Dose-Response Relationships for Carcinogens: A Review

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We review the experimental evidence for various shapes of dose-response relationships for carcinogens and summarize those experiments that give the most information on relatively low doses. A brief review of some models is given to illustrate the shapes of dose-response curve expected from them. Our major interest is in the use of dose-response relationships to estimate risks to humans at low doses, and so we pay special attention to experimentally observed and theoretically expected nonlinearities. There are few experimental examples of nonlinear dose-response relations in humans, but this may simply be due to the limitations in the data. The several examples in rodents, even though for high dose data, suggest that nonlinearity is common. In some cases such nonlinearities may be rationalized on the basis of the pharmacokinetics of the test compound or its metabolites.

Introduction

The primary reason for the authors' interest in dose-response relationships for carcinogens is the need to estimate human risks at low doses. Typically, the only data available are the results of animal experiments at high doses, perhaps augmented by scanty epidemiological information (together with test results from a battery of short-term tests, which are not considered further here). Conclusive demonstration of the existence or nonexistence of certain aspects of dose-response relationships for carcinogens would greatly ease the task of risk assessment, in turn making regulation of carcinogens, and the explanation of such regulation, simpler. For example, if a threshold dose, below which there is no response, exists, then exposure up to that threshold would clearly pose no risk. Evidently, there are many other reasons for study of dose-response relationships at all doses, but in this review we concentrate on the lowest doses that give experimentally measurable response. In particular, we are interested in the extent to which measurable dose-response curves for cancers deviate from linearity.

This review surveys the relevant human ("Human Studies") and whole animal ("Animal Studies") experimental data on carcinogenesis at relatively low doses, with an outline of theories that have been advanced and remain consistent with this data ("Dose-Response For-

mulae"). Of special interest are the observed nonlinearities in dose-response relationships, and the possible explanations for them.

Most models of the cancer process are constructed by describing (mathematically) a set of elementary biological processes which are supposed to be fundamental. The effect of doses of carcinogens on these elementary processes is generally assumed to be the simplest possible (e.g., described by a chemical reaction rate), but the dose-response relationship for the whole model will usually be as arbitrary as the assumptions made for these elementary processes. Many of the mathematical models suggested are expressions of the biological idea of a multistage process. We demonstrate how many different dose-response formulae can be obtained within this one framework, although such formulae might also arise from alternative theoretical frameworks. Consequently, to avoid confusion, it seems preferable to refer to currently used dose-response relationships by some descriptor of the mathematical formulae, rather than by a descriptor of the particular theory or model used to justify them.

"Human Studies" and "Animal Studies" summarize those experimental observations on cancer induction which show most clearly the shapes of dose-response curves. Epidemiological studies and bioassays can use too few subjects to give direct information about doses at which excess tumor rates are below about 1%. Where large numbers of people or animals have been exposed to various levels of a carcinogen we obtain the closest approach to low doses. In addition, studying the outcomes of a large number of small experiments can give

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some statistical information about shapes of dose-response curves.

In "Human Studies" we discuss the small amount of data on dose-response relationships in humans. The best sets of data are those on cigarette smoking in British physicians, with a sublinear dose-response relationship. In addition are the results of a study of lung cancer in those occupationally exposed to coke oven emissions (which contain many constituents present in cigarette smoke). Exposure to radiation is known to produce several human cancers, and there are sufficient data to estimate dose-response curves. The effects can vary substantially, depending on the route of exposure, radiation quality (high versus low linear energy transfer), and target site, leading to highly varied dose-response relationships. Various factors modulate the human response to carcinogens, leading to complications in dose-response relationships. This is illustrated by the case of aflatoxin B_1, where human response appears to be explicable only if various other characteristics of exposed populations are also taken into account.

In "Animal Studies" we discuss animal data. Even though there are many more data than in the human case, they have been gathered in the course of experiments on many more materials, so that there are few detailed dose-response relationships. It is possible to analyze a number of small experiments to determine the dose-response relationships that are consistent with the whole set of data, although this requires assumptions about comparability which may be difficult to justify. Alternatively, analysis of many experiments with substantially the same experimental design may give some insight into the shape of dose-response curves in general. There have been two reported experiments using thousands of animals exposed to single chemicals: one in which mice were exposed to 2-acetylaminofluorene, and one in which rats were exposed either to diethyl- or dimethyl-nitrosamine. Each shows two distinct types of dose-response relationships corresponding to two different tumor sites. In addition, moderately large experiments have been performed on saccharin, vinyl chloride, and radiation. All show nonlinear dose-response relationships, the shape of which may be explained by various biological mechanisms.

We conclude with a discussion of the problems of regulating carcinogens. Although it is clear that nonlinear dose-response relationships are experimentally prevalent and that biologically based theoretical reasoning leads to such nonlinearities, it is rarely possible to identify when and to what extent they will occur in humans. To remain conservative, it is still necessary to assume that dose-response curves may be linear at sufficiently low doses (although even this may fail to be always conservative). However, in analyses that attempt to obtain a best estimate of risk (rather than a conservative one), or perhaps for the legal purpose of assigning causation in a particular case in which there is substantial experimental evidence, a nonlinear extrapolation may be more appropriate.

Dose-Response Formulae

Introduction

In this review we wish to distinguish three concepts that are often confused. The first is the abstract description of biological processes, the second a mathematical model for that process, and the third any formulae that can be used to summarize certain predictions of the mathematical model. In the literature on dose-response relationships, it is often unclear which (if any) of these concepts is intended, since any or all of them may be referred to as a "model."

Knowledge about the processes involved in the ultimate production of cancers has advanced substantially in the recent past. It is clear that a complete description will require knowledge of many processes, including the transport and metabolism of materials throughout the body, damage and repair of DNA and other cellular components, and stimulation and inhibition of cell growth. Such a complete description should, for example, be able to explain the phenomena of background response, promotion, and differences between individual animals. With such a complete description, which lies some distance in the future, it should be possible to predict how the probability of cancer varies with dose of carcinogen (i.e., a dose-response relationship). Figure 1 is one simplified description (1,2) of the processes leading to liver cancers. We display it not to endorse its correctness, but to illustrate the complexity to be expected in such descriptions, and the many places at which a xenobiotic might exert influence; the ultimate dose-response relationship for that xenobiotic may be the result of interactions at any one or all of these places.

For any abstract description of a biological process, it is possible to write down mathematical models that incorporate in a parametric way all the known infor-
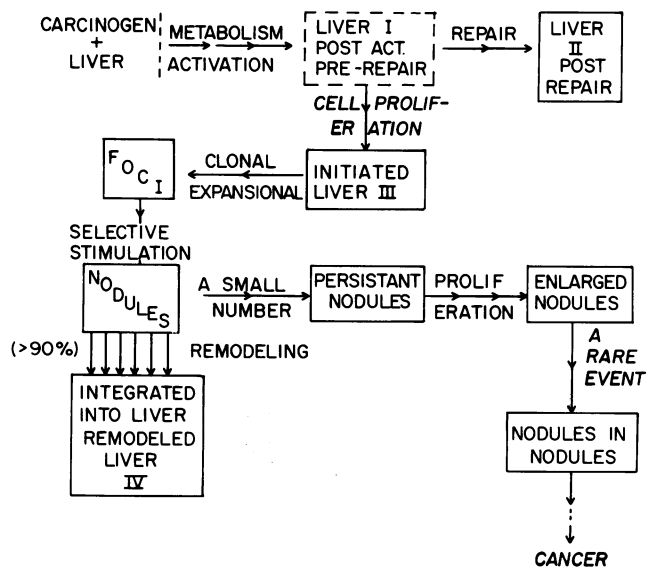

FIGURE 1. One biological description of liver carcinogenesis (1,2).

mation about the process. Such models may differ in formalism (but be mathematically equivalent), or may differ in content if different assumptions are made about any unknown details of the process. Since the known information comes from experiments, which are necessarily subject to some error, it should not be surprising that different mathematical models can be constructed which nevertheless agree in all details with known information. Furthermore, it is possible that the same mathematical model can be used to describe different biological processes, although the parameters of the model would have a different meaning. Recent attempts at developing mathematical models to (among other things) predict dose-response relationships have usually focused on describing in detail one part (or a few parts) of the processes leading to cancer, but the limitations of available data are so great, and the biological processes are so complex, that these attempts have necessarily been incomplete.

