cancer the epidemiological and experimental information available does not suggest the presence of a threshold dose. If initiation is at least partly autonomous, i.e., if it affects a single or very few cells, present understanding of the mechanisms involved suggests that:

(a) The nature of radiation interaction with the cellular target for cancer initiation is stochastic, in the sense that each increment of dose carries a given probability of effective interaction;

(b) The error-free repair of the DNA, which is the most likely target involved, leaves some fraction of the damage unrepaired and the error-prone repair may produce misrepai sed sequences in the DNA structure.

As the product of two probabilities greater than zero cannot equal zero, the presence of a threshold cannot be assumed.
76. A different situation obtains when the lifetime lost per cancer case (instead of the incidence) is related to dose or dose rate. Few malignancies show a definite inverse relationship between dose rate and latent period (see III.B.2), particularly at chronic exposure. In such cases (e.g., bone sarcomas induced by $^{90}$Sr or long-lived radium isotopes) practical thresholds may exist below which no life shortening occurs and a very low probability of tumour appearance applies.

C. UNICELLULAR VERSUS MULTICELLULAR ORIGIN OF NEOPLASTIC GROWTH

77. Under the simplifying assumption that dose-response relationships for radiation-induced cancer reflect mainly the kinetics of initiation, they should include both the dose-dependence of the relevant intra-cellular phenomena and the interactions of individual (contiguous) cells. Indeed the latter play any role in the process [G30]. Therefore, the question whether a malignant clone arises from transformation of a single cell or of several contiguous cells is of critical importance for the formulation of models. Because understanding of the biological process of carcinogenesis is limited, this question cannot be resolved unequivocally at present. However, most experimental and human observations tend to support—even if indirectly—the monocellular origin of neoplasms. Furthermore, the alternative multicellular hypothesis allows the development of models resulting in such a variety of dose-response relationships as to render the exercise of model-fitting totally meaningless.

78. All these problems were reviewed in depth by Fialkow [F3, F4, F6]. Nowell [N5] and others [B60, F13, F14]. It appears from their work that the monocellular alternative is gaining increasing acceptance, except for some rare hereditary tumours in subjects with inborn predispositions (e.g., multiple neuro-fibromatosis [F5]) and other rare tumours, such as condylomata acuminata [F10]. Three main lines of evidence support the unicellular hypothesis [F3, F6, N5]:

(a) The isoenzyme pattern of glucose-6-phosphate dehydrogenase in cancerous cells derived from tumours of heterozygous women, in accordance with the hypothesis that the same X-chromosome of the homologous pair is functioning in all cells of a given neoplasm. A similar pattern was observed recently in 3-methylcholanthrene-induced fibrosarcomas in Pkg-1ª/Pkg-1ª mice carrying X-chromosome inactivation mosaicism for the phosphoglycerate kinase (PGK-1) gene [T17]:

(b) The homogeneity of immunoglobulins produced by almost every case of myeloma;

(c) The common cytogenetic markers frequently detected in the cells of a single tumour.

This evidence is limited, indirect and compatible with the alternative hypothesis that selection of one clone from among several initially transformed cells might lead to an apparently monoclonal composition of the tumour. So far, however, this possibility has not been widely supported.

D. AUTONOMY OF TARGET CELLS FOR TUMOUR INDUCTION

79. Conceptually, even if initiation is taking place in one cell, the multi-stage nature of cancer development and possible influences of tissue and systemic origin could introduce random fluctuations into the expression of cancers in vivo. For radiation-induced cancer, therefore, such regularity and reproducibility of dose-response relationships as that seen for stochastic radiobiological phenomena in cultured cells would not be expected. Much of the information from animal experiments shows nevertheless that dose-response relationships are reasonably reproducible. Proportionality between initiation frequency and expression may therefore be assumed as a plausible hypothesis.

80. On the basis of microdosimetric considerations Rossi and Kellerer [R17], Rossi [R15, R16] and Rossi and Hall [R43] have pointed out that the observed curvilinear dose-response relationships for neutron-induced mammary tumours in rats and other tumours in mice are inconsistent with autonomous response because at low and intermediate doses there should be a direct proportionality (linearity) between dose and tumour incidence. Therefore, if the dose response is in fact non-linear, this must mean that either initiation or expression are modified by dose-dependent factors, implying a lack of "cell autonomy". If such a conclusion should generally apply, theories concerned with the response of single cells may not be relevant to dose-response relationships for cancer induction.

81. In the original experimental data of Shellabarger on Sprague-Dawley rats [S15], 14 months after an acute x-ray exposure, the incidence of all mammary tumours (adenomas plus carcinomas) per rat showed an approximately linear relationship with dose, up to 0.84 Gy, whereas 0.43 MeV neutron irradiation, with doses from 0.001 to 0.064 Gy, resulted in a tumour incidence roughly proportional to the square root of the dose [S15]. Rossi and Kellerer argued [R17] that, since cell killing would be negligible at such low neutron doses, the form of the observed relationship required the interplay of radiation effects in several cells. For mammary fibroadenomas, such an interaction leads, apparently, to a reduction of the tumour rate per unit dose even at neutron doses of a few tens of mGy.

82. Earlier data by Shellabarger et al. [S15] were later extended, with follow-up of the animals to about 1000 days [S37]. Tumours scored included fibro-adenomas and adenocarcinomas, the latter malignant forms being 9-16% of the total. The data expressed as crude incidence or mortality-corrected indices, r(t,D), R(t,D), at various post-irradiation times, essentially confirmed the previous findings. A very high RBE was seen at low neutron doses for both tumour types (about 100 at 0.1 mGy) and the dose-response for the combined tumours was concave downwards for neutrons and approximately linear for x rays. There was also a significant shift of the age-specific r(t) curve in the irradiated animals (acceleration).
83. When the net R(t) values at 800 days (the latest follow-up time for all but one dose groups) were considered separately for fibroadenomas and adenocarcinomas, the following conclusions could be drawn:

(a) For all tumours combined the downward concavity of the curve after irradiation with neutrons results essentially from a low point for the fibroadenomas at 0.064 Gy. Such an effect is not seen for the adenocarcinomas, where the relation is approximately linear, although the experimental scatter precludes accurate assessments in this case:

(b) At 800 days, the departure from linearity of R(t) for the fibroadenomas is much less pronounced than at 400 days [S15] or than that for the incidence of adenocarcinoma [S37]:

(c) No account was taken of cell killing in these experiments, although this may have not been negligible already at 0.064 Gy.

84. In view of their high spontaneous incidence (80% throughout the lifetime in females), the mammary tumours in Sprague-Dawley rats must be regarded as an unusual model. This view is confirmed by the facts that only a minor part of these tumours is histologically malignant [S12, S13, S14, S15, U17, V4, V5] and that the very high sensitivity of the Sprague-Dawley rat to the induction of mammary tumours is not seen in other rat strains [B43, B92, C15, S7, S11, V10].

85. In summary, the shape of the dose-response relationship reported for neutron- and x-ray-induced fibroadenomas at low doses in the mammary gland of Sprague-Dawley rats in one study is rather exceptional among all data on radiation-induced tumours. The low yield of adenomas per unit dose at 0.064 Gy, which has been interpreted to show an inhibitory influence by the neighbouring cells, may also be explained in terms of cell killing. Therefore, the observations discussed do not necessarily imply that malignant tumours arise from more than one initially transformed cell, or from interaction of several cells.

86. Other data on benign lung adenomas in RFM mice [U21, U22] may be discussed in this context. Pathogen-free animals were irradiated locally over the thorax with graded doses of fission neutrons (0.05 to 1.5 Gy) and the number of tumours in dissected lungs was counted 9 months after exposure. The effect was fully manifest at 6 months after 0.5 Gy, and intercurrent mortality was negligible. A peaked response, with a maximum around 0.25 Gy, was seen following single neutron doses. Splitting of the dose into two equal fractions, given at 24-hour or 30-day intervals, resulted in no sparing effect of fractionation. Plotting the mean number of adenomas per animal at 9 months against dose gives a slightly upward concave curve in the range of 0 to 0.25 Gy. A quadratic model fits the data very well (P > 0.99) but non-threshold linearity is not excluded (P > 0.8); also, a threshold of 0.05 Gy cannot be rejected, particularly since the gamma-ray curve suggests such a possibility. It is also possible that increasing doses of neutrons up to 0.25 Gy may have an enhancing effect, interpreted either as acceleration or promotion. Such an effect, however, is the opposite of that seen for mammary fibroadenomas in Sprague-Dawley rats. Results of other experiments with neutron induction of various tumours in BALB/c mice [U23] were also advocated as evidence that induction cannot be treated as a phenomenon related to autonomous cells. However, a correction for sterilization of initiated cells could result in approximately linear dose-response curves up to 0.2 Gy.

87. There is yet another explanation for the above data, i.e., that single-cell initiation is modified by enhancing or inhibiting dose-related effects. The nature of these effects (direct or absorbable, cellular or humoral) cannot be specified at present. Tumours requiring hormonal stimulation for their development (as in the case of the ovary) often have threshold dose-responses. In essence, therefore, none of the data discussed are incompatible with the hypothesis that tumour initiation by radiation may take place in a single cell, which from this standpoint must be considered relatively autonomous.

88. It has been pointed out [G15] that the simultaneous initiation of cancerous growth in several interacting cells would be equivalent to a multi-target event. If so, the sigmoid shape of the curve should become more pronounced with an increasing number of participating cells. There is, however, little data to support such a possibility and no known experimental system to test such hypotheses.

89. It may be concluded that if the curve is not obviously sigmoid or threshold-like upon visual inspection, the idea of a monocellular origin of the tumour may be accepted. The argument of a mono- versus a pluri-cellular theory, as well as the question of the degree of cellular autonomy in the origin of cancer, will not be finally resolved as long as the understanding of tumour pathogenesis remains unsatisfactory. As a rule, however, there appears to be no solid biological evidence against the monocellular origin of neoplasms. The assumptions that malignancy is initiated in a single cell, and that there is a reasonable proportionality between tumour induction and expression, have been incorporated—explicitly or implicitly—into most models of cancer induction by radiation. This view is explicitly accepted here as a working hypothesis, even though it is recognized that different opinions have been expressed [B83, M52, R43].

E. NUMBER OF CELLS AT RISK

90. The number of cells at risk of cancer induction in any organ is not known. When discussing this subject, most authors argue that the number of cells in which initiation takes place must be proportional to the number of cells at risk in a given tissue, and that the latter is proportional to the fraction of tissue irradiated. Most models or theories of cancer induction by radiation have accepted this assumption. It does not follow from it that tumour incidence, spontaneous or radiation-induced, is proportional to the total number of cells in any organ, or in the body of various animal
species [B49, M9], because there is no obvious correlation between body size in various species and incidence of tumours.

91. Epidemiological data on leukaemia in man, after irradiation of various fractions of the total active bone marrow, are broadly consistent with the notion that the probability of induction is proportional to the number of irradiated cells at risk, on the assumption that these are uniformly distributed throughout the marrow. Absolute annual risk coefficients derived from various studies fall within a range which is narrower than the variation of the fraction of the irradiated tissue (annex G in the 1977 UNSCEAR report [U6]). The same conclusion may be derived from experiments on cancer induction by total and partial irradiation of the mammary tissue in rats [B28, S10, S12] and by various areas of skin in mice [H11, H12, H13].

92. An important and related question is the effect on tumour incidence of a non-uniform tissue dose distribution. The extreme case is that of the so-called hot particles, i.e., particles with very high concentrations of radionuclides that are deposited in an organ such as the respiratory tract or the liver. Under such circumstances, the dose averaged over the entire organ would be much lower than that in the immediate vicinity of any such particle. Several authors addressed this question by studying carcinogenesis in the lung [13, L35, S54]. In all cases, as expected from theoretical predictions [M9, M10], hot particles were found to be less effective than similar doses of the same radiation quality distributed in a more uniform manner.

F. POPULATION HETEROGENEITY AND SUSCEPTIBILITY TO CANCER INDUCTION

93. Baum [B16] raised the question of whether linear extrapolation of the risk from the high dose and dose-rate region of low-LET radiation to the low-dose domain is always conservative, in view of a possible difference in susceptibility to cancer induction between groups in the general population. To exemplify the situation, Baum [B16] constructed a model in which three population sub-groups, represented by 1, 10 and 98%, varied in susceptibility (expressed as $D_0$ values) in the ratio of 100 : 1 : 0, respectively. For each of the sub-groups he postulated a dose-response relationship of the form

$$I_x(D_0) = 1 - e^{-(D/D_0)}$$

(2.1)

94. For the most sensitive sub-group (1%) he assumed that cancer induction might be a single-target effect ($n = 1$) with $D_0$ of 0.01 Gy; 10% would exhibit a lower sensitivity ($D_0 = 1$ Gy); and for the rest of the population $D_0 = \infty$. According to Baum, the composite response of the total population is dominated in the low-dose region by the highest sensitivity of the 1% sub-group. In the range from about 0.01 Gy to a few Gy, the relationship would be a curve, concave downwards, with response roughly proportional to $D^{0.5}$. The risk per unit dose above 1 Gy would be less than below 0.01 Gy.

95. It is not easy to envisage why susceptibility between groups should be reflected only by $D_0$ and, if so, why by a factor as large as 100. Moreover, the assumption that the bulk of the population should be totally non-susceptible to induction of malignant disease(s) lacks justification in Baum's paper [B16]. No epidemiological evidence supports this contention, even though it is known that incidence of radiation-induced cancer in man is generally low. Examples of dose-response relationships advocated by Baum to support his argument do not stand up in the face of criticism. The author presented data for acute leukaemia at Nagasaki, and for all cancers, all leukaemias, stomach, lung, and breast cancer at Hiroshima, where incidences rise with a power of dose between 0.1 and 0.8. At present, dosimetric uncertainties preclude a more detailed discussion of atomic bomb survivor data, but preliminary evaluations [K39, S46] of the dose-response relationships for leukaemia, breast cancer and total malignancies for the period up to 1978, using new dose estimates [L27], do not support the above conclusions (this information is still tentative). Therefore, Baum's hypothesis is neither satisfactory on theoretical grounds nor supported by epidemiological observations. The subject itself, however, is obviously important and warrants some exploration.

96. Progress in cancer genetics has shed some light on possible mechanisms of differential susceptibility to cancer development in individuals with various inherited traits [G12, M35]. The relevance of these phenomena to radiation-induced cancer requires attention. The relationship between genetic heterogeneity and incidence of spontaneous cancer, and cancer induced by radiation or other carcinogens, was discussed in detail in annex 1 of the 1982 UNSCEAR report [U24]. It is again reviewed in annex A to this report. The homozygotes for some genes (ataxia telangiectasia, Fanconi's anaemia and several others) have a significantly increased natural incidence of some forms of cancer and an enhanced sensitivity to other cellular and subcellular radiation effects. Various forms of impaired DNA repair are seen in most of these conditions. In view of their rarity, the affected subjects do not pose a public health problem, but the heterozygous carriers of these genes could also have an increased risk of developing spontaneous cancer. Calculations have shown [V6] that a few percent of individuals in the general population should carry such genes in a heterozygous state. If their capability for DNA repair is reduced, this might result in an increased proneness to cancer development. Although it is not known at present whether these heterozygous subjects may also be more prone to radiation-induced cancer, this possibility should not be overlooked.