From the mathematical model, it is generally possible to extract some formulae that summarize the predictions of the model in simplified situations. This may be the only use for the mathematical model, since the parameters which are used in its construction may not be directly measurable. In such a case, the only information the mathematical model provides is the expected functional relationship between various parameters of the model. Specifically, for our purposes, the model would provide information about the relationship between the dose of some material and a probability of cancer. It is important to realize that while any specific model may predict a particular shape for a dose-response relationship, the converse is not true. The same formula for a dose-response relationship can be produced by many different mathematical models (and similarly, by many different biological descriptions). To refer to the use of a particular formula for a dose-response relationship as being the use of a particular model is therefore incorrect, and we try to avoid such usage in this review.

Dose-response models are formulated either to aid in the development and verification of biological theories of carcinogenesis, or as a means of determining safe levels of exposure to carcinogens, and reviews of the literature emphasize either one aspect or the other. In this review we emphasize the second aspect. Several authors have reviewed the models and the corresponding dose-response formulae most commonly used for estimating human risks from animal bioassay data (3–9). Whittemore (10) and Whittemore and Keller (11) give excellent reviews of quantitative models of carcinogenesis based on a multistage description, presenting their historical development and recent formulations. The recent review of detailed multistage models by Forbes and Gibberd (12), who concentrate on age effects, provides a useful update to their work. A guide to the literature on dose-response models is given by Krewski and Brown (13) and the National Cancer Institute (14).

The dose-response models used to gain insight on carcinogenesis are more complicated and require much more data than those presently used by government agencies in setting acceptable human exposures to carcinogens. Yet it is now realized that certain assumptions made in determining the safety of one chemical may not apply to another, so that it may now be more appropriate to choose the dose-response formulae used for standard setting on the basis of models which incorporate more information on biological processes.

Although a simple and precise definition of a carcinogen is straightforward, e.g., "an agent which can cause cancer in the species under consideration," further examination reveals that different types of carcinogens can be usefully distinguished. Distinctions have been drawn between initiators, promoters, cocarcinogens, true carcinogens, primary carcinogens, direct carcinogens, indirect carcinogens, secondary carcinogens, early carcinogens, late carcinogens, and complete carcinogens by one or more authors. These distinctions generally rely on a description or model of carcinogenesis which may or may not have wide acceptance. We discuss the meaning of some of these distinctions later in this section.

Some Constraints on Dose-Response Formulae

When choosing a dose-response formula in order to fit experimentally observed data (on cancer incidence) and to extrapolate to low doses, it is necessary to ensure that the formula is indeed that obtained from a mathematical model of the cancer process. This is not a very strong constraint, since many such models have been proposed, but it does have some consequences. In particular, a background rate of tumors (spontaneous incidence) and the variation of tumor incidence rate with age and time must be consistently incorporated.

Mathematical Model from Biological Description. Consider again the biological description of liver carcinogenesis developed by Farber (1,2), shown schematically in Figure 1. In this description, the first evidence that a step toward the development of liver cancer has occurred is the appearance of nodules of hepatocytes, visible to the naked eye (>2 mm diameter). These nodules are presumed to come from initiated cells (those in which DNA has been modified, possibly by interaction with a xenobiotic or its metabolite). In addition to DNA modification, a cycle of cell proliferation is required. (This proliferation requirement may be the major mechanism of action for those liver carcinogens that are cytotoxic to liver cells, see "True and Secondary Carcinogens.") The initiated cells are supposed to have acquired resistance to the inhibition of cell proliferation exhibited by normal cells. Observable nodules develop rapidly from these resistant hepatocytes (within days of initiation). An assay for the induction of resistant hepatocytes and their subsequent growth into nodules was developed by Farber and colleagues. He notes that although after 5 to 6 weeks from the time of initiation the liver may contain as many as 1,000 nodules, 8 to 10 weeks after this, over 90% have disappeared, having

redifferentiated back into normal-appearing liver tissue. The occurrence of some unspecified but rare event causes new hepatocytes to be formed within the persistent nodules. These new hepatocytes also grow, and thus lead to nodules within nodules, which are the sites of liver cancer.

All of these processes can be described using the mathematical models discussed here (and using other models) to generate a model of the whole process. For example, one might wish to describe the activated liver by the amount of DNA modified. The interaction of a xenobiotic with DNA might be described by a one-hit model to quantitatively predict the number of DNA adducts and thus the total amount of DNA modified. However, obtaining accurate models for some of the subprocesses involved will not necessarily ensure an adequate model for the whole process. Thus, even if the one-hit model holds for DNA adduct formation within the cell, the dose-response relationship for the whole animal could still be highly nonlinear if, for example, repair is dose-dependent, or if nonlinear pharmacokinetics are involved in metabolite formation, or if two dose-related mutations are required rather than the single one implied by the use of a one-hit model.

The usual hope in using a dose-response formula based on a mathematical model is that the whole process is dominated by the part that is modeled. Other processes are known to occur and are not incorporated into the model, but these processes are supposed to have a secondary effect.

Backgrounds (Spontaneous Tumors). It is observed that in any whole animal experiment on a particular xenobiotic, the control animals may also develop tumors. Such spontaneous tumors may be due to other carcinogens than the one of interest, or may arise as a necessary consequence of some normal process and so be truly spontaneous. In fitting dose-response formulae it is necessary to account for such a background tumor incidence. The mathematical problems and the implications for the processes being modeled have been extensively treated (*3,15–17*), and we summarize some major points.

There are many ways of incorporating spontaneous tumor incidence, but they can usually be modeled as an admixture of two particular cases:

1. An independence assumption: The cancers produced by the xenobiotic are completely independent of background tumors. The total probability of a tumor, $p(d)$, assuming a background rate α, is then

$$p(d) = \alpha + (1 - \alpha) \cdot F(d), \tag{1}$$

where $F(d)$ is the probability of a tumor solely due to the externally applied dose d. For example, for the one-hit model this gives

$$p(d) = \alpha + (1 - \alpha)(1 - e^{-\beta \cdot d})$$

$$= 1 - (1 - \alpha)e^{-\beta \cdot d} \tag{2}$$

This procedure is, in essence, that of Abbott (*18*). If

this independence between background and dose-dependent tumors is a correct description, then any nonlinear dose-response function for $F(d)$ will result in a nonlinear relation between the total probability of tumor and dose.

2. An additive assumption: The spontaneous cancers are produced by the same mechanisms as the cancers produced by the xenobiotic. In this case the background tumors may be considered to be produced by an effective background dose d_B, and the total probability of tumor with an external dose d is then:

$$p(d) = F(d + d_B) \approx F(d_B) + d \cdot \left(\frac{\partial F}{\partial d}\right) d_B$$

$$= \alpha + \beta' \cdot d \tag{3}$$

This always gives a response linear in the external dose at sufficiently low doses if the first derivative of F is finite. (Note that the Heaviside function in the threshold model is not continuous, see "Threshold Formulae," and its derivative is not finite at the position of the threshold.) Figure 2 illustrates this extreme case. In practice, the constant of proportionality (β') may be small, so that this linear region of the dose-response function is not experimentally observable. The idea that the carcinogen may add to an effective background dose has been often used to support the claim that a linear dose-response formula is never overly conservative.

Between these extremes are many intermediate possibilities. One is that only cancers arising from the same cell type are similar for the purposes considered here. For very rare cancers, the effective background dose d_B may be practically zero and the shape of the dose-
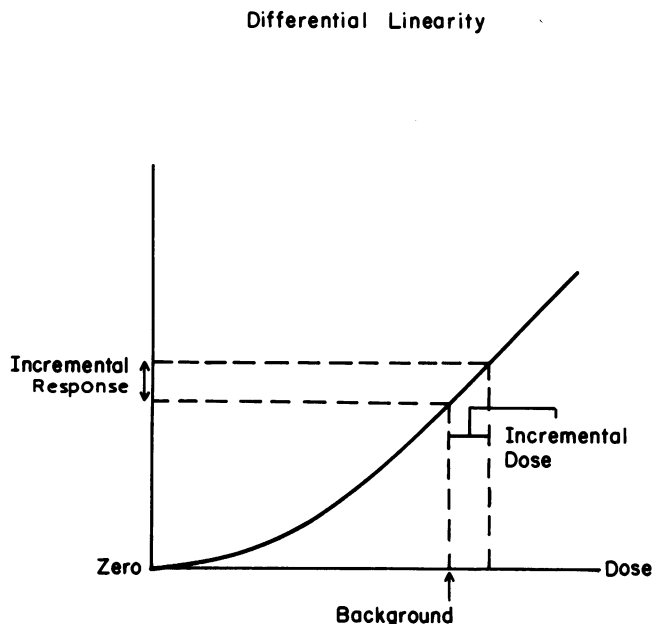

FIGURE 2. Small increments in dose above a background give small, linearly related increments in response, even for nonlinear dose-response functions.

response curve indistinguishable from that expected under the assumption of dose independence. On the other hand, if the cancer in question is common, with d_B relatively large, the response at low doses of the external agent would be linear. Krewski and Van Ryzin (*3*) tested the effect of the additive assumption by incorporating it into three dose-response formulae (extreme value, logit, and probit). Applying these formulae to a series of data sets, effective background doses were estimated and extrapolations down to an excess risk of 10^{-5} were performed. When the observed background tumor incidence was greater than about 1%, the predicted response at low dose was essentially linear in dose. However, for background tumor incidence less than 1%, low dose linearity did not result when the dose-response formula allowed for curvature, and the high dose data exhibited nonlinearity. As a precautionary note, the particular human cancer or cancers that any particular carcinogen might cause are generally unknown, and it may be necessary to assume that it will cause a common cancer if extrapolation of animal results to humans is to be performed.