97. If risk coefficients at low doses were higher than those reported in most studies at about 1 Gy, then perhaps such evidence could be interpreted to suggest the presence of population groups particularly susceptible to cancer development. A higher effectiveness of low dose was claimed by Mancuso et al. [M3, K17, K32], who studied the mortality of workers employed in the Hanford works, Richland, United States, dying
with cancer from 1944, for whom death certificates and data on personal radiation dosimetry were available. This information was reviewed by several authors (A7, D14, G5, G25, H20, M30, R3, S2) who pointed to the low statistical power of the original data and to numerous other difficulties in their interpretation. From careful examination of all the evidence, it appears that these data are at present inadequate to prove or disprove that the risk of radiation-induced cancer at low doses is higher than estimated by UNSCEAR in its 1977 report [U6]. This matter will, however, be kept under review.

G. SUMMARY AND CONCLUSIONS

98. The pathogenesis of tumours, both spontaneous and radiation-induced, is at present poorly understood. The probability of cancer induction cannot therefore be derived from general principles. The data available indicate that carcinogenesis is likely to be a complex, multi-stage process. Initiation at the cellular level is followed by a long period of latency, during which numerous changes take place until the tumour becomes clinically manifest. The probability of some of these changes is very low, and during the intermediate stages numerous exogenous factors could accelerate or inhibit the development of potentially cancerous cell clones.

99. There is considerable variation between organs and tissues in the process of cancer development, presumably related to numerous endogenous factors. Among these, the hormonal and the immunological factors are known to be of importance, at least for some forms of cancer. Significant cell killing in tissues may stimulate compensatory cell proliferation and therefore increase the probability of cancer development. The ways in which, and degrees to which, radiation could modify such mechanisms, particularly in relation to dose, are not well understood. It appears likely, however, that dose thresholds such as those operating for non-stochastic effects might apply. This would imply that low or intermediate doses (below approximately 2.0 Gy of gamma rays or 0.3-0.5 Gy of neutrons) delivered acutely might have little or no modifying effect.

100. Under the circumstances described, for predictive purposes the use of simplified models of tumour induction becomes one of the possible choices, even though the degree of simplification is usually such as to exclude consideration of the above modifying factors. This important limitation of the models should be kept in mind.

101. In view of the multi-stage nature of the phenomena described, and of the small probability of occurrence of each step, the proportion of initially transformed cells giving rise to an overt tumour must be extremely small. It may be postulated that at low doses and dose rates, where the host factors mentioned above are not expected to modify primary events appreciably, there is a broad similarity of relationships between dose and tumour incidence. On the one hand, and between dose and probability of cell initiation and survival, on the other. Exogenous modifying factors (e.g., promoters and inhibitors) could alter the slope, and perhaps the shape, of the dose-response curve in some instances; in man very little is known in this respect, apart from the effects of tobacco smoke on lung cancer induction.

102. Monocellular and monoclonal hypotheses are usually assumed in models of radiation carcinogenesis. Most biological data support the notion that cancer, as a rule, starts from a single cell, but unequivocal, direct evidence to support this point is lacking, and alternative interpretations are possible. The monocellular hypothesis is critical in extrapolating the risk from high to low doses. The alternative hypothesis, that concomitant initiation or interaction of several cells is required, lacks sufficient experimental support and makes extrapolation from intermediate to low doses difficult or impossible at present. The postulate of monocellular origin of cancer must be treated, for the time being, as a working hypothesis.

103. The number of cells at risk of radiation-induced malignant transformation is usually assumed to be proportional to the fraction of an organ or tissue irradiated. The experimental evidence in favour of such an assumption is very strong, but, again, it must be regarded simply as a plausible working hypothesis. Gross non-uniformities of the tissue doses, such as those resulting from deposition of the so-called hot particles, normally result in a lower tumour incidence than for comparable doses distributed uniformly.

104. It has been proposed that sensitive sub-groups in the general population might invalidate any linear extrapolation of cancer risk to low doses of radiation, a procedure hitherto believed to provide upper values of the risk. It is known that the very rare homozygous carriers of particular genes (e.g., ataxia telangiectasia, Fanconi's anaemia) are more prone to cancer and that their cells are particularly sensitive to other effects of radiation, such as cell killing. It is also true that the healthy carriers of these same genes in the heterozygous form (a few percent of the general population) are at greater risk of developing spontaneous tumours and also have a slightly enhanced sensitivity to other radiobiological effects. Whether these same individuals may also be at a higher risk of radiation-induced cancer cannot be proved at present and this question requires further study. The epidemiological evidence in favour of the notion that higher risk coefficients per unit dose of low-LET radiation may apply at low, rather than at high, doses appears at present unconvincing.

III. DOSE-RESPONSE MODELS OF RADIATION-INDUCED CANCER

105. Although in principle a large variety of mathematical expressions and models could be fitted to experimental and epidemiological data on radiation-induced tumours, for the purpose of this annex the Committee decided to limit the analysis only to those models that appear to be supported by general knowledge of cellular and subcellular radiobiology.
A. INTRODUCTION

106. When either the crude or the actuarial incidence of various radiation-induced experimental tumours is plotted against a wide range of doses, then, for low-LET radiation, a peak incidence is normally seen at intermediate doses, followed by a decline at high doses. For high-LET particles, the essentially linear initial slope gradually decreases with dose. In some cases, it even becomes negative. The bulk of data is consistent with this generalization [U6]. Evidence in man is much less certain because only a few series cover a sufficiently wide range of doses. Nevertheless, the incidence per unit dose of bone sarcoma [M29, R18], lung carcinoma [K22, S3] and cancer of the breast [S16] decreases at very high doses. Thus, a model of radiation-induced cancer should allow for two opposing trends: a rise of probability of induction with dose and then a decline, resulting presumably either from killing of irradiated potentially malignant cells [G16, M52, R2], or, in some cases, from increased mortality rate at high doses [I5, J7]. Mathematically, this trend may be simulated by multiplying the induction dose-response function by a term describing the probability of cell (or organism) survival as a function of dose.

107. Conceptually, there are two possible approaches to the formulation of models. The first is based on radiobiological considerations, relating, by analogy, the radiation-induced cancer incidence and the influence of various parameters (dose, dose rate, fractionation) to other effects in single cells. Broad similarities have been observed in numerous cases. The alternative approach is an empirical one, aimed at approximating the data (incidence versus dose) by simple mathematical functions. In practice, the two approaches are often applied in combination.

108. There is no reason why any model might be valid for all radiation-induced malignancies: actually, a more reasonable approach would be to determine separately which model is applicable to which type of cancer, a conclusion supported by the bulk of experimental data. There are examples of radiation-induced cancer with pronounced thresholds (e.g., the thymic lymphoma of the C57BL mouse, ovarian tumours in many mouse strains). Indirect mechanisms (destruction of an overwhelming part of target cells in the thymus: killing of a large fraction of hormonally-active cells in the ovary) may explain such sigmoid relationships. Similar data are exceptional in man (see chapter V). Thus, even if threshold-type relationships cannot absolutely be excluded, the most easily induced human tumours, such as those of breast, thyroid and lung (undifferentiated small cell type), as well as leukaemia, do not indicate the existence of a threshold. Therefore, models used for these malignancies do not postulate a threshold. In principle, the models discussed below have been formulated in terms of the absolute risk, but they seem equally applicable to a relative risk increment.

B. DERIVATION AND CHARACTERISTICS OF DOSE-RESPONSE MODELS

1. The linear model

109. The simplest and oldest linear model was developed for X-ray-induced point mutations in Droso-
ophila [G24, M46]. It was based on the postulate that each energy deposition in a cellular target carries a probability of changes in the genome (mutations) and that these may be either irreparable or the result of misrepair. Since radiation energy is transferred in discrete events, all effects should ultimately be linear with dose at low doses where only few cells absorb some energy and their number is directly proportional to dose. The probabilities of an effect assumed to be an all-or-none phenomenon in relation to each dose increment should be additive, and dose rate or fractionation would not be expected to alter the magnitude of the final effect.

110. In the course of time it became obvious that the linear model must be limited to effects showing single-hit kinetics after low- or high-LET irradiation. However, the postulated additivity of effects and the dose-rate independence does not always apply to single-hit effects [E10, H35], showing that in such cases repair processes must also operate to make an otherwise linear relationship dose-rate sensitive. It appears that when a track passing through the sensitive volume is not 100% effective, then its effects may be subject to repair. In other words, the presence of dose-protraction or dose-fractionation effects may not be incompatible with single-hit kinetics.

111. For high-LET radiation, the linearity of the initial slope, modified by phenomena of saturation or cell inactivation (killing), has been widely accepted [B20, 11, M66, U6, U7, U9, U10]. The dose response is described by the following equation:

\[ I(D) = a_1D e^{-\alpha D} \]  (3.1)

If cells are sensitive to inactivation, the relationship will depart from linearity, even at low or intermediate doses, owing to cell killing. The lower the mean lethal dose \((1/\beta)\), the more downwards concave the relationship will be. Recent experiments on neutron-induced tumours in animals (see chapter IV) suggest that this phenomenon—or others [S37, U23, U25, U26]—does in fact operate, causing departure from linearity, even at a few tens of milligray or a few tenths of a gray. This is explained by the fact that cell killing by high-LET particles is very effective with exponential non-threshold survival curves.

112. If equation (3.1) reflects the kinetics of tumour incidence as a function of the high-LET dose, extrapolation to low doses would tend to underestimate the risk by a factor of \(e^{-\alpha D}\). Examples and limitations of this postulate will be discussed in chapter IV, in the light of available experimental data.

113. Alternatively, the data can normally be fitted over some range of doses by an equation of the type:

\[ I(D) = a_1\sqrt{D} \]  (3.2)

Such a purely descriptive model implies no linear slope at low doses, even for very radioresistant cells. This seems unlikely, however, on radiobiological grounds. In this latter model, as in any other model involving a downward concavity of the dose-response curve, dose fractionation would produce an increased
incidence of tumours if the intervals between fractions were sufficiently long for the effects of each fraction to be considered independent [R37]. By a similar mechanism, the effects at low dose rate could also be enhanced, in comparison with the high dose rate.

114. An interesting variant of the linear model is that developed by Mayneord and Clarke [M9]. They discussed dose-response curves and their relationship with time, in regard to both dose rate and latency in the appearance of tumours. This is of importance because irradiation time and latency may be comparable with the life span of an exposed individual and could, therefore, affect the manifestation of the response. Their point of departure is that irradiation confers a probability of cancer appearance in the future [B48], following a stochastic process. This probability may be estimated by integrating the response of single cells as a function of dose over the number of cells at risk. A linear relationship between the probability of malignant cell transformation and dose was assumed at low doses where cell killing is irrelevant. Because much experimental evidence from chemically- and radiation-induced tumours [B48, E3, E4, F8] pointed to an increase in latency with decreasing dose rate, this particular feature was also incorporated into the model [M9, M10].

115. The result of the analysis was that in all cases, assuming a linear relationship between each increment of dose and risk (the latter distributed in time), non-linear (concave upwards) dose-response relationships were obtained, often with an apparent threshold. This is due to the inverse relation assumed between time of tumour appearance and the dose. According to this model, therefore, in most situations a linear extrapolation of the risk from high to low doses and dose rates is likely to yield a conservative risk estimate. The dose-response relationships should tend to linearity for: (a) long observation times, compared with the median latent period; (b) very heterogeneous populations, i.e., populations with a wide distribution of the latent period after single irradiation; (c) little variation of the median tumour appearance time with the dose. In general, however, the theoretical analysis of Mayneord and Clarke [M10] does not support an overall linear relationship between dose and cumulative tumour rate over finite time intervals in populations having a standard age distribution.

116. Inverse relationships between the mean latent period (from the start of exposure to tumour diagnosis) and dose (or dose rate) are seen mostly for irradiations of long duration, for instance in dogs and mice injected with $^{226}$Ra and $^{90}$Sr [M33, R28, R32]; in Chinese hamsters, in which the latent period of liver tumours decreases with increasing activity of injected $^{239}$Pu [B87]; in rats injected with thorotrast, which produces tumours of liver and spleen [W15]; or rats exposed over periods of weeks or months to various concentrations of radon resulting in lung carcinomas [C34]. Finally, a negative correlation of latency versus mean dose rate was seen in human dial painters with $^{226}$Ra and $^{228}$Ra body burdens [E3, E4, M14, M11, R18]. It is interesting to note that the inverse dose-latency relationship was much less pronounced for induction of bone sarcomas after single injections of short-lived $^{226}$Ra in rats [M67]. For brief exposures such as for atomic bomb survivors, there is, at least in the younger cohorts, a suggestion that the mean induction period for leukaemia is negatively correlated with dose [L32], although the reference in question seems to compare mostly radiogenic cases with mostly naturally occurring ones. Such a relationship was not detected for breast, stomach and lung cancer in atomic bomb survivors [B17, L32, M17, K39, T12] and for breast and thyroid cancers resulting from medical irradiation [B26, S16, S38]. Moreover, of all cases showing an inverse relation of the latent period against dose, an unequivocal departure from linearity was found only for $^{90}$Sr-induced bone sarcomas. The situation for human osteosarcomas induced by $^{226}$Ra and $^{228}$Ra is not totally clear, and the dose-response relation for leukaemia in atomic bomb survivors is still not available. In conclusion, the inverse relationship between latency and dose does not apply to many tumours. Thus, an upward concavity of the dose-response relationship as a result of this factor alone cannot be viewed as a general phenomenon.

2. The linear-quadratic model

117. For the bulk of experiments on single-cell systems, and for many end-points, the prevailing form of the dose-response curve after acute low-LET irradiation is concave upward. The response rising with a power of dose of between 1 and 2. Broadly similar shapes were also observed for tumours induced by the same radiation. This form of the response is thought to imply that for the occurrence of the final effect the interaction of two elementary effects, usually referred to as sublesions [K4], is required. Repair phenomena and dose-rate effects are usually accounted for by the interaction of these sublesions in time and space [L4, S39, S40], but other interpretations were also proposed [G29, G32]. For effects showing curvilinear responses to low-LET irradiation, linear dose-responses are frequently observed with densely-ionizing particles,

(a) The microdosimetric approach

118. Microdosimetric considerations are important because the distribution of absorbed energy divided by the mass in the volumes believed to be the primary targets of radiation action (i.e., cells or cell nuclei) is very inhomogeneous at low and intermediate doses [F7]. Thus the distribution of specific energy, z (energy absorbed divided by the mass of these volumes), may be more relevant for the analysis of the effects than the organ or tissue mean dose, D.

119. Many experiments indicate that for a number of effects the probability of occurrence, E, after low-LET radiation, rises with a power of dose close to 2. This may be accounted for by the theory of dual radiation action which assumes that the induction of two non-specified sublesions is necessary for the production of the effect (lesion). In the approximation that was first developed in detail [K4], it was assumed that the probability of sublesion interaction does not depend

185
on their distance within a certain volume (the site). Consequently, the probability of effect should rise in proportion to the mean of the square of the specific energy, i.e., \( z^2 \) in the site. It was shown also that \( z^2 \) is a function of the absorbed dose, \( D \):

\[
z^2 = \zeta D + D^2
\]

(3.3)

where the \( \zeta \) is a weighted average of the specific energy deposited by single events (energy depositions in the critical structures by individual charged particles). Thus, the probability of a radiobiological effect, \( E \), should be

\[
E = K(\zeta D + D^2)
\]

(3.4)

The quantity \( \zeta \) has the dimension of dose and its value denotes the dose at which the contribution of the linear equals that of the quadratic term. For x and gamma rays, and for sites of 1 \( \mu \)m diameter, the value for \( \zeta \) falls within the range of 0.3-0.8 Gy; for medium-energy neutrons, it is as large as 16 Gy. For smaller sites, the values of \( \zeta \) are considerably larger, but the ratio of the values for neutrons and for sparsely ionizing radiation is less dependent on site size.

120. Consideration of the values of \( \zeta \) for radiations with different values of LET and their comparison with biological data, indicates that intracellular distances at which sublesions can interact must be of the order of 0.1 to 1 \( \mu \)m [K4]. High values of \( \zeta \) for neutrons and alpha particles (relative to x and gamma rays) account for a bulk of radiobiological observations. They show that the RBE of high-LET radiations varies as an inverse function of the square root of their dose and this can be understood on the basis of microdosimetric concepts. This type of dependence can hold down to doses where the response to both radiations becomes linear and the RBE reaches a plateau.

121. Suitable time functions can be inserted into the basic equation (3.4) to describe the interaction probability during the time interval between the formation of two sublesions [R12]. One of the conclusions is that the linear term for single events in equation (3.4) should be independent of dose rate. Therefore, any effect of time distribution may only result in a modification of the quadratic term. This relationship between irradiation time, \( T \), and mean time between induction of a sublesion and its repair, \( \tau \), is reflected by a reduction factor, \( G \), that affects the quadratic term:

\[
G = 2(\tau/T)^2(T/\tau - 1 + e^{-\tau/T})
\]

(3.5)

In the presence of recovery, the curves of RBE versus absorbed dose do not change in shape, but are shifted towards higher doses by a factor depending on the dose distribution in time and the recovery time, \( \tau \). For a constant dose rate, if the exposure time is much longer than the recovery time, the yield of elementary lesions becomes simply proportional (linear) to dose.

122. The theory of dual radiation action has been applied by several authors to the interpretation of biological results, for example to cell inactivation by ionizing radiation [B62, F7]. In this model, the slope of the survival curve depends explicitly on radiation quality through the quantity \( \zeta \) [K4]. The theory has also been used as a theoretical basis for the linear-quadratic model for many effects, including mutations, chromosome aberrations and initiation of oncogenic transformation. However, experiments with ultrasonic x rays [C23, G9, G10, G11] and with spatially correlated ions [B88, G31, G32, K41, R38] indicate a strong dependence of the interaction probability of the sublesions on distance, with a dominance of short-range interactions (much less than 0.1 \( \mu \)m) for densely ionizing radiation. The applicable version of the theory [K5] is a model of higher complexity that is less easily tested experimentally.

123. Alternative explanations were considered for the commonly observed RBE-dose and dose-response relationships for low- and high-LET radiation. In agreement with earlier ideas of Haynes [H27], Goodhead et al. [G29, G32] put forward the notion that a number of cellular and subcellular radiation effects depend both on the production of the primary lesions and on a dose-dependent inactivation of repair mechanisms. Whereas the probability of the primary lesions is taken to increase linearly with dose, impairment of the repair system would depend on absorbed dose and specific energy. It has been postulated that the degree of impairment of repair should display a pronounced dependence upon dose and/or dose rate of low-LET radiation. For high-LET particles there would normally be no error-free repair, independently of dose. Therefore, basically linear dose-responses would be expected for induction at the cellular level of each of the various effects separately.

124. The above concepts have not been widely tested. A variety of factors have been reported that may influence the expression of potential damage, and thus change linear dose-effect relationships to curvilinear ones, and vice versa [F18, H22, L26].

(b) Characteristics of the linear-quadratic model

125. Upward concave dose-response curves that fit well the cumulative cancer incidence as a function of acute doses of low-LET radiation are in themselves no proof that cancer is actually initiated in accordance with a linear-quadratic relationship. Curvilinearity could well result from an inverse relationship between dose and latent period [M10]. However, when follow-up data show a constant latent period with dose, it may be assumed, as a working hypothesis, that the upward concavity reflects a linear-quadratic kinetics of initiation.

126. A generalized linear-quadratic dose-response relationship for radiation-induced cancer would be as follows:

\[
l(D) = (a_0 + a_1D + a_2D^2)S(D)
\]

(3.6)

where \( l(D) \) is the incidence (in an ideal case corrected for intercurrent mortality); \( D \) is the dose; \( a_0 \) is the spontaneous incidence; \( a_1 \) and \( a_2 \) are coefficients for the linear and dose-squared terms of cancer initiation;
and $S(D)$ is the probability of survival of transformed cells and may be written as

$$S(D) = e^{-\psi D + \beta D} \quad (3.7)$$

In the equation (3.6), initiation may also be treated as a simplification of a more general expression, which includes some repair at low and intermediate doses and dose rates [B95].

127. To test compatibility of the model with experimental and epidemiological data, the parameters in equation (3.6) must be assigned some value. This can only be done by: (a) reasonable analogy with cellular radiobiological effects; or (b) deduction of the values from empirical studies. As to the parameters for cell survival, these are known for many cell lines (e.g., Barendsen [B5, B6, B9-B12, B45]; Goodhead et al. [C23, G9, G10]; Elkind [E2]; and others [A17, B19, B27, C1, C37, D3, D8, F2, F19, H2, H6, J6, K2, K24, L6, M16, M60, R39, S33, T18, T19, W6, W7]), but are not necessarily applicable to specific tumours.

128. The number of unknown coefficients in equations (3.6) and (3.7) is too large to permit their calculation without some constraint. One of these is that all parameters should be positive or equal to zero [C29]. However, this is often insufficient for reasonably stable estimates of $\psi$ and $\beta$, particularly when $\alpha_1$ and $\alpha_2$ are estimated at the same time. Attempts were made, therefore [B7, B47], to derive independently the quotient $\alpha_1/\alpha_2$, which facilitates calculations by lowering the degrees of freedom.

129. Assuming that the theory of dual radiation action [K4] applies to the initiation of cancer, there must be a subcellular structure in which the sublesions appear and an interaction distance below about 1 $\mu$m where the final lesion leading to malignant transformation is produced. Kellor and Rossi [K4-K7, R12] did not specify the relevant structure for cell transformation, but the most likely target of radiation action appears to be the genome. In the light of this assumption, and of available evidence, it is not unexpected that models postulating a linear-quadratic relationship presuppose a direct action of radiation on DNA or make reference to phenomena involving DNA (chromosome aberrations, mutations) for derivation of the relevant parameters.

130. Barendsen [B7] selected the values for $\alpha_1$ and $\alpha_2$ on the basis of dose-response relationships for chromosomal aberrations in lymphocytes and the rounded values of $\alpha_1/\alpha_2$ for x and gamma rays were set at 0.5 and 1.0 Gy, respectively. On a similar principle, Abrahamson [A3] and Brown [B47] assumed that, if human radiation-induced cancers are due to mutations or chromosome aberrations in single cells, then information on the quotient $\alpha_1/\alpha_2$ could be derived from extensive data available in the literature. However, the large variability of the quotient precluded the use of numbers at their face value (e.g., mean or median). Brown showed also an inverse relationship between the DNA content per haploid genome in cells of various species and the $\alpha_1/\alpha_2$ quotient, the values extending over ranges of $10^3$ and $10^4$, respectively.

131. For mammalian cells, $\alpha_1/\alpha_2$ values for mutations and chromosome aberrations, as reviewed by Brown [B47], clustered around 1 Gy (geometric mean, 1.27 Gy). The values of the $\beta_1/\beta_2$ ratio for cell sterilization were generally much higher, with a geometric mean of 7.76 Gy. The difference is due mainly to higher values of the linear parameter for cell killing, in accordance with conclusions [B9, B11] that at low doses there must be a substantial component in cell killing that cannot be identified with the induction of chromosomal aberrations. Brown concluded that if the initial radiation lesion triggering a human cancer is of mutational or cytogenetic nature, then risk estimates derived at 1-2 Gy of low-LET high-dose-rate exposure would, as a maximum, over-estimate the cancer risk at low doses and dose rates by factors of about 2 and about 4, respectively.

132. Concerning cell survival, it has been pointed out repeatedly [B7, E2, F2, K2, M21, S17] that the low-LET survival curves can be described by various models and, among others, by functions of the "single-target" plus "multi-target-single-hit" type

$$S(D) = e^{-\psi D} [1 - (1 - e^{-\psi D})^n] \quad (3.8)$$

where $S(D) = S_D/S_0$, $S_0$ and $S_D$ are mean inactivation doses for single and multiple targets, respectively, and $n$ is the target multiplicity. Alternatively, the linear-quadratic form may be applied, as in equation (3.6).

133. Whether for each cell type there is an initial slope [B1 in equation (3.7) and (e$^{-\psi D}$) in equation (3.8)] is still a matter of debate, but this question is rather immaterial in the present context as both functions describe satisfactorily the experimental data for surviving fractions between 1.0 and 0.1 [B9, B11, F2, M21]. Equation (3.6) has been most commonly used in relation to the linear-quadratic model of cancer induction (see [U6, annex G] and [B7, C29, M31]). For high-LET radiation the contribution of the quadratic term is negligible.

134. As a first approximation, for low and intermediate doses, particularly of low-LET radiation, the killing term $S(D)$ may be neglected. This could be justified under the assumption that repopulation of the target cells after irradiation has a promoting effect on the development of radiation-induced cancer roughly proportional to the degree of cell killing [P22]. If, in addition, some of the terms in equation (3.6) and (3.7) are omitted, a transition is possible from the linear-quadratic model to the pure linear or pure quadratic ones (Figure IV). As can be seen from equation (3.6), the model does not assume the presence of a threshold.

135. There are many methodological and biological factors [B10, C1, D8, H6, K24, L6], which make it difficult to select appropriate survival parameters. In particular, cells irradiated in situ might exhibit higher survival capacity than when irradiated in culture or in suspension [G14, H8, H10, M60] (however, for a contrary view, see [A17]). By using a range of survival parameters suitable to describe the most and least
sensitive cell lines, one may examine the degree to which cell killing may affect the shape of the tumour incidence data and, hence, the reliability of extrapolation from the intermediate to the low doses. This should provide some indication of the degree of over- or under-estimation of the risk, within the range of values selected for the parameters in question.

136. In an independent analysis, UNSCEAR selected two values of the $a_1/a_2$ quotient applying to x and gamma rays: 0.5 and 2.0 Gy. For survival, the bone marrow stem cell was selected as the most sensitive. Its survival curve is described by $\beta_1 = 4 \times 10^{-1} \text{ Gy}^{-1}$ and $\beta_2 = 8 \times 10^{-2} \text{ Gy}^{-2}$ [B6]. For the least sensitive cell, a hypothetical line was assumed with survival parameters $\beta_1 = 10^{-1} \text{ Gy}^{-1}$ and $\beta_2 = 8 \times 10^{-2} \text{ Gy}^{-2}$ [B6]. In addition, other survival parameters for radiation-induced human leukaemia cells (Table 1 from [U17]) were used, as follows: $\beta_1 = 3 \times 10^{-1} \text{ Gy}^{-1}$, and $\beta_2 = 10^{-1} \text{ Gy}^{-2}$. To normalize the calculated data, it was further assumed that the lifetime cumulative incidence at 3.0 Gy may be 15,000 cases per $10^6$ (5000 $10^{-6} \text{ Gy}^{-1}$). The results in terms of the yield of cases from $10^{-3}$ to 3 Gy for all combinations are plotted in Figure V.

137. From such an analysis, the following tentative conclusions may be drawn. First, the sensitivity to cell killing has a more pronounced effect on the shape of the dose-response relationships than the $a_1/a_2$ quotient. (Similar conclusions were reached by Mole [M31].) Secondly, for the cells most sensitive to killing, the

Figure V. Expected cumulative incidences of a radiation-induced cancer according to a linear-quadratic model with $a_1/a_2$ quotients = 0.5 and 2 Gy and cell survival functions $S_{\text{max}}$ and $S_{\text{min}}$, as given in paragraph 136. Incidence normalized to 15,000 cases per $10^6$ at 3 Gy. For full explanation, see paragraphs 136 and 137.
relationship is concave downward, with maxima at 2-2.5 Gy. Since such curves are not observed for human cancers after low-LET irradiation, it is possible that the assumed sensitivity is too high for in vivo irradiation. This would be in accordance with the lower sensitivity of single cells killed in situ, which is due mostly to a wider shoulder of the survival curve [D7, G14, H8, H10, M60, S26, S27]. Thirdly, for the cells least susceptible to killing (the carcinomatous cells according to Barendsen [B6, B7]), the overestimate of the tumour yield per unit dose from 1-2 Gy to 1-10 mGy (relative to linear extrapolation) ranges from 4.0-2.6 at $a_1/a_2 = 0.5$ Gy, to 1.6-1.3 at $a_1/a_2 = 2$ Gy, respectively. The maximum over-estimation of the risk will result from totally neglecting the cell-killing effect. In such a case, extrapolation from 2 and 1 Gy down to about 10 mGy would involve the following over-estimate:

\[
\begin{array}{ccc}
\text{Over-estimate by a factor of:} & \text{from 2 Gy} & \text{from 1 Gy} \\
(a_1/a_2) & 0.50 & 4 & 3 \\
2.00 & 2 & 1.5
\end{array}
\]

The above differences for $a_1/a_2$ of 0.5-2 Gy could not be easily—if at all—detected between estimates derived from 1 or 2 Gy and the exceptional estimates available for doses of a few tens of mGy per single dose. The lack of statistically significant difference is therefore compatible with both the linear or the linear-quadratic model.

138. Inspection of equations (3.6) and (3.7) indicates that cell killing may alter significantly the incidence of a given cancer through interaction with the spontaneous incidence, $a_0$. At very high levels of the latter and with high sensitivity of the cells to sterilization, a dose-related decrease of the incidence would be expected. This in fact was seen in experiments with animals, showing a negative slope of the dose-response relationships for the so-called reticulum cell sarcoma [M19].

139. The slope of the dose-response curve in the complete linear-quadratic model will be described by a differential:

\[
d\frac{dD}{dI(D)} = \left(\frac{a_1 + 2a_2D}{a_0 + a_1D + a_2D^2}\right) e^{-\theta_1D + \theta_2D^2}
\]

(3.9)

As $D$ approaches zero this equation simplifies to:

\[
d\frac{dD}{dI(D)} = a_1 + 2a_2D - \beta_1a_0 - D\beta_1a_1 + 2\beta_2a_0
\]

(3.10)

To examine the interaction with dose of $a_0$, $a_1$, and $\beta_1$ and its influence on the curve at low dose, two human cancers were selected: breast carcinoma, with a high spontaneous incidence and moderate sensitivity to cell killing of initiated cells; and leukaemia, with rather low values of $a_0$ and high sensitivity to sterilization. The notional parameters selected were:

**Breast cancer:**

- $a_0 = 8 \times 10^{-4}$ a$^{-1}$
- $a_1 = 3.2 \times 10^{-4}$ a$^{-1}$ Gy$^{-1}$
- $a_2 = 3.7 \times 10^{-4}$ a$^{-1}$ Gy$^{-2}$

- $\beta_1 = 2 \times 10^{-1}$ Gy$^{-1}$
- $\beta_2 = 8 \times 10^{-2}$ Gy$^{-2}$

**Leukaemia:**

- $a_0 = 5 \times 10^{-5}$ a$^{-1}$
- $a_1 = 1 \times 10^{-4}$ a$^{-1}$ Gy$^{-1}$
- $a_2 = 1 \times 10^{-4}$ a$^{-1}$ Gy$^{-2}$

- $\beta_1 = 4 \times 10^{-1}$ Gy$^{-1}$
- $\beta_2 = 8 \times 10^{-2}$ Gy$^{-2}$

When the values of $d/dD (D)$ were calculated at 1, 10 and 100 mGy, their ratios to the respective values of $a_1$ were:

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>0.01</th>
<th>0.01</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemia</td>
<td>0.80</td>
<td>0.82</td>
<td>0.94</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>0.50</td>
<td>0.52</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Thus, at very low doses interaction of cell killing, predominantly with the spontaneous incidence, would introduce some degree of over-estimation of the risk, if this interaction should be neglected in the extrapolation procedure. It appears unlikely, however, that this over-estimation might exceed another factor of 2, over that estimated in paragraph 137.