Time and Age. Most uses of dose-response formulae, in particular for estimating risks at low doses, have ignored the age and time dependence of cancer incidence. This is sometimes adequate if the experimental data under analysis are for subjects exposed continuously for a fixed time to a fixed level of carcinogen and if the age distributions of subjects in the various dose groups, including the controls, do not differ. Often, however, this is not the case.

AGE CORRECTIONS. First, in estimating risks, there is the problem of competing risks. Smokers die before their time not only of lung cancer; they are also far more likely than nonsmokers to die of heart disease, and consequently there are fewer old smokers. Similarly, in other epidemiological studies, those in the most highly exposed groups are often the oldest; such is the case with underground miners who are exposed to radon gas, a lung carcinogen (see "Lung Cancer from Radon Gas"). In bioassays, treated animals suffer reduced survival due to chronic toxic effects, as well as cancer, so their survival curves can differ substantially from control animals. Discrepancies in longevity among dose groups can lead to a spurious change in the shape of dose-response curves, so some adjustment is needed. Nonparametric techniques that do not require the assumption of any specific dose and age response function are frequently applied. The techniques of Kaplan and Meier (*19*) can be used to estimate the probability that animals in a treatment group would have had a specific cancer if that group had the same age-specific noncancer mortality as the untreated animals. There are some technical disadvantages with this approach, and so Peto and colleagues have proposed an alternative nonparametric approach (*20*). In each treatment group, the observed number of people or experimental animals with tumors is compared with the number expected if the exposed groups had the age-specific tumor rates of all groups combined. The ratio of observed-to-expected number

obtained using this procedure is approximately proportional to the response curve required, allowing any curvature of this dose-response relationship to be observed, although there may be a bias toward flattening of the curve at high doses. This technique was used to analyze the dose response curvature of the large diethyl- and dimethyl-nitrosamine experiment (see "*N*-diethyl- and *N*-dimethyl-nitrosamines"). In estimating carcinogenic potency it is sometimes more useful to apply a parametric technique, i.e., to specify a dose-age-response model and then to estimate the parameters of this model from the observed data. A mixed technique has also been used (*21*), in which the dose dependence of tumor incidence was specified parametrically, but the age dependence was estimated nonparametrically. This was the approach used to compile a consistent set carcinogenic potencies from an exhaustive review of the literature (*22,23*).

VARIED DOSING SCHEDULE. A second problem in estimating risks is that the exposure often is not constant. This is always the case for occupational exposures and frequently occurs in animal bioassays. Misleading dose-response relationships can be obtained when the age variation of the dose rate is ignored and modeling is done using either estimates of cumulative exposure, that is, total exposure throughout an individual's lifetime, or the average exposure rate (cumulative dose divided by lifetime). A model that takes into account both age and dose dependencies is needed to determine a more realistic dose-response relation. The formulae that result from such models can be divided into two classes: in the first, $I(d,t)$, the incidence at age t and dose d, also called the hazard function, can be separated into a product of an age-independent function of dose [e.g., $g(d)$] and a dose independent function of age [e.g., $f(t)$], so that $I(d,t) = g(d) \cdot f(t)$; in the second, this decomposition of the hazard function is not possible. Spurious dose-response relationships can be obtained when, for example, the dose rate varies, and a separable form (using only cumulative dose) is used to analyze the data. Many dose-response formulae that include a specification of dose and age dependence can be derived from some specialization of multistage models, and are briefly discussed in "Multistage Models."

Particular Models and Formulae

The Linear and Linear Exponential Formulae. Perhaps the simplest dose-response relationship used is that based originally on the one-hit description and corresponding model of radiation carcinogenesis formulated by Crowther (*24*). The biological idea is that a cell is damaged by a random hit (in the original formulation, from radiation; by extension, possibly from a chemical molecule) and that it then grows inevitably to form a cancer. A mathematical expression of this idea leads to the formula,

$$\text{Excess number of cancers in a population} = K \cdot \text{Exposure}$$

It is necessary to allow for the fact that organisms only die once, so that the probability of getting cancer [$p(d)$] when exposed to a cumulative exposure or dose d becomes

$$p(d) = 1 - \exp(-\beta d) \longrightarrow \beta \cdot d \quad \text{for small } d \tag{4}$$

The biological one-hit model that leads to this formula may be disputed even for radiation carcinogenesis. The charged particle background due to cosmic rays is, at the earth's surface, $10^4/\text{min-m}^2$-ster, or about $10^3/\text{min}$ over the human body. Each of these charged particles causes ionization as it passes through tissue. Even if sensitive cells occupy only 1/1,000 of the body, this still means that cosmic rays alone would initiate 1 cancer/10 min, or 10^5 in a lifetime—a factor 10^6 higher than the observed cancer background rate. To bring the description into agreement with observation, one must postulate either that the cells must suffer ionization damage in a very specific way, or that there are repair mechanisms. More recent knowledge of carcinogenesis suggests that there is more than one stage in the progression to a cancer, so that a one-hit model cannot be anything but an oversimplification. However, if the fraction of cells that are repaired is independent of the dose, and if the target site of attack is independent of dose, and if ionization at only one specific target site is required, the one-hit model might be an adequate description.

Nevertheless, the linear formula

$$p(d) = \beta d, \tag{5}$$

and its modification for high doses

$$p(d) = 1 - \exp\left(-\beta \cdot d\right) \tag{6}$$

and the equivalent formula modified to include a background cancer rate

$$p(d) = 1 - (1 - \alpha) \exp\left(-\frac{\beta \cdot d}{1 - \alpha}\right)$$

$$\text{or} \quad 1 - \exp[-(a + b \cdot d)] \tag{7}$$

are often used to analyze data. The modifications for high doses are for the probability of dying of cancer or the probability that any individual animal has at least one tumor. One can still postulate strict linearity [$N(d) = \beta \cdot d$] between the total number of tumors and the dose at high doses: one animal can have several tumors, but the animal is counted only once. Postulating a one-hit model thus has implications for another observable measure, the number of tumors per animal, as well as implying the formulae given for the probability of a tumor.

However, as discussed previously, using the formulae displayed does not imply the postulation of a one-hit model. As discussed below, the same formulae can be derived from, for example, various specializations of multistage models. Furthermore, the use of these for-

mulae for data analysis also gives upper bound estimates of risk for a wide variety of models. We will therefore refer to these dose-response formulae as "linear" (or "proportional") (Eq. 5) or "exponential linear" (Eqs. 6 and 7), although we are not particularly careful in distinguishing these two, since they are equivalent at low enough doses.

Because of the simplicity and importance of these formulae, others are often compared to them. A sublinear dose-response formula is one in which the curve, fixed to be equal to the proportional (or exponential linear) formula at each end, lies always below the proportional curve; a superlinear formula is one in which the curve, also fixed to the proportional (or exponential linear) one at each end, lies above the proportional curve. Examples of super- and sublinear curves are given in Figures 5 and 6, respectively. Such curves will also be described as cases of downward curvature and upward curvature, respectively, to indicate the shape of the curve as the dose (on the x-axis) is increased.

Threshold Formulae. It is generally accepted that organisms have a certain tolerance for poisons; exceeding some threshold dose will result in overt effects. An extreme form of such a threshold dose-response relationship can be expressed as

$$p(d) = H(d - d_T) \tag{8}$$

where H is the Heaviside unit function, and d_T is the threshold dose (Fig. 3). Above the threshold a response is certainly exhibited; below the threshold no response is exhibited. More generally, a threshold-type dose-response relationship has some particular dose d_T, the threshold dose, below which the response of interest may be considered negligible, and above which the response grows rapidly.

Schaeffer (*25*) has argued that the dose-response relationship for chemical carcinogens must include a threshold because carcinogenesis involves chemical reactions, which are subject to free energy and entropy constraints. The "reactions will exhibit the usual mass requirements of more common chemical reactions: the single molecule causing cancer theory is chemically and biologically unrealistic." These ideas were incorporated into a threshold model, the "filter model" (*4*), derived from thermodynamic ideas to describe tumor response in whole animals, and the resulting dose-response formulae were used to analyze animal data at high doses.
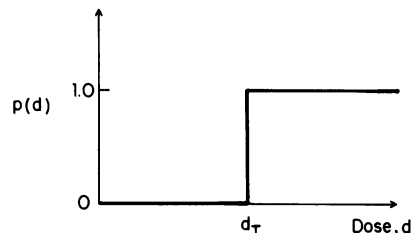

FIGURE 3.   The prototype threshold dose-response function.

However, it seems unlikely that such thermodynamic thresholds are high enough to be of any consequence, even at very low doses, as can be seen from the observations on DNA carcinogen adducts.

It is now possible to measure the concentration of such adducts at very low levels (*26*). Extraordinarily small concentrations of adducts have been associated with very small exposures to carcinogens. The relationship between exposure and adduct concentrations for two potent carcinogens, aflatoxin and benzo[a]pyrene, appears to be linear, indicating the potential for nonthreshold biological activity at very low doses.

The Probit Formula. GENERAL. There are certainly variations in individual tolerances to poisons. The probit model (*27*), used to estimate the LD_{50} (the acute dose at which 50% mortality occurs within a short time in a test population) from mortality data on animals, may be derived by assuming that a threshold dose (d_T) exists for each individual, but that this threshold dose differs for different individuals in the population. If the individual threshold doses are lognormally distributed with a logarithmic mean $\mu = \log(LD_{50})$ and logarithmic standard deviation σ, then the expected fraction [$p(d)$] of animals dying at some dose d is given by

$$p(d) = \frac{1}{\sqrt{2\pi}\sigma} \int_{-\infty}^{\log d} \exp\left\{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2\right\} dx, \tag{9}$$

which can be rewritten as

$$p(d) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{a+b\cdot\log d} \exp\left\{-\frac{1}{2}y^2\right\} dy.$$
$$\equiv \Phi(a + b \cdot \log d) \tag{10}$$

where $\Phi(z)$ is the Standard Normal Integral (Fig. 4).

When this formula is graphed as a function of dose (d), it is seen to have an effective threshold, since the expected fraction of animals affected rapidly becomes negligible (faster than any power of dose) as the dose is decreased. It has enjoyed great success in, and is the standard for, the analysis of acute toxic effects. It contains two constants, and thus may be expected to fit small data sets better than one parameter formulae (like the proportional or threshold formula), but for such acute effects it would be difficult to find a better formula. (In all cases, inclusion of a background response requires at least one more constant, but for modeling acute mortality the background is effectively zero.)

THE MANTEL-BRYAN PROCEDURE. The probit model was derived for use on noncarcinogenic responses, but Mantel and Bryan (*28*) suggested its use for modeling response to carcinogens in order to provide a method of setting a safe exposure. Their suggestion was generally accepted, and until recently, a procedure based on the probit model, the "Improved Mantel-Bryan Procedure" (*29*), has been widely used (e.g., by the FDA until 1977).
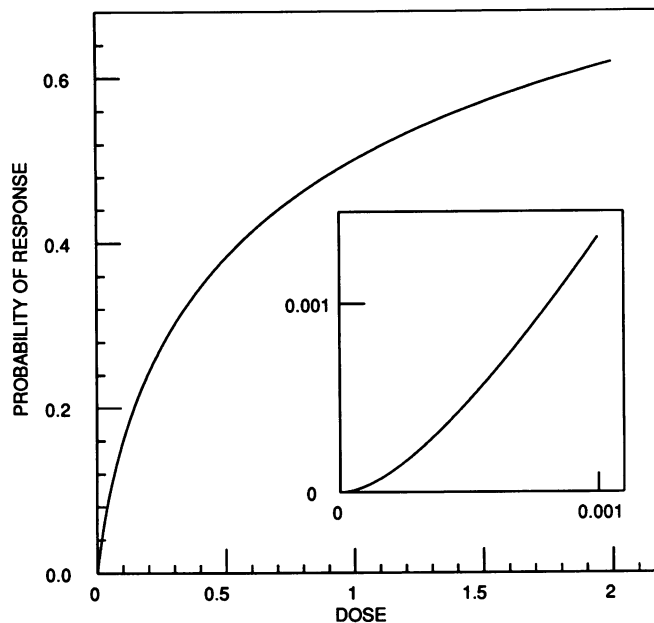
For conservatism, the slope parameter (b in Eq. 10)

FIGURE 4. A probit dose-response function (Eq. 11 with $a = 0$ and $c = 0$). Inset is the same function at much lower doses, showing curve rapidly becomes sublinear at low doses.

was assumed to be unity when base 10 logarithms were used, so that every 10-fold decrease in dose corresponded to a unit decrease in normal deviate. This choice was made from experience with experimental data and reduced the number of free parameters in the formula. However, to account for spontaneous tumors, Mantel et al. introduced an additional parameter, "*c*," the expected (spontaneous) incidence in untreated animals. This they assumed to be independent of the incidence induced by the carcinogen, and so Abbott's correction is employed. The resulting formula for probability of cancer as a function of dose is

$$p(d) = c + (1 - c) \cdot \Phi(a + \log_{10} d) \tag{11}$$

It was suggested that maximum likelihood procedures be used to determine a, c, and their confidence bounds from experimental data, and also that high dose groups be progressively eliminated to obtain the lowest of the upper confidence limits on a, call it a^*. The safe level, d^*, corresponding to a predetermined *de minimis* excess risk, say r^*, is then given by solving

$$r^* = \Phi(a^* + \log_{10} d^*) \tag{12}$$

using the estimated value of a^*.

The Gamma Multihit Model and Weibull Formula. The gamma multihit model may be derived as a generalization of the one-hit model. Instead of just requiring a single hit on a cell, it is postulated that some critical number, k, of hits are required before a cell becomes cancerous. Rai and Van Ryzin (*30*) derive a dose-response formula by assuming that the probability for any single hit is proportional to dose, and the probability

for a given number of hits follows a Poisson distribution. If each of k hits required is indistinguishable, and if all are equally likely, then the probability for at least k hits is given by

$$p(d) = \sum_{i=k}^{\infty}(\lambda d)^{i}\exp(-\lambda d)/i! \qquad (13)$$

which can also be written as

$$p(d) = \int_{0}^{\lambda d}\frac{u^{k-1}e^{-u}}{\Gamma(k)}du \qquad k \geq 0 \qquad (14)$$

where $\Gamma(k)$ denotes the gamma function. The parameter λ is the proportionality constant between probability of any hit and dose. Although this formula was derived for an integral number k of hits, it can be unambiguously extended to nonintegral values using the integral formulation (Eq. 14), although the relation to the original description is now unclear. For this dose-response formula, the probability of cancer, $p(d)$, is proportional to d^{k} at low doses.

The gamma multihit formula is mathematically attractive for simple data fitting (without assigning physical or biological meaning to the parameters) because it has the two parameters λ and k which are continuously variable. It can be fit to data exhibiting strong superlinearity (Fig. 5) or strong sublinearity (Fig. 6) very well. However, the formula must be used with caution. If the best fit is $k < 1$, the predicted low dose response will be much larger than that predicted by most other formulae, since the derivative dp/dd is then infinite at zero dose. Figure 5 is an example where the experimental outcomes (liver angiosarcomas in rats, produced by vinyl chloride inhalation) can be analyzed with a gamma multihit formula to produce practically meaningless predictions for low doses. The best fit is with $k \approx \frac{1}{2}$ (8). This seems impossible to interpret in terms of any biological description because half a hit makes no

FIGURE 6. A gamma multihit formula fitted (8) to experimental data (bladder tumors caused by NTA) showing sublinear behavoir.

sense. Fits to sublinear dose-response data can also give peculiar results. For example, for a cancer bioassay on NTA (Fig. 6), which perhaps acts as a promoter, the best estimate for k using a gamma multihit formula is 28, implying 28 hits—surely an overestimate.

A similar effect can occur when a Weibull formula is used to analyze data. The Weibull formula is simply

$$p(d) = 1 - exp(-d^{k}) \qquad (15)$$

and so corresponds to continuing the low-dose behavior of the gamma multihit formula to higher doses (although this is not the only way it may be obtained). Cothern et al. (31), in discussing trichloroethylene in drinking water, attempt to conservatively estimate low dose risk by using a Weibull formula, but in this case obtain $p(d) \propto \sqrt{d}$ at low doses. While this procedure may give a conservative estimate, the values obtained will have little relation to actual risks. Only by using further biological information or using more data can sensible conclusions be drawn about risks at low doses.