140. When extrapolation is made from intermediate or high doses of low-LET radiation delivered at low dose rates or in small fractions (of size much below $a_1/a_2$), then, due to repair processes, the quadratic terms of equation (3.6) should be reduced. Under these circumstances, dose-response relationships approaching linearity would be expected in which the risk coefficient per unit dose is the same over the range of low and intermediate doses. For high-LET radiation, the contribution of the dose-squared component for most effects in single cells is negligible up to a few tens of Gy. Therefore, the model may be treated as linear, although for some effects the slope of the line may not be dose-rate independent (see III.B.1). Such observations cannot be explained solely on biophysical considerations.

141. It has been pointed out [U17] that the linear-quadratic model of cancer initiation is a simplistic concept and not a pathogenetic theory. The great complexity and the interaction of various phenomena at the cellular level preclude its acceptance as a generalized theory of cancer initiation in all tissues and under all circumstances. The dose dependence of the many modifying mechanisms operating at the tissue and systemic level is still too obscure to allow quantitative predictions over the entire range of doses. However, as discussed in chapter II, it may be postulated that the influence of modifying factors might not be very important in the range of doses up to 1-2 Gy of low-LET radiation (or their high-LET equivalent). Under these circumstances, appropriate extrapolation may be acceptable down from the dose region of 1-2 Gy of x or gamma radiation (or an equivalent dose of neutrons) to obtain risk estimates for the low-dose domain.

142. With all these reservations in mind, the linear-quadratic model has been applied to the results of selected animal tumour experiments where dose, dose rate, dose fractionation, dose protraction and LET were systematically investigated (see chapter IV). In several cases [B7, P22, U2, U3, U25, Y11] the results were in basic agreement with the model's expectations.
3. The quadratic model

143. This model is based on the assumption that for the induction of a given effect two consecutive events are necessary, or two concurrent events separated in space in such a way that their production by a single ionization track is extremely unlikely. Except for irradiation of flattened bone lining cells (see V.E.2.), this assumption is difficult to justify on microdosimetric grounds for low-LET irradiation of normal cells in situ. In tumour radiobiology, this concept may be traced back to the theory of bone sarcoma induction by β emitters [M26, M45, M47]. Proportionality to the square of the dose has been noted for various cancers [H12, H13, M26, M52, R18]; this does not exclude the presence of a linear component, but simply assumes that it may neither be demonstrated nor ruled out on statistical grounds. Therefore, if present, such a component must be relatively small.

144. Recently, Mole et al. [M52] have shown that dose-response relationships for radiation-induced acute leukaemia in CBA/H mice may be characterized by an approximately pure quadratic or linear initiation term for x rays or neutrons, respectively (see IV.B.1). On the basis of microdosimetric considerations the apparent absence of a linear term in the dose-response equation for x rays is difficult to explain when the diameter of a nucleus is of the order of few μm. The proposed hypothesis [M52] was that the interaction of two neighbouring cells is necessary for the initiation of this cancer. Initiation by neutrons is in direct proportion to dose and this was explained by the fact that a single recoil track could cross the nuclei of two neighbouring cells. However, for neutrons of maximum effectiveness (400 keV) the range of the secondary protons is less than most nuclear diameters.

145. The experimental basis of these hypotheses is still debatable, and needs further experimental testing. Moreover, the postulates are difficult to reconcile with the results of transformation experiments of cells in vitro, irradiated at low density (see chapter IV) where it is unlikely that two neighbouring cells may be crossed by a single recoil track or may interact during oncogenic transformation by x rays. Explanations other than the microdosimetric ones must be found to accommodate all these facts.

146. When fitted to the experimental data obtained at intermediate or high doses, the quadratic model will predict effects at low doses to be much less than implied by the linear or the linear-quadratic models within the usual range of α₁ and α₂ values. The influence of the time pattern of dose administration cannot be specified a priori for the quadratic model; if repair is fast and dose-rate-dependent, dose progression or fractionation could lead to a considerable reduction of the effect, and zero effect could not be excluded.

147. The quadratic model has been used by the Committee on the Biological Effects of Ionizing Radiation (BEIR Committee) [C29] to obtain the lower boundary of likely linear-square dose responses at low doses. Three special models have recently been proposed which assume, or derive from experiments, the basic postulate of the dose-squared concept. They refer to leukaemia in CBA mice [M52], skin cancer in rats [V1] and bone sarcoma in man [M44]. They are reviewed in chapters IV and V, respectively.

C. SUMMARY AND CONCLUSIONS

148. Quantitative models of radiation-induced cancer must allow for two facts: first, that tumour incidence rises with dose at low doses; and, second, that at high doses the effect usually declines (either in absolute terms or per unit dose). This shape of the curve may be modelled by the product of two terms: an initiation term increasing as a function of dose, and a cell-survival term declining with it. The latter term can be neglected at low doses, but this simplification may lead to misinterpretations as the dose increases. There is no reason why any one model should be universally applicable to all radiation-induced types of cancer. Models should be tested in each instance by statistical criteria. In each given tumour system, they should allow for the effect of the main radiobiological variables, such as dose, dose rate, dose fractionation, radiation quality.

149. The linear model implies direct proportionality between dose and the probability of tumour induction. Absence of dose-rate and fractionation effects was thought to be an inherent feature of the linear model, because the biological lesion is assumed to result from a single-track effect. However, examples are known where this is not true, and linear dose-response relationships show shallower slopes with decreasing dose rate. There are few examples of cellular or carcinogenic effects following linear kinetics after low-LET irradiation, while the effects produced by high-LET particles often conform to these characteristics. However, there are examples where dose-rate effects for neutrons are found, in an opposite direction (enhancement) from those normally seen for low-LET exposure. The linear model may be regarded as an upper boundary for the more general linear-quadratic model when the doses at which the linear equals the dose-squared components are high or very high.

150. The model of Mayneord and Clarke assumes linearity between dose and cancer initiation at the cellular level. It also assumes a log-normal distribution of the latent periods for overt tumours and an inverse relationship of latency to dose. Solution of this model for observation times comparable to human life expectancy results in dose-response relationships which are upward concave, sometimes with apparent threshold. The critical assumption leading to such results is the shortening of the mean latent period with dose. If this were a general rule, then extrapolation of risk from high to low doses would always lead to over-estimates. However, for some radiation-induced tumours in man (thyroid, stomach, breast, lung), the latent period is not obviously dose-dependent; also, curvilinearity is not seen in several cases where there is an inverse relation of the latent period with dose. Thus, it is impossible to generalize the notion that just for this reason curvilinear responses would normally apply.

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151. When corrected for cell killing, numerous biological end-points induced by low-LET radiation in single cells show an exponential dependence on dose with exponents of between 1 and 2. On the contrary, the same end-points for high-LET radiation show essentially linear dose-responsive. Consequently, the RBE is an inverse function of dose. Low-LET radiation shows pronounced influences of dose rate and fractionation, in contrast with high-LET, for which such phenomena are seldom observed or are observed to a lesser extent. Most of these facts may be accounted for by microdosimetric considerations. They predict that, as a rule, linear-quadratic dose-responses should be expected, where the relative contribution of the two terms is comparable (for low-LET). This prediction is in accordance with numerous experimental data on single cells, and so are the effects of dose rate and dose fractionation that may be deduced from the kinetics of repair of the sublesions postulated in the linear-quadratic model.

152. Recent data on ultra-soft x rays, however, do not conform to the predictions of the theory of dual radiation action in its early approximate formulation. The disagreement could be reconciled in the generalized version of the theory [R88], assuming that some parameters of the biological effect previously taken to be invariable are actually subject to some variability: or that, in case of low-LET radiation, the rate of repair depends on dose and on dose rate. Which of these two alternatives is likely to apply remains to be answered.

153. The linear-quadratic model may be characterized by the quotient of the induction constants ($a_1/a_2$), which varies with the radiation quality and the specific biological effect. For high-LET particles, this quotient is so high that the contribution of the dose-squared term may normally be neglected. The model then becomes linear. For low-LET radiation (considering chromosomal exchanges, mutations and induction of some malignancies) the $a_1/a_2$ quotient is between 0.5 and 1.5 Gy. The over-estimation of the probability of effects at about 10 mGy from single-dose data at 1-2 Gy (acutely delivered) by linear (as opposed to linear-quadratic) extrapolation would vary from 1.5 to 3.0 for an assumed reasonable set of parameters.

154. Similar over-estimations would apply to the effects of low-LET radiation delivered at low dose rates, irrespective of the total doses below the level when cell killing becomes important. This would result from the disappearance of the quadratic component of the dose-response curves owing to repair of sublesions.

155. Introducing a cell-killing term into a linear-quadratic model leads, in general, to a lesser degree of risk over-estimation at low doses. The effect of such a term may be analysed by applying a range of experimental cell-survival parameters. Since the killing effect of a given dose might be less in vivo than in vitro, the over-estimation involved in the extrapolation to low doses and dose rates would probably fall between that obtained from typical cell survival parameters in vitro and that obtained by neglecting cell killing entirely.

156. For fission neutrons and alpha particles, the $a_1/a_2$ quotient is of the order of several tens of Gy and the microdosimetric linear-quadratic model predicts a linear curve, relatively independent of dose rate. At doses where departure from linearity becomes substantial, a significant under-estimation of the risk must result from linear extrapolation to zero doses. Fractionation or protraction of dose may result in enhancement of the effect even at low and intermediate doses, contrary to what is seen at intermediate doses for sparsely-ionizing radiation, where the effect decreases upon fractionation and protraction. These observations, related to high-LET particles, cannot be explained on biophysical grounds.

157. Dose-response curves after low-LET irradiation have been reported for some tumours that suggest either the absence of, or a very small, linear term. In such cases, the curve approaches the purely quadratic, indicating perhaps a very low probability of interaction of lesions in two targets crossed by a single ionizing track, or the presence of highly efficient dose-dependent repair processes. Quadratic models have been proposed for leukaemia in CBA mice and cancer of the skin and bone. They predict a very pronounced reduction of the effect by lowering the dose rate or by dose fractionation. Substantial over-estimation of the risk would derive in these cases from linear extrapolation to low doses and dose rates from high or intermediate single doses.

IV. DOSE-RESPONSE RELATIONSHIPS IN EXPERIMENTAL SYSTEMS

A. PHENOMENA OBSERVED AT THE CELLULAR AND SUBCELLULAR LEVEL

158. In recent years, there has been good progress in understanding the role of oncogenes, and of related phenomena at the macromolecular level, in the causation and development of cancer. The newest data are reviewed in annex A to the present report. This progress, however, has left a considerable gap in the understanding of how these phenomena may be linked to mechanisms of radiation carcinogenesis, and what are the relevant molecular targets [G34]. From qualitative analysis of the available information it appears that a rather wide spectrum of radiation interaction with genetic material should be considered.

159. The present section, therefore, discusses dose-response relationships for radiobiological effects, at the subcellular and cellular level, that might be relevant to the induction of malignancy. Some effects, such as in vitro cell transformation, are probably closely related to in vivo carcinogenesis; the relevance of others is not yet well established, but is not implausible. Among the latter effects are mutations, because there is strong evidence of involvement of the genome in both the process of malignant transformation and of cancerous growth (see II.A). Mutational events that could be linked with carcinogenesis may include point mutations, chromosomal deletions and
translocations, as well as transposition of the genetic material [B71, B72, C32, F14, S43, S56, Y3]. It has been postulated [F21] that “tumour specific” symmetrical chromosomal translocations may be particularly relevant for two reasons: first, there are cancers whose cells consistently show aberrations of this type (e.g., chronic myelogenous leukaemia, Burkitt’s lymphoma); second, this chromosomal aberration is compatible with the cell’s continuous ability to divide, a pre-condition for a transformed cell to develop into a cancerous clone.

1. Mutations in germ cells

160. The frequency of point mutations as related to dose in germ cells of male and female mice, and of other species, was reviewed in depth in annex H of the 1977 UNSCEAR report [U6] and in annex I of the 1982 UNSCEAR report [U24]. The most recent findings are dealt with in annex A to this report. In summary, there is an unquestionable effect of dose rate and fractionation upon specific locus mutations induced in germ cells of male and female mice by low-LET radiation. Interpretations of these findings differ considerably. Abrahamsson and Wolff [A2, A3] postulated that mutational lesions are both one- and two-track events, and tried to fit linear-quadratic equations to the data. The attempt was criticized by Russell [R24, R25, R26] who has long been advocating an alternative hypothesis, namely, that mutations themselves are single-track phenomena, and the apparent multiple-track events reflect damage and saturation of the repair processes at higher doses and dose rates. UNSCEAR reviewed both hypotheses in detail and, in the light of present evidence, accepted that of Russell as a more likely explanation.

2. Mutations in somatic cells

(a) The Tradescantia system

161. The data obtained on plant cells of the species Tradescantia are very valuable because: (a) mutations can be scored down to 3 mGy of x rays (and correspondingly lower doses of neutrons); (b) the amount of data on the effects of dose, dose rate and radiation quality is far better than for any other cellular system: (c) the effect is evidently a somatic mutation (although its molecular mechanism is not clear) and the underlying lesion is most likely chromosomal; and (d) the form of the dose-response curve is similar to that for dicentrics and deletions in mammalian T lymphocytes.

162. Dose-response relationships for somatic mutations in Tradescantia, induced by acute 250-kVp x rays, are plotted on a log-log scale in Figure VI [N2, S20, U19]. Below 0.1 Gy, the curve is linear, but then its slope increases up to 1 Gy, where a dose-squared component sets in, leading to an upward concavity of the curve. Above 1-2 Gy, the curve bends and declines, reflecting, perhaps, preferential killing of the mutated cells (the effect is scored in terms of frequency per surviving cell). The data points below 1 Gy are well fitted by a non-threshold regression equation of the type \( L = a_1 D + a_2 D^2 \), where \( a_1 \) and \( a_2 \) are constants, presumably for single- and two-track events. Mean values of \( a_1 \) and \( a_2 \) deduced from three studies [S20, N2, U19] amounted to 5.1 (range 4.6-6.4) \( 10^{-2} \text{Gy}^{-1} \) and 6.5 (5.5-7.3) \( 10^{-2} \text{Gy}^{-2} \), respectively [N1]. In terms of the linear-quadratic model, the \( a_1/a_2 \) quotient for x rays varied from 0.33 to 0.83 Gy. For gamma rays, \( a_1 \) and \( a_2 \) are 2.1 \( 10^{-2} \text{Gy}^{-1} \) and 5.2 \( 10^{-2} \text{Gy}^{-2} \), respectively [N1]. Studies with monoenergetic neutrons (0.065-13.4 MeV) produced a linear dose-response up to 0.05-0.1 Gy [S19, U13] of the form \( L = a_1 D \). At high and intermediate doses, the RBE of neutrons versus x rays was inversely proportional to the square root of the neutron dose.