Multistage Models. HISTORICAL DEVELOPMENT. The evolution of multistage descriptions for carcinogenesis and the corresponding mathematical models have been described in extensive reviews by Whittemore (10) and Whittemore and Keller (11). Table 1 outlines some of the history, beginning with the singlestage model of Iverson and Arley (32), which describes the transformation of a single normal cell to one that divides into proliferating daughter cells. Even this model can be traced even earlier to the one-hit model of Crowther (24). Once the colony of abnormal cells reaches a certain size, it is recognized as a tumor. This theory could not adequately explain why the incidence of human cancers generally increase with the fifth or sixth power of age, so two extensions were proposed. According to the first, by Fisher and Holloman (33), tumors only develop if a number of cells (~ 6) have been independently transformed. Muller (34) and Nordling (35) offered an alternative biological theory, later quantitatively expressed by Stocks (36) and Armitage and
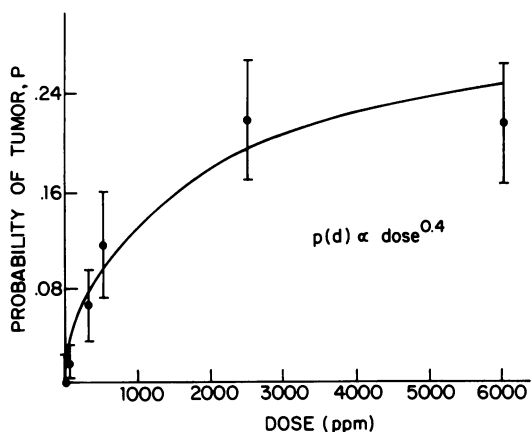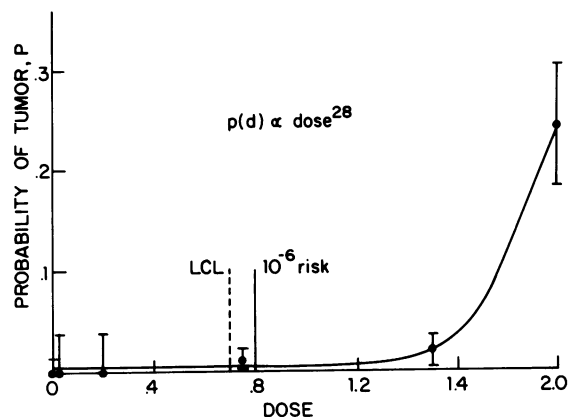

FIGURE 5. A gamma multihit formula fitted (8) to experimental data (liver angiosarcoma caused by vinyl chloride) showing superlinear behavior.

Table 1. Historical development of quantitative theories of carcinogenesis.[a]

A. Crowther (24): One-hit model of radiation carcinogenesis
 —$p(d) = 1 - \exp(b \cdot d)$

B. Iverson and Arley (32): Single stage theory; single cell theory
 —a single transition from normal cell to transformed cell with transition rate $\lambda(t)$
 —detectable tumor is a mass of n_0 cells which are descendants of a transformed cell
 —growth of cell mass follows a pure birth process with birthrate β

 —$I(t) = N\int_0^t \lambda(\tau)\exp\left\{-\int_0^\tau \lambda(s)ds\right\}\beta(n_0 - 1)e^{-\beta(t-\tau)}(1 -$

 $e^{-\beta(t-\tau)})n_0^{-2}d\tau, \ (\lambda(\tau) = a + b \cdot d(\tau))$

C. Fisher and Holloman (33): Multicell theory
 —six or seven different transformed cells needed for tumor development
 —time from tumor detection to death negligible so that mortality from cancer is the same as the rate of appearance of tumors
 —exposure of carcinogen from birth to death a constant at dose d
 —tissue with N cells
 —$I(t) \sim N \cdot (N\lambda)^r t^{r-1}/(r - 1)!$ for $\lambda t \ll 1, \ N \gg r$
 —$I(t) \sim N \cdot (Nbd)^r t^{r-1}/(r - 1)!$ for $\lambda = bd$

D. Stocks (36); Nordling (35); and Muller (34): Early multistage theory
 —single cell origin of tumor
 —only one mutation experienced by a cell in one year
 —transition rates the *same* for all stages, leading to $I(t) \propto d^r$

E. Mixed multistage/multicell theory
 —e.g., two kinds of transformation required: type 1 and type 2
 —for tumor to develop, r cells must have transformation 1; $n - r$ cells must have transformation 2
 —$I(t) \sim Nr(a_1 + b_1d)^r(a_2 + b_2d)^{r-n}/(n - r)!n!$

F. Armitage and Doll (37): The basic multistage framework
 —tumor develops from a single cell which has undergone a number (say, r) different transformations
 —transition rates not necessarily the same for all stages
 —order of transitions could be important
 —simplified expression: For continuous exposure d, $\lambda_i = a_i + b_id$, with $a_i \geq 0$ and $b_i \geq 0$.

 $I(t) \sim N \cdot t^{r-1}/(r - 1)! \cdot \prod_{i=1}^{r}(a_i + b_id)$.

 —for 2 stages affected by the carcinogen, $I(t) \sim Nt^{r-1} \cdot (\alpha_1 + \alpha_2d + \alpha_3d^2)/(r - 1)!$

[a] $I(t)$ is the incidence rate; N, the number of cells in the tissue in question; λ, the probability per unit time of a transition; r, the number of stages required for tumor development; and, d, the dose rate. Most of the information taken from Whittemore and Keller (11).

Doll (37): cancer originates with a single cell that has undergone a number of changes. Stocks' formulation has each of the required changes affected by the presence of a carcinogen to the same degree. However, experimental evidence shows the incidence of several cancers to be proportional to the dose or the dose squared, but not to higher powers of the dose, so Armitage and Doll allow for different transition rates λ_i between the required stages and also for the possibility that some transitions are not affected by the presence of the carcinogen. Thus, when mutations (or some other transition event) are rare and the transition rates remain constant,

they give incidence at age t, $I(t)$, for a seven-stage process as

$$I(t) = k\lambda_1\lambda_2\lambda_3\lambda_4\lambda_5\lambda_6\lambda_7 t^6, \tag{16}$$

where k is a constant. With these assumptions, tumor incidence is directly proportional to the concentration of the carcinogen if the probability of one mutation is proportional to the carcinogen concentration and different factors are required for the other transitions. In general, if a cell has to pass through r stages before becoming overtly tumorous, then the probability per unit time per cell for the transition to a tumorous state is (providing I is small enough)

$$I_r(t) = \left\{\prod_{i=1}^{r}\lambda_i\right\}t^{r-1}/(r - 1)! \tag{17}$$

and the probability that any given cell is tumorous by age t is just the integral of this:

$$p_r(t) = \int_0^t I_r(s)ds. \tag{18}$$

If, as Stocks suggested, the probability of occurrence of each mutation is proportional to dose, then each term λ_i would be proportional to dose and the overall probability $p_r(t)$ would have a component proportional to $(\text{dose})^r$.

For a total of N cells in an organ, the probability that k have transformed to a tumorous state is just (provided that tumorous cells act independently)

$$p_k(t) = \binom{N}{k}[Np_r(t)]^k[1 - Np_r(t)]^{N-k}$$

$$\approx \frac{[Np_r(t)]^k}{k!}\exp[-Np_r(t)] \tag{19}$$

where the Poisson approximation is effectively perfect, since N is very large. Thus the probability of one or more tumorous cells in the organ at age t is just

$$p(t) = 1 - \exp[-Np_r(t)]. \tag{20}$$

If all but one of the λs remain constant in time, and one, say λ_s, varies when a total of r stages are required, the incidence I can be obtained as

$$I(t) = \frac{1}{(s - 1)!(r - s)!}\left(\prod_{i=1,i\neq s}^{r}\lambda_i\right)$$

$$\int_0^t \lambda_s x^{s-1}(t - x)^{r-s}dx \tag{21}$$

This expression can be useful in analyzing data from experiments with discontinuous dosing (38–40).

For example, if the carcinogen only affects the sth stage, and the effect on this stage is linear in some effective dose rate $[d(t)]$, which may vary with time, combining the last two equations gives

$$p(d,t) = 1 - \exp\left\{-\left(\frac{t}{t_0}\right)^r\right.$$
$$\left(a + \frac{b}{B(r-s+1,s)} \int_0^1 d(vt)(1-v)^{r-s}v^{s-1}dv\right)\right\}$$

(22)

where t_0 represents a nominal lifetime, $B(\cdot)$ is the Euler beta function, and a and b are constants (related to background tumor rate and potency, respectively). This formula is linear in the dose-rate function $d(t)$, but this effective dose could be a nonlinear function of applied dose; for example, if there was a nonlinear pharmacokinetic relation between the two.

Formulations like this, based on the idea of a single cell going through five or six stages, are limited in that they do not adequately describe how the fully transformed cell develops into a tumor. Subsequent biological theories of carcinogenesis have described cell transformation as a two-stage, or at most, a three-stage process. In the first stage the cell is initiated, a process usually thought to require a mutation; in the later stage(s) the cell becomes fully transformed through a process of promotion, for which many different mechanisms have been proposed. This leads naturally to attempts to divide carcinogens into initiators and promoters. Initiators are assumed to act on the normal cell, or on an early transformed stage, and may thus be described as early carcinogens; promoters are assumed to act on a later stage and are late carcinogens. Further, initiators are expected to cause mutations in appropriate test systems, but promoters are not. A complete carcinogen can act at each stage, either because it is a mixture (such as cigarette smoke) of initiator(s) and promoter(s), or because even as a pure chemical it can perform either function.

Armitage and Doll (41), Moolgavkar and Knudson (42), and others have developed two stage models in which cells which are not fully transformed can proliferate. These are discussed in the reviews mentioned previously (10–12).

GENERALIZED MULTISTAGE.   Various generalizations of the multistage idea can be attempted, but their relevance to analysis of dose-response data from experiments can be questioned. To illustrate, consider the following generalization.

Assume that a cancer arises after a sequence of n events have occurred in a cell—possibly a sequence of DNA damage events incorporated into the genome. If four possibilities are allowed for a given cell (mitosis, death, another event in the sequence, and repair of the previous event), one can write the probability of one or more cells being in the state in which k events have occurred by age t as $Q_k(s,t)$ evaluated at $s = 0$, where $Q_k(s,t)$ satisfies the partial differential equation:

$$\frac{\partial Q_k}{\partial t} = [\phi_k(1 - e^{-s}) + \lambda_{k-1} +$$
$$\mu_k(1 - e^{+s})]\cdot\frac{\partial Q_k}{\partial s} - \lambda_{k-1}e^{-s}\left\{\frac{\partial Q_{k-1}}{\partial s}\Big|_{s=0}\right\}$$

(23)

Here ϕ_k is the probability per unit time for binary division; μ_k is the probability per unit time for cell death; λ_k is the difference between the transition rate from state k to state $k + 1$ and the repair rate from state $k + 1$ to state k.

This model incorporates repair only between adjacent states, but it could clearly be extended to deal with more than two types of damage at the same time. The transition rates between states may depend on the dose of carcinogen, the division rate, and other factors. Formulae including some of these complexities have been written down by several authors. This one is written here simply as an illustration, not to encourage its general use. To use it would require an enormous amount of information, on at least $3r$-2 parameters, all as a function of age (and in the case of the transition rates, and possibly death rates, as a function of dose also). Obviously, there would never be enough data to estimate all the parameters from a statistical fit to experimental bioassay data. To even attempt use of the formula would require finding far more information (for example, on cell division rates), most of which is not currently known.

POLYNOMIAL FORMULAE.   Many of the dose-response formulae, especially those used for extrapolating risk estimates to low dose, can be derived as special cases from multistage models. There are two common ways of modifying the simple formula given by Armitage and Doll in their 1954 paper (Eqs. 16 and 17).

1. Write transition rates explicitly as linear functions of the carcinogen dose rate (d):

$$\lambda_i = a_i + b_i d$$

(24)

[Armitage and Doll (37) suggested the use of a linear relationship, but did not write the explicit formulae.] If a particular stage can occur only in the presence of the carcinogen, [i.e., the background rate for the stage is zero $(a_i = 0)$], then $\lambda_i = b_i d$; conversely, if the carcinogen cannot affect the occurrence of a particular stage, then $b_i = 0$ and $\lambda_i = a_i$. Substituting $a_i + b_i d$ for λ_i in Equation 17 gives

$$I(t,d) = \left(\prod_{i=1}^r (a_i + b_i d)\right) t^{r-1}/(r-1)!$$

(25)

It takes time for a transformed cell to give rise to an observable tumor. Ignoring this consideration, an expression for the probability of a cancer by age t can be obtained from Equations 18, 20, and 25,

$$p(d,t) = 1 - \exp\left\{ -k \left(\prod_{i=1}^{r}(a_i + b_i d) \right) t^r \right\} \tag{26}$$

where extra constants have been absorbed into k and it is assumed that the dose rate d is constant over the lifetime.

When an analysis is always performed with data from lifetime experiments, the age t in Equation 26 may be treated as a constant and the dose-response formula simplified to

$$p(d) = 1 - \exp -(q_0 + q_1 \cdot d + q_2 \cdot d^2 + \cdots)$$

$$q_i \geq 0 \tag{27}$$

which at low doses reduces to a simple polynomial

$$p(d) = u_0 + u_1 \cdot d + u_2 \cdot d^2 + \cdots \qquad u_i \geq 0 \tag{28}$$

Strickly speaking, Equation 27 is only an approximation to Equation 26, since it does not take account of all the relationships expected between the q_i obtained when the polynomial in Equation 26 is expanded. However, it is used as a heuristic dose-response curve, not in order to obtain the a_i and b_i in Equation 26.

2. A second common modification of the Doll-Armitage formulation explicitly includes the time it takes for a single transformed cell to give rise to an observable tumor. Most analyses assume this time to be independent of dose, and very often the time-to-tumor or latency period is assumed to be a constant, w. If in addition, the time it takes to fully transform a normal cell follows the Doll and Armitage form given in Equation 17, the probability of tumor at time t is given by

$$p(d,t) = 1 - \exp\left\{ -k \left(\prod_{i=1}^{r}(a_i + b_i d) \right) (t - w)^r \right\}, \tag{29}$$

where the product is often treated as before as a simple polynomial with positive coefficients. Once again, derivation of this formula has assumed constant dose rate, and once again, if the age t is assumed to be constant, the term in the exponent is reduced to a polynomial in dose rate.

THE EPA'S MULTISTAGE APPROACH. The multistage formula used by the EPA to determine safe exposures to carcinogenic chemicals is that given in Equation 27. Computer programs are available to solve for the parameters a_i in Equation 27 (*39,43*). If the animal bioassay data under analysis has n dose groups, it is possible to fit terms in the polynomial up to d^{n-1}, with each term constrained to be positive. However, if the resulting dose-response formula does not fit the experimental data very well, the results from the highest dose group are omitted, and the procedure repeated until a satisfactory fit is obtained (*44*). The excess risk caused by a dose d in Equation 27 is dominated by the term

linear in dose at sufficiently low doses, so the EPA approach is to find an upper confidence bound on the estimate of q_1, and use this to extrapolate to low doses. The EPA selected this approach because of its built-in conservatisms, but the dose-response formula used clearly cannot adequately represent some (observed) dose-response relationships. For example, Equation 27 implies that the probability of tumor will approach unity at sufficiently high doses. Vinyl chloride provides a counter-example for this feature of the formula. The bioassay data showed a maximum probability of tumor (all tumors) of about 40% at high doses, and a maximum probability for angiosarcoma alone of about 20% (Fig. 5).

TIME-TO-TUMOR MODELS. Latency periods can be fairly easily incorporated into multistage models; Equation 29 represents one way of incorporating a fixed period between the causal event and its manifestation. If the latency period can only be specified by a probability distribution, with density function $g(\tau)$ at latency period τ, the resultant model can be obtained as a convolution of this density function with the incidence function for fixed latency period. For example, if the incidence for fixed latency period τ is

$$I(\tau,t) \sim f(d)h(t - \tau) \tag{30}$$

[cf. Eq. 29 where $h(x) = x^{r-1}$ and $f(d)$ is a polynomial in dose rate] then the incidence with a distribution $g(\tau)$ for latency periods will be

$$I(d,t) \sim \int_0^t f(d)g(\tau)h(t - \tau)d\tau$$
$$= f(d)G(t) \tag{31}$$

where $G(t)$ will depend on the parameters describing $g(\tau)$ and thus may be estimated from data. This description assumes a constant dose rate, and $f(d)$ might well be a polynomial in this dose rate d. [For examples of such forms, see Peto and Lee (*45*) and Hartley and Sielken (*46*).] If the dose rate is not constant, the formulation is more complicated to write (although still mathematically straightforward), and much more difficult to use in the analysis of data.

Mixed Approaches

The current standard practice in estimating cancer risks is to postulate first, that one of the formulae described above (or a similar one) can adequately represent a dose-response relationship over some range of doses where excess tumor incidence is high enough to be observable, and second, that at lower doses this formula will provide either a good estimate or an upper bound to the excess probability of tumor. Using experimental data then allows estimation of the parameters of the chosen formula, which in turn allows extrapolation (strictly, an interpolation; we shall not distinguish the two here) to the lower doses.