163. Reduction of the neutron dose rate had almost no effect upon the yield of mutations [N2, U13]. When the x-ray dose rate was reduced from about 1 Gy min\(^{-1}\) to 0.5 Gy min\(^{-1}\), the frequency of mutations was an increasingly linear function of the dose, approaching a linear response of the form \( L = a_1 D \). When the dose rate of x or gamma rays was progressively lowered [M18], and a constant dose of 0.8 Gy delivered over increasing time intervals, lower mutation frequencies were observed approaching asymptotically the limiting value of the respective \( a_1 D \) for gamma rays. Fractionation of an x-ray dose of 0.6 Gy into two acute fractions of 0.3 Gy each [M18] produced no reduction of the mutation yield up to a 15-minute fractionation interval, but the yield declined with further spacing of the fractions up to 24 hours. Splitting an x-ray dose of 0.05 Gy into two 0.025 Gy fractions given at 5 hours from each other did not reduce the response any further [U27].

164. The influence of fractionation of two x-ray doses (0.7 and 4 Gy) was studied upon induction and repair of two types of somatic mutations (pink and colourless) in this system [W16]. The intervals between
the two fractions varied from 0.5 to 12 hours. Recovery for the two types of mutations differed. in that pink mutations showed recovery at both doses, whereas colourless events showed some recovery at lower doses, but an increased effect at higher doses. Thus, two types of mutations induced by x rays in the same system may have different dose and time kinetics.

165. In summary, the effects of dose, dose rate, fractionation and quality of radiation upon Tradescantia mutations provide, in general, a good example of the linear-quadratic model of radiation action.

(b) Mammalian somatic cells

166. In recent years, significant progress has been made in the methodology of studies of point mutations in somatic cells in culture. Enzyme deficiencies are induced by mutagenic agents, and mutant cells are screened for resistance of developing clones to substances that are toxic to normal cells, e.g., to 8-azaguanine, 6-mercaptopurine or 6-thioguanine in hypoxanthine-guanine-phosphoribosyltransferase (HG-PRT) deficient mutants. Biochemical mechanisms and methods of investigation were reviewed in detail in annex H of the 1977 UNSCEAR report [U6] and in annex I of the 1982 UNSCEAR report [U24] and are discussed again in annex A to the present report.

167. In summary, both linear and curvilinear (concave upwards) relationships have been observed for induction of HG-PRT deficiency in various cell lines by sparsely-ionizing x or gamma rays, and the character of the dose-response curve is evidently related to the genome of the cell studied and not only to the quality of radiation applied [A13, A14, B42, C10, C23, C24, E8, K16]. In cases of curvilinear dose-response relationships, after acute x or gamma irradiation there was also a pronounced reduction of the yield of mutations by dose fractionation. For densely-ionizing radiation (alpha particles, helium, boron and nitrogen ions), the dose-response relationship for induction of HG-RPT deficiency, both in human and hamster cells, was essentially linear [C20, T18]. Induction of forward mutations in Chinese hamster cells by x rays resulted in curvilinear dose-response relationships (concave upwards): moreover pronounced dose-rate effects were observed [S25].

168. Experiments on radiation-induced mutations resulting in HG-PRT deficiency show some correlation between the shape of the survival curve for low-LET radiation and the kinetics of induction of somatic mutations in the same cell lines. This observation has been generalized by Thacker et al. [T10, T9, T8], Munson and Goodhead [M36], Leenhouts and Chadwick [L3, C2] and Goodhead et al. [G29] on different radiations and cells in various stages of the cell cycle, in the sense that there is a relationship of the type \( \ln S = -K \cdot M \) where \( S \) is the cell survival (\( SP/S0 \)), and \( M \) the frequency of induced mutations. This correlation is corroborated by other studies (e.g., [R5]). Thacker [T8] has shown that it applies to exponentially growing cells of several species (man, hamster and mouse) with the same proportionality constant K. However, the mutation frequency per lethal lesion seems higher for high-LET radiation and lower for ultrasoft x rays [G34].

169. When Thacker et al. studied the same relationship [T19], by irradiating V-79-4 hamster cells grown to plateau phase without re-feeding (most cells in the G1 phase), they found a similar association of frequency of induced mutations with (log) survival. This was true both after high (1.7 Gy min\(^{-1}\)) and low dose rate (down to 3.4 mGy min\(^{-1}\)) gamma irradiation. There was considerable repair of sub-lethal and sub-mutational damage with decreasing dose rate. However, when the cells were held for 5 hours after irradiation, before trypsinization and seeding, the potentially lethal damage was effectively repaired, whereas that leading to mutation (HPGPRT locus) remained unaffected. Goodhead et al. [G29] interpreted all this to show that the average number of mutagenic lesions in a cell is proportional to the average number of lethal lesions induced by a given radiation. However, results on repair of the potentially lethal damage show that phenomena involved in cell killing and mutation induction can be separated.

170. This conclusion remains to be confirmed. However, if the correlation were generally valid, a linear relationship between frequency of induced mutations and dose of low-LET radiation would be exceptional, because exponential cell-survival curves for x and gamma rays are also exceptional [A17, B63, C9, C21-C23, F2, K21, N8, P7, P15, S33, W6, W7], sigmoid cell survival generally being the rule [B5, B9-B12, B19, B27, B45, C1, C37, D3, D8, E2, F1, F2, F19, H2, H6, K2, K24, L6, M16, M60, S33, T18, W6, W7]. In most cases studied after neutron and alpha-particle irradiation, the survival of diploid mammalian cells is, with few exceptions [H1, C20], a simple exponential function of dose [B5, B9-B12, B29, B45, J6, K2, K3, R39, T18].

3. Chromosome aberrations

(a) Translocations in germinal cells

171. As discussed in annex H of the 1977 UNSCEAR report [U6], the frequency of chromosomal translocations induced by x and gamma rays in spermatogonia, spermatids and oocytes shows a pronounced dose-rate and dose-fractionation effect, and data can be fitted to a linear-quadratic equation. On the other hand, neutrons produce essentially linear dose-reponse relationships without any significant protraction or fractionation effect. These conclusions have been confirmed by more recent data reviewed in annex I of the 1982 UNSCEAR report [U24] and in annex A to the present report.

(b) Somatic cells

172. Studies of chromosomal aberrations in human lymphocytes have been reviewed in the past by UNSCEAR (1969 report, annex C, [U9]), and by others [B15, L13, L12, D4]. In most cases, the yield of
easily identifiable dicentric chromosomes and acentric fragments, induced in G0 cells, was studied against the dose of various radiations. The asymmetric exchanges are not compatible with cell survival over many generations: however, the yield and kinetics of induction are accepted as representative of symmetrical chromosomal exchanges. The latter may be relevant to present considerations. The subject of dose-response relationships for induction of dicentrics has been thoroughly reviewed by Lloyd and Edwards [L36]. The review included re-calculation of the dose-response functions from original data (62 experiments) using a standardized maximum likelihood method for fitting a linear-quadratic equation. The main conclusions are:

a) For gamma rays and high-energy electrons, the value of \( a_1 \), in the region of 2.2-2.5 \( 10^{-2} \) dicentric per cell and gray, appears to be consistent with most observations;

b) For x rays (180-250 kVp), a representative value of \( a_1 \) of about 5 \( 10^{-2} \) dicentric per cell and gray seems to apply;

c) The \( a_2 \) coefficients for gamma rays, x rays and electrons show less variation than the individual reported values of \( a_1 \), and they cluster within the range of 4.8 \( 10^{-2} \) dicentric per cell and gray square;

d) For fission neutrons, no significant \( a_2 \) term was observed. Typical \( a_1 \) values would be 40-90 dicentric per cell and gray. The coefficient tends to increase with lowering of the particle energy, to a peak at about 350 keV (about 160 dicentric per cell and gray);

e) In several studies [K36, V11, Z1], the yield of chromosome aberrations in human lymphocytes was studied after doses well below 0.5 Gy of x rays. When the data on dicentrics from these studies were pooled [Z1], they fitted a linear function of dose passing through the origin, as would be expected from the values given above. The response for acentric fragments was similar, though less regular:

f) At neutron energies above 15 MeV, a significant value of \( a_2 \) appears:

g) For protons with energies > 7.4 MeV, the \( a_1 \) coefficients are very similar to those for x rays. The \( a_2 \) values are rather lower than for typical low-LET radiation:

h) For alpha particles, all dose-response relationships (excluding one study with possible methodological complications) are linear, but \( a_1 \) values scatter widely, owing probably to systematic dosimetric inaccuracies;

i) When the value of \( a_1 \) is correlated with LET, the coefficient increases with the latter above 5 keV/\( \mu \)m to a maximum at about 70 keV/\( \mu \)m, and then falls off sharply at still higher values of the LET. The \( a_2 \) value is roughly constant for low-LET radiation, but virtually absent for high-LET particles.

174. The RBE of neutrons versus x or gamma rays is inversely proportional to the square root of the neutron dose. The limiting value at low dose rates may be obtained from the ratios of the relevant \( a_1 \) coefficients.

175. This was also noted when whole blood was irradiated by adding tritiated water prior to initiation of blastic transformation. Doses between 0.2 and 4 Gy \( \beta \) radiation were delivered over 30 minutes or 24 hours. Whereas the dose-response curve for 30-minute exposures was practically the same as that after acute x rays, the response was close to linearity after longer exposures (the linear component remained unchanged while the quadratic one decreased by a factor of about 3 [P20]).

176. Similar relationships were obtained for dicentrics in the lymphocytes of all mammalian species studied so far (maromset [B41], Chinese hamster [B41], rabbit [B1, B2, S5], potteroo [S5]. swine [B1, B41, L7], sheep [L7], goat [L7], mouse [B23, B41], rhesus monkey [B61], and other primates [H9, M37]). With protraction or fractionation of x- or gamma-ray doses, the linear term prevails [S4, L9]. With irradiation extending 24 hours the relationships for dicentrics after exposure to gamma rays tend to approach linearity.

177. For acentric fragments, a linear-quadratic relationship is usually observed, but the results are less reproducible [B2, L13]. As these aberrations include both interstitial and terminal deletions, classified together, the relationship does not contradict linearity for single-break aberrations.

178. In conclusion, induction of chromosomal aberrations of the one- and two-break type. In somatic cells in G0 or G1, is in broad agreement with the linear-quadratic model for low-LET and with the linear models for high-LET particles. This agreement refers to dose and to the effects of dose rate and fractionation. For single-break aberrations, a linear model should fit the data, but methodological problems make the demonstration difficult.

4. Oncogenic transformation of cells in vitro

(a) General

179. Studies of transformation from the normal to the oncogenic state, on cells in vitro, are very relevant
to the purposes of the present annex. They allow investigation of the mechanisms of cancer initiation without interference of other factors at the tissue and systemic levels. In addition, killing and transformation may be measured in the same target cells, allowing the relative importance of the two effects to be studied at the same time. Moreover, in vitro experiments are less expensive and time-consuming than studies on irradiated animals and are easier to analyse, owing to the lack of competing risks and the higher precision of measurements. On the other hand, lack of close intercellular contact and systemic regulation during culture growth is an artifact compared with the situation in situ and could change—at least quantitatively—the reactions of cells studied. Details of cell handling, such as whether trypsinization has been applied to the cultures, or not, in the course of the experiments, may also substantially modify the results. So far, observations on cell transformation have been limited almost exclusively to fibroblasts, whereas tumours of epithelial origin prevail among radiation-induced cancers in man.

(b) Dose-response relationships

180. Transformed cells are assessed by scoring characteristic colonies recovered in a culture. This directly measures the transformation frequency per surviving cell. Most experiments are reported in this way, but with some additional effort, information can be obtained on cell survival as a function of dose, and on the plating efficiency. The transformation yield per initial cell at risk may be calculated by correcting the transformation frequencies per surviving cell for the respective survival values and plating efficiency. This latter measure of transformation can seldom be computed from the frequencies per survivors reported in the papers, which often makes it difficult to give a precise description of the dose-response relationships. Many features of the dose-response relationships depend strongly upon the time interval between the establishment of a culture (seeding of cells) and irradiation: new and established cultures should therefore be dealt with separately.

(i) Irradiation of freshly established cultures

181. Borek and Hall [B33] x-irradiated early-passage golden hamster embryo cells in vitro, 24 hours after trypsinization and seeding, with doses from 0.01 to 6.0 Gy (a point at 0.003 Gy x rays was added later [B76]). A dose-response relationship per surviving cell is presented in Figure VII, showing a rise with dose up to 1 Gy, a plateau between 1.5 and 3.0 Gy, and a decline thereafter. The dose-response relationship from the same and subsequent experiments was also expressed per initial cell at risk [B29]. It is impossible to say whether a linear or an upwards concave line would be a better fit of the experimental points along the ascending portion of the curve. A linear fit was assumed by the authors themselves but a linear-quadratic fit was also attempted by Chadwick and Leenhouts [L3]. In a linear plot the line tends to pass through the origin, suggesting no threshold. Below 0.5 Gy of x rays and down to 0.01 Gy the yield of transformants appears to rise with a power of dose of 1. The transformation frequency per Gy of x rays below 1 Gy is of the order of $10^{-2}$ per cell, which is several orders of magnitude greater than for any somatic mutation [U6] but still an order of magnitude lower than the total yield of chromosomal aberrations induced by comparable doses of x rays in mammalian lymphocytes [D4]. In a comparison of 300-kVp x and $^{60}$Co gamma rays [B76] the RBE of the latter varied from 0.85 at about 1 Gy to 0.5 at a few tens of mGy.

182. Borek, Hall and Rossi [B29] and Borek and Hall [B79] irradiated hamster embryo cells with monoenergetic neutrons (0.43 MeV) and compared the dose-response relationship with that for x rays. The curves representing the relationships, both per surviving cell or per initial cell at risk, were peaked in shape and roughly parallel. A fit to the neutron data similar to that for x rays, but shifted toward lower doses, was obtained (Figure VII) with no suggestion of a threshold. Below 0.1 Gy, the transformation frequency could also be interpreted as rising with a power of dose lower than unity. The RBE of neutrons, at transformation frequencies of $10^{-3}$ and $5 \times 10^{-3}$, was about 12 and about 6, respectively. Measurable transformation was induced even by 1 mGy of neutrons. Accelerated argon ions (429 MeV per atomic mass unit) were roughly as effective as neutrons [B29].

183. Data were also obtained for cell survival [B29]. For x rays a sigmoid survival curve ($D_0 = 1.47$ Gy; $n = 6$), and for 430-keV neutrons a purely exponential curve ($D_0 = 0.5$ Gy), were reported. The RBE of neutrons varied approximately with the inverse square.
root of the neutron dose, with values from about 30 at doses below 0.1 Gy to about 5 at 1.5 Gy. At doses above 2-3 Gy of x rays, cell killing was appreciable, but the decline of transformation yield per unit dose above 1 Gy of x rays and 0.25 Gy of neutrons was explained by assuming that transformed cells were preferentially killed by radiation. An alternative explanation of this phenomenon was also proposed [C7, L3].