In addition to data from long-term animal studies, there is much information relevant to dose-response modeling which is now formally ignored because it is unclear how it should or could be included. For example, some chemicals are known to increase rates of cell division but apparently do not act as initiators (they are not mutagenic, or only very weakly so, in current test systems). If the multistage description is to be credible, such chemicals should affect tumor rates only indirectly, and the formulae derived by assuming a direct effect on transition rates between stages should not be applicable. However, it is still unclear whether the models, and hence the formulae, should be modified for these chemicals; in particular, it is unclear under precisely what conditions such modifications should be made. Another example of information that is currently not incorporated in models is that on the pharmacokinetics of carcinogens. This information is generally acknowledged as important, but its use in estimating risks from carcinogens is still controversial. However, there has been much recent interest in formally incorporating known pharmacokinetic details in cancer models.

The Pharmacokinetic Models. The formulae obtained above have been derived to allow interpolation between doses. The structure of such formulae were guided by mathematical models based on descriptions of the biological processes involved, but in most cases the parameter values in the formulae were ultimately to be obtained from experimental data on tumor incidence alone. The aim of a pharmacokinetic model, in contrast, is to describe some parts of the process using pharmacokinetic parameters measurable in subsidiary experiments; in particular, the model aims to obtain a better approximation to an effective dose rather than relying on the applied dose. The hope is that there is a simple relationship between excess tumor incidence and the effective dose, a relationship that extends even to very low effective dose. This approach has been used to explain, for example, the observed dose-response relationship for vinyl chloride. The first priority was to understand the metabolism of vinyl chloride (47–50), then to measure in auxiliary tests on rats the production of metabolites as a function of vinyl chloride dose. It was found that the amount of vinyl chloride metabolized does not increase proportionately with the inhaled dose. An increasing fraction is exhaled because the oxidation pathway responsible for the activation of vinyl chloride to a chemically active metabolite becomes saturated. When the fraction of animals with angiosarcoma was plotted as a function of the metabolite concentration, an apparently linear relationship (without saturation) was obtained, although Gehring et al. (51,52) report that this effective dose versus response plot was also consistent with a probit formula. However, the concentration of active metabolite may or may not be linear with applied dose at low doses, for no adequate measurements of the metabolite at exposures comparable to environmental exposures have been made. In addition, the analysis did not consider the high mortality in high treatment groups due to causes other than angiosar-

coma; this appears to have contributed significantly to the downward curvature in the dose-response curve (N. Gravitz, personal communication).

The relationship between applied dose and effective dose (in this case, metabolite formation) is usually unknown at low doses. Michaelis-Menten kinetics might be assumed, and used to extrapolate to low doses. However, as Hill et al. (53) point out, experimental results are frequently inconsistent with the Michaelis-Menten formulation, especially when a wide range of concentrations are used. As a general approach, the pharmacokinetic approach is clearly desirable as part of any attempt at low dose extrapolation, but it requires a knowledge of the pharmacokinetics at low doses, identification of the metabolite(s) that cause the tumors, and still calls for the choice of a formula to represent the relationship between metabolite concentration and response. This information is also needed in applications of the more general physiologically based pharmacokinetic model(s) (54), which take into account processes of transport, absorption, and excretion, in additon to metabolism.

Some of the Biological Information Required for a Full Description. The multistage framework can incorporate pharmacokinetic mechanisms as well as several additional ideas about carcinogenesis. Pharmacokinetic descriptions are usually used to estimate the concentration of the ultimate carcinogen at the target site, and the time dependence of that concentration, as a function of the exposure, and so can be taken into account in the modeling of the first stage of a multistage process. The multistage model can then be further extended to include more detail on cellular processes that influence the genesis of cancer.

A complete formulation would include, at the very least, details of the following:

1. Entry of the original material into extracellular fluids and transport around the body.
2. Receptors on and transport through cell membranes.
3. Receptors within the cytoplasm and transport within the cell.
4. Reactions producing the actual carcinogen, e.g., cytochrome P-450 enzyme activation reactions, as well as deactivation reactions.
5. Transport of the carcinogen throughout the cell.
6. Transport of the carcinogen across cell membranes.
7. Transport of the carcinogen through extracellular fluids.
8. Transport of the carcinogen through target tissue cell membranes.
9. Receptors within the target cells and transport throughout such cells.
10. Action on the DNA and other cellular components.
11. Repair mechanisms (e.g., for DNA and other targets) and their failures.
12. Cellular division rates, which may be affected by the cytotoxicity of the chemical, and mechanisms which act to prevent subsequent repair of damage.

13. Other necessary effects before a cancerous cell is generated.
14. Immune system responses of the whole organism to the altered cells or products of such cells.
15. Cell to cell communication.
16. Growth rates of damaged cells.

This must not be considered a complete list; it simply shows some of the expected complexity of the processes which should be modeled. Clearly, many of the processes can contribute to a nonlinear dose-response relationship.

Steps 1 to 3 of this list represent initial entry and transport of the material of interest. Step 4 is an activation step (if necessary). Steps 5 to 9 are some stages of the transport of the carcinogen that may differ significantly between species. Steps 10 through 15 represent the mechanisms that go into the microscopic dose-response relationship. If the chemical of interest is not an initiator and/or does not interact with DNA, it may nevertheless still be a promoter and so steps 1 through 9 are still relevant, as are steps 12 through 16.

One would expect to see quantitative species differences in rates of transport and in activation rates, for example, but there may also be qualitative differences. Steps 1 through 9 may combine in such a way that the effective microscopic dose to the affected cell has a different relation to the externally applied dose in different species. Then the shape of the dose-response relationship may differ from species to species. For example, if the enzymes necessary for an activation step are available only at much lower levels in one species, they may be saturated in that species at much lower doses.

DNA Adduct Formation as a Measure of Effective Dose. Much of the work now in progress on pharmacokinetics is on the study of steps 1 through 9 of the schema described. If carcinogenesis is indeed due to direct action of the carcinogen on DNA, then it might be hoped that a measurement of this action would give a very good measure of effective dose. This would allow a better description of steps 1 through 9, and, paradoxically, might remove the necessity for modeling these steps to obtain dose-response formulae by allowing direct measurement of the effective dose instead of the applied dose. One measure of the action of a material on DNA is the production of DNA adducts, and such a measure is one possibility for an effective dose.

The usually observed linearity between DNA adduct formation and applied dose could be interpreted as showing that in practice the effective dose is indeed proportional to the dose applied to the animal. On the other hand, Hoel et al. (55) suggests that the relation between DNA adducts (possibly specific adducts rather than total adducts) and cancers is likely to be linear, but that the relation between applied dose and the effective dose (measured by DNA adduct concentration) might be nonlinear because of nonlinear kinetic mechanisms. There are cases where there appears to be a nonlinear relationship between DNA adduct formation and applied dose. Figure 7 shows this relationship for
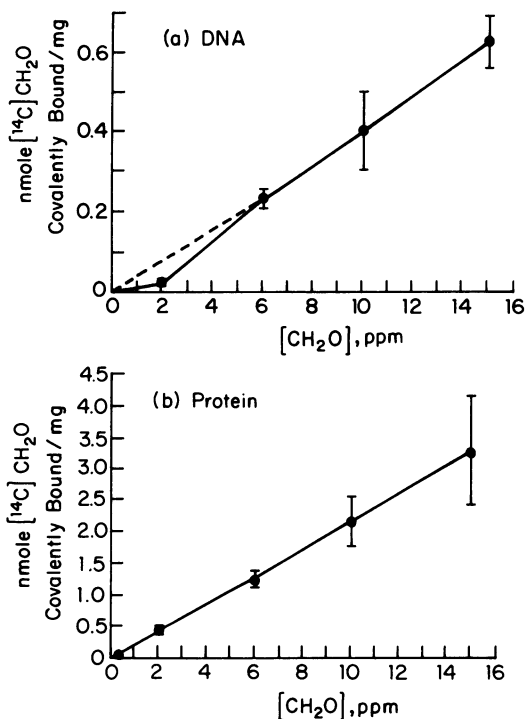

FIGURE 7. Mean concentration of covalently bound formaldehyde (*209*) in (*a*) the DNA, and (*b*) proteins, from the respiratory mucosa, after exposure to varying concentrations of formaldehyde (CH_2O).

formaldehyde, where the DNA adduct concentration is sublinear at low doses, even though the corresponding curve for binding of formaldehyde to protein stays linear.