184. The dose-response relationship for x-ray-induced transformation of C3H10T1/2 cells irradiated 24 hours after seeding was also studied [T7] and expressed per surviving cell (at cell densities below 3.8 cell cm$^{-2}$; 100-kV x rays, 0 to 15 Gy). The transformation frequency rose rapidly with dose, up to 4 Gy, and reached a plateau at about 15 Gy. At about 1 Gy, the transformation frequency was about 0.5 $10^{-4}$, roughly 2 orders of magnitude lower than for hamster embryo cells [B33]. The doses required to double the transformation yield along the ascending branch of the curves for these two types of cells were 1.0 and 0.1 Gy, respectively. The dose-response curve for C3H10T1/2 cells showed no apparent threshold and was concave upwards, the effect rising with a power of dose $>$ 1 and close to 2.

185. In a later study, Miller et al. [M23, M41] examined, under a similar experimental protocol in the same C3H10T1/2 cells, the effect of single and split doses of 300-kVp x rays. The single-dose response curve had a complex, curvilinear shape. The transformation frequency was less than proportional to dose between 0.1 and 0.3 Gy, almost flat between 0.3 and about 1 Gy, and then rising with the square, or higher, power of dose. A plot of the data at 0.1-10 Gy on a log-log scale, per surviving cell, is given in Figure VIII. Interpretation of the shape of this dose-

response relationship is very difficult, particularly in the dose range below 1 Gy.

186. Yang, also, studied the transformation of C3H10T1/2 cells by x rays and energetic silicon ions (initial energy 320 MeV per atomic mass unit. residual range in water 4.5 cm, dose average LET = 88 keV/μm) [Y5]. This radiation was more effective than 225 kVp x rays (RBE not given). Cells kept in a highly confluent state for some time after irradiation were able to repair part of the transformation damage induced by x rays, but not that induced by accelerated silicon ions.

187. When C3H10T1/2 cells were irradiated with neutrons (35-MeV $d^-$-Be), the results were as in Figure IX [B79]. The dose-response curve is shifted towards lower doses with a similar dose-independent plateau as for x rays. The RBE increased with decreasing dose and was similar for both transformation and cell killing.

188. Lloyd et al. [L15] studied transformation in C3H10T1/2 cells by irradiation with 5.6-MeV alpha particles, showing a higher effectiveness as compared with x rays (the relationship of irradiation- and seeding-time is not available). For alpha particles, the dose-response relationship is of a grossly curvilinear (concave upward) character, the effect rising with approximately the cube of the dose. When expressed per surviving cell, the maximum transformation rate was seen at about 2 Gy.

189. C3H10T1/2 cells were transformed by 100-kVp x rays, fast fission neutrons (mean energy 0.5 MeV, 8-20% gamma contribution) and cyclotron neutrons (mean energy 38 MeV, 8% gamma contribution). The dose ranges were: 1.5-11 Gy, 0.5-5 Gy, and 1.5-6 Gy, respectively [B89]. The cells were irradiated 36 hours after seeding. For fission neutrons, the response per survivor was most effective and the initial portion of the curve was only slightly concave upwards. X rays were least effective and 38-MeV neutrons produced curves very similar to those of x rays. For both radiations, the dose-response curves were grossly concave upwards at their ascending portions. A plateau of effect at high doses was reached for all radiations tested. It is worth mentioning that for each radiation the survival curves had pronounced shoulders, which were roughly proportional to the degree of curvilinearity of the dose-transformation curves. The RBE relative to x rays at the transformation frequency of $5 10^{-4}$ was 1.2 and 3.8 for 38-MeV and 0.5-MeV neutrons, respectively.

190. Transformation of BALB/3T3 cells by $^{238}$Pu alpha particles and x rays was studied by exposure of freshly seeded single cells or confluent cell monolayers [R39]. The dose ranges were 0.5-2.5 Gy and 0.5-5 Gy for alpha particles and x rays, respectively. When calculated per surviving cell the rise of transformation frequency with dose was exponential (steeper than proportional). However, when the results were expressed as transformants per exposed cell, for alpha particles the highest yield was induced already by a dose of 0.5 Gy, with a slight decline thereafter. This
Figure IX. Pooled data obtained for the fraction of transformants per surviving C3H10T1/2 cell following irradiation with x rays or neutrons. [B79]

may be attributed to highly efficient cell sterilization by alpha rays. A high effectiveness of the alpha particles, relative to x rays, was noted. The precise shapes of the curves at the lower end of the dose scale cannot be established. Little studied the transformation by x rays of freshly seeded cells (20 hours prior to exposure) of BALB/3T3 mouse [L10]. Although there was some indication of an upward concave curve, the statistical uncertainty of the data does not allow precise conclusions.

191. Golden Syrian hamster embryo fibroblasts, seeded about 12 hours before, were exposed for 17 hours to varying concentrations of methyl ³H-thymidine [L33]. The number of transformants per surviving cell (and mutants at HGPRI locus) showed a proportionality to the concentration of the substance. Tritiated uridine, which is not incorporated into DNA, was ineffective in inducing both oncogenic transformation and mutations.

192. BALB/3T3 embryo fibroblasts, synchronized in the S-phase, were incubated for 16 hours with the DNA precursors ³H-thymidine and ¹²⁵I-iododeoxyuridine (¹²⁵I-dUrd) [L34]. The incorporation of the radionuclides into cellular DNA was proportional to concentration in the culture medium. The transformation frequency per survivor, versus concentration of activity per cell, was linear in both cases—without suggestion of a threshold—up to a frequency of about 5 × 10⁻⁴. The ratio of the initial slopes of ¹²⁵I-dUrd versus ³H-thymidine dose-response curve was about 25. In the same experimental series, the transformation by x rays was somewhat irregular, with an approximately exponential increase of the transformation frequency with dose over the whole range of doses (0.5-6 Gy). The high effectiveness of ¹²⁵I-dUrd was explained by the release of a cluster of low energy Auger electrons (about 25 per decay), leading to a very high energy deposition in the vicinity of the DNA double strand.

(ii) Late irradiation of cultures

193. Dose-response relationships for in vitro transformation of cell line C3H10T1/2 by acutely delivered x rays (50-kVp, 0.18 mm Al filtration) and neutrons (fission, mean energy 0.85 MeV) were reported by Han and Elkind [H1]. In contrast with the experiments reviewed above, cells were irradiated 48 hours after seeding. When the transformation rate was expressed per initial cell at risk, the decline of the yield beyond the maximum had the same slope (or D₀) as for the killing of non-transformed cells, suggesting that sterilization by high doses of normal or transformed cells was similar. On linear co-ordinates (Figure X), the ascending portions of the dose-response curves (per initial cell at risk) for x rays was concave upwards (at least above 1.5-2 Gy) but details cannot be established from the graphs. For neutrons, the ascending portion of the curve appears to be close to linearity. The RBE of neutrons increased with declining doses, and the maximum attainable transformation frequency by neutrons was higher by about 50% than that for gamma rays (per initial cell at risk). In contrast with the data of Miller et al. [M23] (Figure VIII) there was a significant flattening of the yield above 4 Gy of x rays, when the data were expressed per surviving cell [H14] (see Figure XI).

194. Watanabe et al. [W18] irradiated golden hamster embryo cells 72 hours after establishing the culture. The exposures varied from 50 to 600 R at exposure rates of 5, 75 and 600 R/min. Additional exposures (1-50 R) were applied at the dose rate of 5 R/min. The
Terasima et al. [24] studied C3H10T1/2 cells, x-irradiated (0.23 to 9.3 Gy) in the plateau phase of growth (contact inhibited). The dose response (per surviving cell) was very similar to that found by others for the same cell line. After a steep exponential increase, up to about 1.8 Gy, there was a gradually slower increase of the transformation frequency with a plateau above 6-8 Gy. Recent data on in vitro transformation of C3H10T1/2 cells by 31-MeV protons were published by Bettega et al. [B3], showing that the transformation frequency per surviving cell had a marked change in slope at about 2 Gy.

(c) Effects of dose fractionation and protraction; repair of sub-transformation and potential transformation damage

(i) Irradiation of freshly established cultures

196. Irradiation of cultures seeded 24 hours before exposure with two doses of 4.5 + 4.5 Gy of x rays at various intervals (zero to five hours) showed that survival of C3H10T1/2 fibroblasts nearly doubled when the interval exceeded 2 hours, with no further increase for longer intervals [T4]. For doses of 1.5, 3.0 and 8.0 Gy in two equal fractions at five-hour intervals, the transformation frequency per surviving cell remained unaltered at the lowest dose, but declined significantly (p = 0.005) at the two higher doses.

197. Hamster embryo cells in culture (seeded 24 hours earlier) showed a doubling of the effect after split, as opposed to single, doses of 0.5 or 0.75 Gy [B90]. At the same time, the survival of irradiated cells increased only marginally. When the experiment was extended [B64] by studying the effect on the transformation frequencies of splitting 1.5, 3 and 6 Gy of x rays, no enhancing effect of \( \times 0.75 \) Gy, as opposed to \( 1 \times 1.5 \) Gy, was noted. Fractionation of 3 and 6 Gy led to a reduction of the total effect.

198. Miller and Hall [M23] studied dose fractionation on C3H10T1/2 fibroblasts, plated 24 hours before x-ray treatments (0.3 to 8.0 Gy). Their results confirmed the sparing effects of splitting the dose above 1.5 Gy (with a possibly incidental deviation of the points at 4 Gy) and an enhancement of transformation below that dose. The experiment was repeated [M41], and the dose range expanded to include 0.1 and 10 Gy. The results are presented in Figure VIII. In addition, a dose of 1 Gy was split into 2, 3 or 4 equal fractions [H21]. An almost proportional (2-, 3- and 4-fold) enhancement of transformation was observed. Also, when a dose of 1 Gy of \(^{60}\)Co gamma rays was delivered at a low dose rate (over 6 hours) the effect was significantly enhanced, by a factor of about 3, by comparison with acute exposure (over 10 min) [H21]. In conclusion, using this experimental protocol in the range 0.3-1 Gy there is an enhancement of the effect by splitting the dose into two equal fractions within 5 hours or by protracting the dose over a similar interval. At 2 Gy, there is no effect. There is a decline of the yield after fractionation at higher doses. Approximately the same trend is seen when the data are expressed per initial cell at risk.
199. Little [L10] reported dose-fractionation experiments on BALB/3T3 cells seeded 24 hours before the first exposure with essentially identical results. The fractionated doses ranged from 0.1 to 3.5 Gy, and similar observations were also made in still another study by Suzuki et al. [S44]. It may be concluded, therefore, that enhanced transformation (per survivor) below 1.5 Gy of x rays after fractionation within 5 hours is a phenomenon observable in all cell lines studied so far, irradiated in freshly established cultures. Such an effect might be deduced from the curves in Figure VIII [M23]. In fact, if an independent action of the two dose fractions is assumed, the decrease above 2 Gy, where the response rises with D^2 or with a higher exponent, is easily understood. Similarly, below 1 Gy, over the plateau, where the effect is roughly independent of dose, fractionation should increase the yield, as observed.

200. Figures VIII and IX show clearly that a linear extrapolation down from intermediate (1 Gy) and high (2-3 Gy) doses of x rays would lead to an underestimate of the real transformation frequency, particularly with respect to the effect of split doses in the low-dose region. The greatest underestimate, by a factor of 4-6, would be encountered when the effect of single doses in the range 1-3 Gy is linearly extrapolated to predict effects of split total doses of 0.1-0.3 Gy. The relevance of this finding to tumour induction in vivo has been commented upon by several authors [B76, H21, M41].

(ii) Late irradiation of cultures

201. Data on fractionation were reported by Han and Elkind [H1] for x rays (0.75 Gy) and fission neutrons (3.8 Gy) on C3H 10 T1/2 cells exposed 48 hours after seeding. The transformation rate after doses of this magnitude was on the declining part of the curve when expressed per initial cell at risk. Fractionation of the neutron dose within 0 to 16 hours had little, if any, effect on single-dose survival and caused a slight reduction of the transformation yield, perhaps within the limits of experimental error. With x-ray fractionation times up to 5 and 10 hours, the frequency increased and then declined to the level of the nonfractionated exposure at about 16 hours. The increase was within a factor of 2 and its statistical significance uncertain. When expressed per survivor, the transformation frequency after x irradiation declined steadily to a plateau at 12-16 hours fractionation interval, reflecting essentially an expected and considerable effect upon survival of the non-transformed cells. This observation, therefore, confirmed other findings [M23, M41] that fractionation of high x-ray doses in this cell line reduces the transformation frequency when expressed per surviving cell.

202. Han and Elkind also irradiated C3H 10 T1/2 cells, 48 hours after plating, with 60Co gamma rays at high (1.0 Gy min^-1) and low (0.05 Gy min^-1) dose rate [H14]. The range of doses varied between 0.2 and 13 Gy. The transformation frequency per survivor (Figure XI) was consistently reduced at the lower dose rate, as was cell sterilization. The reduction in transformation yield was by a factor of about 5 above 6 Gy and by a factor of about 3 at 2 Gy, and was still present at doses of 0.5 and 0.2 Gy [E9, H26].

203. Hill et al. [H30] studied the effects of gammaray fractionation in the same system. Throughout the dose range tested (0.25-3 Gy), fractionation of the dose into 5 daily fractions resulted in a significant reduction of transformation frequency. Up to 1.5 Gy delivered unfractonated, and up to 3 Gy fractionated regimes, the dose response could be fitted by a straight line passing through the origin (Figure XII). The yield of transformants per unit dose was 3 times lower after fractionated than after single doses, and very similar to the dose-response curve for the lower dose-rate exposure referred to above [E9, H26]. Increasing the fractionation intervals beyond 24 hours, and reducing the dose per fraction, led to a further slight reduction of the effect. This suggested that there may be a limit beyond which further dose protraction cannot reduce the transformation frequency. Trypsinization per se, or delivery of the dose within 7 days of growth in culture, did not affect the yield of transformants per unit dose, provided they took place at least 40 hours after seeding. Confluence of the cells was excluded as a possible reason for the observed fractionation effect. These results of dose fractionation and protraction below 1.5 Gy are in striking contrast with those observed when the cells were irradiated 24 hours after seeding [H21, M23]. The difference was attributed to atypical conditions of cellular growth early after plating and to the action of radiation on para-synchronous cells [H30].

204. Watanabe et al. [W18] irradiated golden hamster embryo cells, sub-cultured 72 hours earlier, with x rays
at various exposure rates: 5, 75 and 600 R/min. Throughout the whole range of exposures tested (50-600 R), the lower dose rates produced a reduced yield of transformed cells, even within the 50-100 R range where the dose-response relationship was practically linear. At equal survival levels (which were inversely related to dose rate), the transformation frequency was higher in cells irradiated at higher than at lower dose rates. Terasima et al. [T24] studied the effect of fractionating x-ray doses (0.93, 1.86 and 3.72 Gy) in C3H10T1/2 cells irradiated in the plateau phase. Equal fractions were spaced at intervals of 3, 10 and 15 hours. There was significant reduction of the yield at all doses tested, as compared with single doses, the maximum reduction being achieved already at the 3-hour interval.

205. Hill et al. [H26, H34] worked with fission-spectrum neutrons (mean energy 0.85 MeV) at various dose rates in C3H10T1/2 cells irradiated 48 hours after seeding. There was no discernible effect upon cell survival of neutrons delivered at a low rate (0.43-0.86 mGy min⁻¹) versus a high rate (0.103-0.38 Gy min⁻¹). At the same time, the low-rate neutron irradiation enhanced significantly the transformation frequency at doses below 1 Gy (by a factor of about 9 at 0.025-0.1 Gy) (Figure XIII), an effect that cannot be explained on biophysical grounds. Above doses of 1.5-2.0 Gy, the effect of the dose rate disappeared and dose-response relationships converged to a plateau of $5 \times 10^{-3}$ transformants per surviving cell.

206. The influence of dose fractionation (5 fractions over 4 days) of fission-spectrum neutrons, delivered at a rate of 0.1 Gy min⁻¹, was studied by the same authors. Over the whole range from 0.103 to 1.12 Gy, an enhancement of effects by fractionation was observed. The ratio of linear slopes of the dose-response curves at low doses was about 8. The increased effectiveness of the low dose rate and of the fractionated neutron irradiation was explained either by the induction of more efficient error-prone repair or by facilitation of the expression of "sub-effective transformation damage", whatever this means [H37].

A recent study on transformed mammary epithelial cells transplanted in vivo to virgin female BALB/c mice suggests that the effect of fractionated neutron exposures may be analogous to chemical promotion and is due to an enhanced expression of the transformation damage [U28]. The effects of fractionation schemes applied to the cells in an asynchronous growth indicate that x and gamma iradiation—by analogy with sublethal damage—result in error-free, repairable, sub-transformation damage. The transformation damage induced by neutrons either does not undergo repair, or is subject to error-prone repair [E10, H37]; however, why the latter should be more effective at protracted or fractionated low doses is not clear.

207. Terzaghi and Little [T5] found that irradiating C3H10T1/2 cells with x rays in a density-inhibited state, and delaying plating up to 48 hours substantially increased survival rates. Delay for 2 to 4 hours caused marked increase of the transformation rate. Further delay of plating reduced the yield of transformants to very low levels after 48 hours. Terasima et al. [T24], however, did not observe any initial increase of the transformation frequency, as the potential transformation damage fell over the first 3 hours of delayed plating. The rate of potential transformation repair in C3H10T1/2 cells depended strongly on a particular batch of fetal calf serum used in the cell culture medium, both before and after release from confluence [T11]. Lack of serum was accompanied by a high degree of repair (80% over 6 hours) after keeping the irradiated cells (x rays, 3.7 Gy) in the non-proliferating state. Some batches of serum totally prevented the occurrence of repair. No repair of potential transformation could be found after irradiation with alpha particles [R39] and accelerated heavy ions [Y5].

208. When potentially lethal damage was studied by holding these cells in confluence after irradiation for varying lengths of time, a pronounced repair was observed for x rays and none for alpha particles [R39].

209. The results of other experiments [L23] suggest that two separate processes may be involved in the recovery of damage: first, DNA repair leading to enhanced survival; and, second, a slower error-prone repair process responsible for changes in DNA and leading to mutation and transformation. It should be stressed that the frequencies of transformation reported for most in vitro experiments apply to actively proliferating cell populations and may not be representative of situations where the overwhelming majority of cells is in the resting state, as in most organs in vivo.
(d) Other factors affecting oncogenic transformation in vitro

210. Numerous factors affect the yield of in vitro oncogenic transformation. Among these, a basic role is played by both the time and the density at which cells are seeded.

211. Studies on three cell lines (hamster embryo, C3H10T1/2 mouse embryo and mouse BALB/3T3 cells), have shown [B34, K12, L10, T6] that several post-irradiation cell divisions—4 to 6, depending on the cell line studied—are necessary for expression or fixation of the transformed state. Of these, at least one must take place in the first 24 hours post-irradiation.

212. Terzaghi and Little [T7] investigated the influence of cell density on the transformation frequency per surviving cell. Above 400 viable cells per dish (about 5 cell cm⁻²), there was a steep decline from plateau values of transformation observed at lower cell densities. The authors suggested that this effect could be the result of an incomplete expression of transformation due to early confluence, which lowers the number of cell divisions during exponential growth below that necessary for full expression of transformation [B71, B72, L26]. Han and Elkind observed, in the same cell line, a similar relationship between the density of seeded cells and the transformation rate after x-ray and neutron irradiation [H1]. Direct cellular contact cannot operate at such cell distances, but a diffusible mediator substance might prevent occurrence or growth of the transformed clones [L15].

213. Lloyd et al. [L16] experimented on co-cultivation of transformed C3H10T1/2 cells with non-transformed ones. When transformed cells were seeded at low densities, together with normal cells, at some ratios of the two cell types (1:50 to 1:500, respectively), expression of the malignant state could be completely prevented. Adding non-transformed cells in greater numbers again elicited this response. The experiment points clearly to a very complex nature of the interaction between transformed and non-transformed cells, even in vitro.

214. The data by Kennedy et al. [K26] are of importance because they throw some light on the complexity of the phenomena leading to oncogenic cell transformation in vitro and could modify substantially the current interpretation of this radiation effect. These authors studied the influence of re-suspending C3H10T1/2 cells after irradiation, once confluence was reached. When dilutions at the time of re-seeding were progressively increased, the number of originally irradiated and inoculated cells per plate decreased to very low values. In spite of that, after a constant dose of 4 Gy of x rays, the number of transformed foci per plate remained practically constant. This indicates that the number of transformed foci per plate is apparently independent of the number of cells initially irradiated. It is interesting to note that similar results were obtained when the effect of inoculum size was studied on the appearance of tumours, after in vitro irradiation of monodispersed thyroid or mammary cells injected into the fat pads of rats [G30]. These observations are in direct conflict with the expectation that—if initiation of a cell is a rare phenomenon—the probability of observing a tumour should rise in proportion to the size of the inoculum.

215. It has been suggested [K26, G30] that exposure to radiation results in some change in the cellular state in many or, after high doses, in all cells. Gould postulates that initiation is not an all-or-none phenomenon, but that its intensity rises with dose up to a saturation level, which is reached in C3H10T1/2 cells at 5-6 Gy after 60Co gamma irradiation or 3 Gy of neutrons. The change is probably transmitted to the whole progeny of surviving cells. The nature of initiation—genetic or epigenetic—is unknown, but the latter would presumably be suggested by these observations. This interpretation is not easily reconciled with the demonstrated direct involvement of DNA in the carcinogenic process [B14, L33]. Only a second rare phenomenon (perhaps mutation), would lead to a phenotypically recognizable transformation at confluence of C3H10T1/2 cells. The probability of this second phenomenon should be in some proportion with the degree of initiation.

216. A report by Hall et al. [H28] challenged the hypothesis that commitment of irradiated cells to the transformed state takes place at confluence. By re-plating cultures at different times after irradiation and testing for distribution of transformed foci among the plates, the authors could show that commitment takes place within 1 week of exposure. This observation is in agreement with a study [B91] on in vitro oncogenic transformation of C3H10T1/2 cells by 7β,8a-dihydroxy, 9a,10a-epoxy, 7,8,9,10-tetrahydrobenzo(a)-pyrene. The results of this study showed that acquisition of the ability to form transformed foci occurred within 2 days of exposure to a carcinogen. Hall’s experiments [H28] also showed that increasing the density of cells in culture probably suppressed expression of the transformed state. These results do not contradict the fact that at 4 Gy of x rays the probability of initiation per cell is close to 1, but they have been interpreted to challenge the postulated non-stochastic nature of initiation [K26].

217. The presence of thyroxin in the culture medium at a physiological concentration was shown to be a prerequisite for transformation to occur in Syrian hamster embryo and C3H10T1/2 cells [B73, G26, G27]. Thyroxin is required for protein synthesis only within a few hours of irradiation and its action can be reversed by an agent blocking protein synthesis, e.g., cycloheximide.

218. Other factors were shown to enhance transformation in culture during or after irradiation. Extensive investigations were made on a well-known promoter of in vivo carcinogenesis, the active ingredient of croton oil, 12-O-tetradecanoyl-phorbol-13 acetate (TPA). By adding TPA to the cultures within 96 hours of irradiation, Kennedy et al. [K10] showed enhancement of the x-ray transformation frequency (or its expression) in C3H10T1/2 mouse embryo cells.
The effect was particularly marked after doses of 0.5-1.0 Gy. These data indicate that promoting agents can increase the levels of x-ray-induced transformation in vitro, just as they enhance carcinogenesis in vivo. It was also shown [K27] that the most effective period of exposure to TPA is that of exponential growth. However, a delay of TPA application, e.g., addition to cultures re-seeded after confluence, did still lead to a significant enhancement of oncogenic transformation.

219. When TPA was added for six to seven weeks (at 0.1 µg/ml of medium) to cultures of C3H10T1/2 cells irradiated with graded doses of x rays (0.25-4.0 Gy), there was not only an absolute increase of transformation frequency, but also a change in the shape of the dose-response relationship, from curvilinear (upward concave) after x rays alone to linear (Figure XIV). The

![Graph](image)

**Figure XIV.** Influence of post-irradiation incubation with the tumour promoter TPA on x-ray transformation of mouse C3H10T1/2 cells. C x-irradiation only; Δ TPA (0.1 µg/ml) added for the entire period of expression. [L26]

action of TPA was especially pronounced at doses in the low and intermediate range [L26]. A very similar effect of TPA on the shape of the dose-response curves for x-ray-induced and neutron-induced transformation of C3H10T1/2 cells was also seen in another study [H22].

220. Numerous mechanisms of action for TPA have been advocated, including inhibition of the cell-to-cell communication [H22, T15]; enhancement of chromosomal rearrangements [K28]; production of short-lived radicals [B76]; and action via mechanism of cellular differentiation [B74, S43]. Increase of cellular proliferation is a phenomenon that often accompanies promotion [F14], but it is doubtful whether it is at all necessary, or sufficient [D13, K27]. The TPA promoting effect is apparently not related to any of the known DNA repair processes [K27].

221. Enhanced transformation of various cell lines was also noted after addition to the medium of the epidermal growth factor, a natural hormone-like polypeptide [F15]: bromodeoxyuridine [R30]; high concentrations of insulin [U29]; cortisone [K30]; and interferone [B46]. The particular batch of fetal calf serum used may also have a profound influence on the transformation frequency eventually observed [T11]. A new promoter, dihydrotestosterone, an antibiotic derivative recently discovered, has a promoting capacity per unit mass about 100 times higher than that of TPA [H31]. A synergism between x rays and a food pyrolysis product 3-amino-1-methyl-5H-pyrrolo-(4-3b)indol (Trp-P-2), itself a mutagen and carcinogen isolated from boiled meat and fish, has also been shown [B76] in Syrian hamster embryo cells. Hyperthermia (43°C for 60 minutes or 45°C for 15 minutes before irradiation) caused some increase of the x-ray-induced transformation frequency in C3H10T1/2 cells [C33]. The nature of most enhancing agents mentioned above suggests that the mechanisms of action are epigenetic rather than likely to cause structural changes in the genome itself.

222. There are also suppressing (inhibiting) factors. Kennedy and Little [K35] and Little [L26] described suppression by the protease inhibitors antipain and leupeptin. In other studies [B65, G28] it was shown that antipain added to cultures before irradiation enhanced the yield of transformants. While addition shortly after exposure reduced the transformation frequency, and addition 1 or 2 days later was ineffective. Inhibition of radiation-induced oncogenic transformation was also observed when inhibitors of poly(ADP-ribose) synthesis (benzamide, 3-amino-benzamide) were added to cultures of C3H10T1/2 and golden hamster embryo cells [B96].

223. An inhibition (by a factor of 3) of transformation in C3H10T1/2 cells was observed by adding 24 hours before exposure. A non-toxic derivative of vitamin A, trimethyl-methoxy-phenyl, analogue of N-ethyl retinamide (Ro-H-1430) and other retinoids [B73]. The retinoids irreversibly suppressed oncogenic transformation when present in culture for only a few days after irradiation. Later addition of TPA to the culture remained ineffective. The retinoids may act by suppressing the carcinogen-induced progression of the neoplastic process [B76].

224. Selenium compounds [B73], actinomycocin D [K31] and lymphotoxin [D19, E6, R44] also inhibit radiation-induced transformation. When superoxide dismutase was added to irradiated C3H10T1/2 cultures from irradiation to expression of transformation, the frequency of radiation-induced transformants was
greatly reduced, whether or not the drug was present during x-ray exposure \[M49\]. Quantitatively, the effects of oxygen and misonidazol upon oncogenic transformation and cell killing of C3H10T1/2 cells are very similar \[B97\].

(e) Conclusions

225. The oncogenic transformation in vitro of hamster embryo cells and several established mouse fibroblast lines by ionizing radiation shows numerous similarities in respect of dose-response relationships, effects of dose fractionation, and influence of cell density upon transformation frequency.

226. The mechanisms of transformation are still imperfectly understood and several methodological questions are not yet fully resolved. The subject has been thoroughly reviewed \[B71, B76, G34, L26, Y3\]. Transformation is probably a complex multi-stage phenomenon that includes initiation, progression and final expression of malignant foci. A number of promoting and suppressing factors may affect the frequency of transformation and therefore the shape of the dose-response relationships.

227. For these reasons, analysis of dose-response relationships for low- and high-LET radiation must, for the time being, remain descriptive. After single doses of x rays or neutrons, the dose-response curves have some features in common with those seen in vivo for many experimental tumours. For example, when expressed per initial cell at risk, transformation shows an initial rise of the frequency with dose, then the curve reaches a peak and the yield declines at still higher doses. The lower efficiency of gamma rays at the low, as opposed to high, dose rates in cultures established at least 40 hours before irradiation is also in accordance with most observations in vivo. The same applies to the RBE values of neutrons and alpha particles, which are definitely higher than unity and tend to decline with increasing dose, in agreement with the general understanding of cellular radiobiology \[B11, K7\]. Neutron dose fractionation upon oncogenic transformation in vitro produces effects that are similar to those seen recently in some—but not all—experiments in vivo. There are, however, differences, particularly when comparisons are made with cells plated shortly before irradiation. The most important is the rise of the transformation frequency with a power of dose less than 1 at intermediate doses \(<1.5\,\text{Gy}\). This shape of the curve may be linked with the enhancing effect of fractionation of x ray doses in the same region, a phenomenon observed in all cell lines tested so far.

228. If enhancement of transformation by fractionation applied in vivo, the complex nature of the low-LET dose-response relationship for single doses might imply an under-estimation of cancer induction when risk coefficients for man are extrapolated from the intermediate down to the lowest doses. Similarly, in the case of fractionated exposure, there might be an even greater under-estimation, owing to the enhancement produced by dose fractionation and protraction in some trans-

formation experiments with low-LET radiation. However, in the light of present knowledge—which is still insufficient—such conclusions are premature, for the following reasons:

(a) No dose-response relationship has been observed for tumours in animals with a plateau below 1 Gy after single acute doses of x or gamma rays. It is also quite clear that no enhancement of tumour induction by fractionation or protraction of external low-LET irradiation has ever been noted at doses below 1 Gy (with one possible exception, see IV.B);

(b) The dependence of the dose-response relationships on the length of the culture period before irradiation shows that the irregular shape of the dose-response curves and the enhancement of fractionation in freshly plated cultures result from irradiation of para-synchronous cells and may therefore be considered an artifact \[H37\];

(c) The effects of low-LET dose fractionation and protraction on established cell cultures are in general agreement with observations related to other effects at the cellular level (induction of mutations, chromosome aberrations, cell sterilization).

B. TUMOURS IN EXPERIMENTAL ANIMALS

229. In its 1977 report \[U6\], UNSCEAR reviewed experimental data on radiation-induced tumours, including an examination of the dose-response relationships then available. The essential conclusions were that the peculiarities of each tumour model were such as to prevent large generalizations. There were difficulties in interpreting tumour induction curves on the basis of simple mechanisms of action, in view of the complex interplay of primary and secondary contributing factors. With few exceptions, the data came from observations at doses above 0.5 Gy. Data were insufficient to define, unambiguously, dose-effect relationships in the low and sometimes also in the intermediate dose region.

230. With the exception of the mammary tumour of the Sprague-Dawley rat, dose-incidence curves obtained at high dose rates with low-LET radiation showed a slope that increased with increasing dose, not incompatible with a linear-quadratic trend. With high-LET radiation, on the other hand, dose-effect relationships tended to be more linear and their initial slopes showed relatively little change with dose rate and fractionation. However, with low-LET radiation, a decrease in dose rate led mostly to a decrease of the oncogenic effect, following some inverse function of the exposure time. It appeared difficult to quantify this decrease because the shape of the dose-induction relationships was often altered by the change in dose rate. It was clear, however, that the sparing effect of low dose rate or fractionation was higher for low- than for high-LET radiation. Occasional departures from this general scheme were attributed to the peculiarity of the model systems tested, rather than to real exceptions of established radiobiological mechanisms.
The National Council on Radiation Protection and Measurements [N1] evaluated the influence of dose and its distribution in time on the induction curves for tumours, with different radiations and a wide range of dose rates. The ratio of the linear non-threshold curves fitted to the high- or to the low-dose-rate experimental points was taken to be a measure of the "dose-rate effectiveness factor" (DREF). DREF values referring to 10 different experimental tumour systems varied from 1.1 to 6.7, the majority of the values clustering between 3 and 5. This analysis led to the conclusion that in most cases the risk of low-LET radiation would be significantly underestimated if non-threshold linear extrapolation of the data for high-dose-rate were applied to low-dose-rate irradiation. Moskalev et al. [M61, M68] came to the same conclusion when reviewing the available scientific literature. New data that have become available since 1977 are reviewed below.

1. Myeloid leukaemia

Robinson and Upton [R7] re-analysed part of the original data on radiation effects in RF mice [U14-U16], correcting for competing risks. About 2000 male mice, irradiated with 250-kV x-rays at doses between zero and 4.5 Gy, were selected. Early causes of death (myeloid leukaemia, M, and thymic lymphomas, T) or late causes (reticulum cell sarcoma, L, or others, R) were analysed separately by a non-parametric Kaplan-Meier survival function and its logarithmic transform (the cumulative force of mortality, cum. F.M.). Models were set up for treatment of these two categories, on the assumption of independence between the various causes of death. For causes M and T, there was a significant decrease of the latent period with dose up to 3 Gy. When the effect of dose on the integral tumour rate of M (which corresponds to the cum. F.M.) was studied, the form of the relationship for this cause of death peaked, with a maximum between 2 and 3 Gy and a further decline up to 4.5 Gy, depending on the age at irradiation of the animals (which was 5-6 weeks for group A and 9-10 weeks for group B) (Figure XV). The model for fitting the experimental data was such that the estimate of the ultimate value of the cum. F.M. corresponded to the number of leukaemogenic cells per animal. These were assumed to have a linear-quadratic dose dependence for induction, and linear-quadratic kinetics for killing. Following Barendsen [B7], the authors took an \( a_1/a_2 \) quotient of 0.5 Gy and a \( \beta_1/\beta_2 \) quotient of 3 Gy. The fit of the data actually produced a \( \beta_1/\beta_2 \) quotient of 2.4, which is in reasonable agreement, considering the assumptions involved. It was concluded that the data were consistent with the postulated linear-quadratic dose-response model.

Petoyan and Filyuskin [P22] tested another model on x-ray- and neutron-induced (high dose rate) leukaemia in RF mice [U16]. The basic assumptions of the model were:

(a) Initiation in the bone marrow stem cells is a two-track phenomenon, perhaps a symmetrical chromosomal translocation;
(b) The sensitivity of initiated and non-initiated stem cells to killing by radiation is the same;
(c) Most of mitotic cell death is due to asymmetric chromosomal exchanges, which have a functional dose dependence basically similar to that of the symmetrical translocations. Therefore, the shape and parameters of the dose-response functions for induction of symmetrical exchanges and cell death should be similar for a given cell line;
(d) The promoting influence of radiation is brought about through an enhancement of mitotic activity and, therefore, the degree of promotion should be inversely proportional to the post-irradiation survival of stem cells;
(e) The probability of scoring an overt malignancy during the remaining life span is a complex function of the "spontaneous" incidence of a given malignancy.

The parameters of the model were defined by selecting from independent sources the survival functions of bone marrow stem cells irradiated with x-rays and neutrons. The model was fitted to experimental data on the incidence of leukaemia in the RF mouse [U16] by the maximum likelihood method, using a single set of two adjustable parameters; the same set was applied to x-rays and neutrons. A good fit was obtained, by visual inspection, and the dose-response curve showed a slight upward concave curvilinearity and linearity of the initial ascending part of the curve for x-rays and neutrons, respectively. Linear extrapolation from 1.5 Gy to the low-dose region would lead to a small over-estimate of the risk for x-rays, but would be adequate for neutrons. The assumption that the dose-response functions for the initiation and cell killing are similar could be criticized on the basis of available information [B9, B11], but the postulate may be accepted as a first approximation to reality.

Mole et al. [M2, M52, M64-M66] studied the induction of acute myeloid leukaemia by x-rays, gamma rays and fission neutrons in male CBA/H mice that have almost a zero incidence of this disease. With little intercurrent mortality from competing causes of death, this tumour model is also less sensitive to systematic bias from such causes than that on RF mice [R7].
236. Mice were exposed to one of ten doses of x rays (from 0.25 to 6 Gy, delivered at the rate of 0.5 Gy min⁻¹) and then followed until they died [M52, M64]. Median survival in all groups was very similar. There was essentially no association between latency and dose. The results were fitted to a four-term polynomial of the general form \( I = (a_1D + a_2D^2) \exp(-\beta_1D + \beta_2D^2) \) as well as to four simplifications with three or two parameters only. All fits were acceptable in a statistical sense (\( P \) for goodness of fit = 0.34-0.44), but only two equations had all parameters positive and significantly larger than zero (\( P < 0.05 \)). These were: \( I = a_1D e^{-\beta_1D} \) and \( I = a_1D^2 e^{-\beta_1D} \). The former was rejected on the ground that cell survival depending solely on the square of dose is normally not found. The observed incidence could best be fitted by the latter relationship, as shown in Figure XVI. In another recent study of the induction of myeloid leukaemia in CBA male mice by whole-body x irradiation (250 kVp, HVL 1.5 mm Cu) [D3], the age-corrected incidence after doses of 1, 3, 5 and 7 Gy was essentially superimposable on the data by Moe [M52] (see Figure XVI). A purely exponential survival for haemopoietic cells is probably a simplification of reality, but survival curves for bone marrow stem cells usually show only a small shoulder at low doses. For in vivo irradiated bone marrow stem cells McCulloch and Till [M16] found \( D_0 = 0.95 \) Gy and \( n = 1.5 \). Mean values for 17 measurements of \( D_0 \) for spleen-colony-forming units in different mouse strains irradiated in vivo with x rays were in the range 0.95-1.61 Gy [H32]. The value of \( \beta_1 (0.7-0.11 \text{ Gy}^{-1}) \) found by Moe [M52] is compatible with this range.

237. A contribution of a dose-linear term \((a_1D)\) to the dose-response relationship in Figure XVI cannot be excluded on purely statistical grounds. The \( a_1/a_2 \) quotient estimated for the complete four parameter model was negative, but the upper 95% confidence limit was 1.29 Gy; and therefore, values of 0.3-0.5 Gy cannot be excluded as very unlikely. Moreover, when the data were tested on three models with a number of adjustable parameters greater than two, one or two of the parameters were always insignificant. From the simplified two-parameter models, one had to be chosen on the basis of additional information, and this implies a constraint a posteriori even if none had been chosen a priori. In summary, it appears that complete parameters for cell initiation and survival cannot be estimated, independently and simultaneously, solely from the data. However, when a likely shape of the survival function is assumed, the kinetics of initiation for low-LET radiation appears, in this case [M2, M52, M64], to be concave upwards with a pronounced \( D^2 \) component.

238. Induction of myeloid leukaemia in CBA/H mice was also studied after brief (0.25 Gy min⁻¹) and protracted exposure to \(^{60}\text{Co} \) gamma rays (1.5, 3.0 and 4.5 Gy) [M65]. Protracted exposures were delivered either as daily fractions (0.25 Gy min⁻¹, 5 days per week for 4 weeks) or at a constant rate of 0.004-0.11 mGy min⁻¹. The latter two modes of exposure did not differ in their effectiveness and gave a rather constant response at 5-6% incidence for doses above zero. The shape of the dose-response curve is very similar to that observed for leukaemias induced by fractionated x-ray treatment for ankylosing spondylitis [S49]. The response after acute irradiation was higher by a factor of 2.2, 2.8 and 5 for the three doses, respectively. The most effective gamma-ray dose for leukaemia induction was higher than that of x rays.

239. Irradiation of CBA/H male mice with fission neutrons was performed at high rate (exposure times, 2-20 minutes) with 7 air-midline kerma values in the range from 0.02 through 2.0 Gy [M66]. The observed incidence of acute myeloid leukaemia was fitted by the equation \( I(D) = (4.5 \pm 1.25) \times 10^{-11}D e^{-(1.01 \pm 0.28)D} \) (\( P = 0.25 \)). Neither a purely linear model without correction for cell killing, nor the dose-squared model that fitted the x-ray data, could be satisfactorily interpolated to the neutron data (\( P \) for goodness of fit in either case < 10⁻⁴).

![Figure XVI. Dose-response relationship for incidence of myeloid leukaemia after brief exposures of male CBA mice to 250-kVp x rays. Closed circles, data from Mole [M52]; Open circles, data from Di Maio et al. [D3].](image-url)
240. In experiments by Ulrich and Storer [U20], specific-pathogen-free (SPF) RFM/Un mice of both sexes were irradiated at 10 weeks of age with $^{137}$Cs gamma rays at 0.45 Gy min$^{-1}$, with doses ranging from 0.1 to 3 Gy. Myeloid leukaemia was less frequent in the females, in which the age-corrected incidence reached significance over the control level at 1.5 Gy. Although both a linear and a linear-quadratic model provided a satisfactory fit to the data ($P > 0.5$ and $> 0.8$, respectively), the dose-squared component was not significant and linearity predominated between zero and 3 Gy.

241. In male mice, the incidence was significantly higher than control, even at 0.5 Gy, and the form of the relationship was similar to that in females: it could be described either by a linear ($P > 0.5$) or a linear-quadratic model ($P > 0.35$), with a very small and non-significant dose-squared term. The ratio of the linear slopes for the two sexes indicated that males were more susceptible by a factor of about 5. Low-dose-rate (0.083 Gy per day) gamma exposure [U3] was very much less effective in inducing myeloid leukaemia in the female mice; no significant increase above controls could be reached even at 2.0 Gy. After acute and chronic neutron irradiation, the incidence of the disease was very low and did not permit any detailed study of dose-response relationships. In either case, the peak incidence was observed at 0.47 Gy, but the results were not significantly different from controls ($p > 0.05$).

2. Thymic lymphoma

242. Ulrich and Storer [U2, U3, U5, U20] studied the dose-response relationship and dose-rate effects for $^{137}$Cs gamma rays and neutrons in SPF 10-week-old RFM/Un mice. The response was assessed as age-corrected incidence, standardized to the distribution of the age at death of control animals. In females, thymic lymphoma was efficiently induced by gamma rays at 0.45 Gy min$^{-1}$; the incidence was significantly higher than in controls in all groups receiving 0.25 Gy or more. No simple model could describe the response over the entire dose range (0.1-3 Gy). There was a steep rise in the region below 0.5 Gy, followed by a shallow rise at higher doses. Over the range of 0-0.25 Gy (three points), a dose-squared model provided an adequate fit and linearity could be rejected ($P < 0.06$). From 0.5 to 3 Gy the increase in incidence with dose was compatible with linearity.

243. In males, the significance of the difference above controls was reached only at 1 Gy. A linear model provided a satisfactory fit over the entire range of gamma-ray doses (0.1-3 Gy; $P > 0.4$). Over 0-1.5 Gy, both a linear and linear-quadratic equation (with D$^2$ coefficient not significant) provided a very good fit to the data ($P > 0.95$ and $> 0.80$, respectively). Thus, in males the dose-response relationship for acute gamma rays was predominantly linear over the entire dose range.

244. Lowering the dose rate to 0.083 Gy per day considerably decreased the incidence of thymic lymphoma in the females and changed the form of the curve from quadratic followed by linear to linear-quadratic with a negative linear component. Simple linearity could be rejected ($P < 0.001$). It is quite clear that in the RFM mouse, after low-LET exposure, there is no suggestion of the threshold-type response found by Kaplan and Brown [K33] in C57Bl mice. In those older data there was a statistically verified threshold in the dose-response relationship. As may be seen in Figure XVII (kindly provided by G. Walinder), fractionation of the dose into 1, 2, 4 or 8 fractions with one-day spacing, is without effect upon the incidence of the lymphoma. When plotted against exposure (either simple or fractionated) the incidence may be fitted by a linear function of the form $I = aD - b$ where $a = 0.112$ and $b = -21.6$ (significantly different from zero); the coefficient of correlation is very high (0.903).

245. In experiments by Maisin et al. [M62], 12-week-old male BALB/c mice were exposed to single or to fractionated doses of $^{137}$Cs gamma rays (10 equal doses separated by 1 day) in the dose range from 0.25 to 6 Gy. The dose-response curve for thymic lymphoma was of a threshold type. The actuarial incidence rising above control only at 4 and 6 Gy. Single doses were more effective at 4 than at 6 Gy.

246. Other data on thymic lymphoma induction by x-rays in neonatal (C57Bl/6J Nrs X WHBT/Hc) F1 mice were reported [S48]. Even though the incidence in females and males at three exposure points (200, 400 and 600 R) could be fitted to a linear-quadratic equation with a negative linear term ($P > 0.80$), the data adequately fit a threshold-type response such as that presented in Figure XVII for the C57Bl mice.

247. Fast neutrons (acutely delivered doses at 0.05 and 0.25 Gy min$^{-1}$ and chronic doses at 0.01 Gy per day, with total doses between 0.1 and 3 Gy) induced thymic lymphoma in female RFM mice [U5]. In the range 0.25-0.5 Gy, the RBE with respect to high-dose-rate $^{137}$Cs gamma rays was between 3 and 4. For acute neutron exposures, the dose-response curve was concave downward. In the range 0-0.94 Gy, linearity could be rejected ($P > 0.001$) and a good fit was obtained with the square root of the dose ($P > 0.8$). Up to 0.47 Gy, a linear fit was satisfactory ($P > 0.75$). For chronic neutron exposure, a linear fit adequately described the curve over the 0-0.94 Gy range ($P > 0.8$); and the loss of effectiveness over the acute exposures amounted to about 30%. However, there was a decrease of susceptibility to thymic lymphoma upon acute exposure with increasing age, so that the lower efficiency of the low-dose-rate exposure may in part be due to this latter factor alone. The RBE of acute neutron exposure relative to gamma rays varied proportionally to the inverse square root of neutron dose.

248. Induction of thymic lymphoma was also studied in male BALB/c mice irradiated at the age of 12 weeks with d(30)Be neutrons (modal energy about 23 MeV) [M63] at doses from 0.02 to 3 Gy. The actuarial incidence of thymic lymphoma fitted the same sigmoidal threshold-type curve found for $^{137}$Cs gamma rays [M62].