The studies of DNA adduct formation indicate that for some chemicals the dose-response relation for adduct formation is linear, or very nearly so, over the very wide range of doses from those at which tumors may be observed in experimental animals down to those to which humans may be exposed in the environment (Figs. 8 and 9). Such a result could indicate linearity in the combination of steps 1 through 10 above. However, DNA adducts do not themselves represent a stage in the development of cancer, since they are a measure of the total amount of DNA repair. It is presumably the misrepair or nonrepair of DNA which forms one of the stages in the process, not the total amount of repair. Very small variations between DNA adduct formation and unrepairable DNA damage can lead to very different dose-response relations for tumor formation versus dose (see the precautionary remark below). Without a direct measurement of such unrepairable (or unrepaired) damage, observation of total adduct formation cannot provide sufficient sensitivity to detect possible nonlinearity.

Nonetheless, it would be interesting to compare the production of DNA adducts for different compounds at different sites. The site and number of adducts formed may correlate well with the variation in carcinogenic
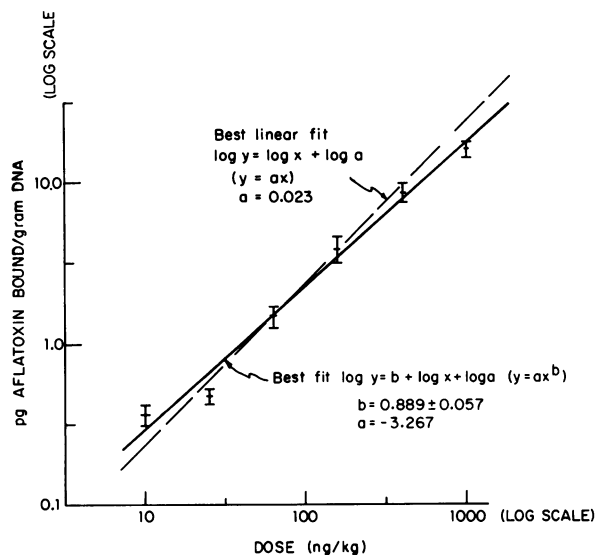
FIGURE 8. DNA adducts of aflatoxin in cells of the liver (158) 1 hr after administration of varying doses of aflatoxin.
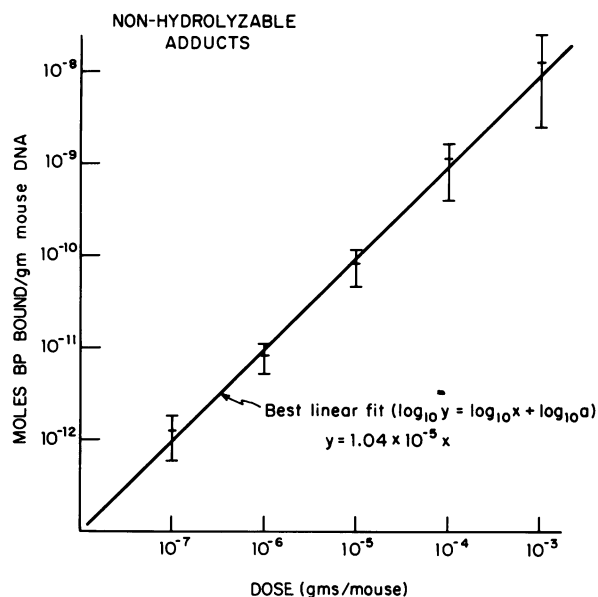

FIGURE 9. DNA adducts of benzo[a]pyrene in cells of the stomach (56) 18 hr after administration of varying doses of aflatoxin. 10^{-3} g/mouse corresponds to a dose of 30 mg/kg.

potency (as computed from high dose experiments in animals). Without such a correlation, with high explanatory power, it would be unbelievable that the dose-response curve for DNA adducts could be used as a surrogate for a cancer dose-response curve. But even if such a correlation exists, one should still be extremely cautious about using such a surrogate to extrapolate to low doses.

DNA ADDUCTS—PRECAUTIONARY REMARK. If an animal is fed a dose of carcinogen which may be expected to produce observable numbers of tumors [say 10 μg of

benzo[a]pyrene (38,56)] in some target organ, it is observed that the number of DNA adducts formed within that target organ is of order 10^{10} (i.e., about one per million molecules of benzo[a]pyrene). The fraction of animals getting tumors in the target organ at such doses is of order 0.1, so there is a ratio of about 10^{11} between adduct formation and cancer as an end point (57). Suppose there is one particular faultily repaired site on the DNA, at which adduct formation and excision necessarily leads to a tumor (or which is linearly related to tumor formation). The dose-response relation for adduct formation at this site, and hence for tumor formation, could be highly nonlinear, for example:

$$n = (d/d_0)^3 \qquad (32)$$

where n is the total number in the target organ of such sites affected at dose d, and d_0 is a typical dose which causes observable tumors. If there are also other, perfectly repairable sites with a linear dose-response relation:

$$N = 10^{11} \cdot (d/d_0) \qquad (33)$$

where N is the total number of sites in the organ affected, then the overall dose-response relation between total adducts and dose would be simply:

$$\text{Total adducts} = 10^{11} \cdot (d/d_0) + (d/d_0)^3 \qquad (34)$$

which is experimentally indistinguishable from linearity (for doses below $10^4 \cdot d_0$, which would probably kill the animal outright).

This example is given solely to point out the possibility that there may be nonlinear dose-response relations hidden in the overall linear observations of adduct formation. It would be evident that one could substitute practically any nonlinear term for the cubic term above with the same result. The enormous factor of 10^{11} between the total adduct formation and the final response of interest can hide practically anything. In particular, the mechanism of this example is not the only one which might be operating to cause a nonlinear carcinogenic response even with linear DNA adduct formation. There is some evidence for a two-step mechanism, with cell division fixing the DNA damage in some way. If the unrepairable DNA damage is produced linearly at a low rate, but the rate of cell division is affected by dose, then one could also obtain a nonlinear dose-response relation for carcinogenesis.

Examples of Mixed Approaches. A COMBINED PHARMACOKINETIC AND MULTISTAGE MODEL FOR VINYL CHLORIDE. Bois et al. (58) describe the body by compartments, into and out of which vinyl chloride is transferred by blood flow. In each compartment the parent compound is metabolized and the metabolites and parent compound may be absorbed, circulated in the blood, excreted, or detoxified. They may also be bound on proteins or DNA. The model also allows for some of the DNA adducts formed to be removed by

repair mechanisms. Michaelis-Menten equations are used to describe activation, detoxification, and repair processes, but binding is considered to be a first-order process. The number of bound DNA adducts is used as the effective dose for the cancer process. The physiologic and pharmacokinetic components of the model are similar to those developed by Ramsey and Anderson (54).

The process of carcinogenesis is modeled by postulating that cells are in one of three states, normal, initiated, or cancerous, with cells in these states growing, dying, differentiating, and changing between states. The transition rates between states are taken to be a function of the DNA adduct level in an organ. The probability that one or more cells are transformed to the cancerous states corresponds to the probability of cancer.

The application of this model to vinyl chloride is discussed in "Vinyl Chloride."

A MULTISTAGE MODEL FOR SACCHARIN-INDUCED BLADDER TUMORS. Greenfield et al. (59) have developed a two-stage model of carcinogenesis, which they apply to the study of bladder carcinogenesis. Three cell populations, normal, initiated, and transformed, increase or decrease in number according to mitotic and transition rates which are allowed to vary in time. Certain predictions of the model are directly verifiable, since it is possible to distinguish transformed cells from the other two types on histological slides, so that mitotic rates and population sizes may be measured. Model predictions can be matched with tumor data using various assumptions for the transitions rates, and this procedure has been used to test various hypotheses on how saccharin increases the activity of FANFT (N-4[-(5-nitro-2-furyl)-2-thiazolyl]formamide) (see "Saccharin").

Remarks

It is a common premise that cancer arises from a single cell and that one or more mutations, caused directly by damage to DNA or by errors during DNA replication or repair, are a necessary initial step in the process of carcinogenesis; but this premise is not universally accepted (60,61). The relationship between effective dose and mutation frequencies is usually presumed to be linear, based on several examples of linear dose-response relationships for mutation in bacterial systems, although there are some noted examples of nonlinearity (e.g., nitrosamines). However, there are other processes, such as mitosis, cell death, and intercellular communication, which are also important in carcinogenesis but which this formulation ignores. Indeed, Rubin (61) believes that mutation plays a minor role and that cellular interactions are much more important. The dose-response relationship associated with such processes would be expected to be highly nonlinear.

Intercellular Communication. Trosko (62) provides a description of how inhibited metabolic transformation might promote the process of carcinogenesis. An inhibited cell in the healthy environment of normal cells is unlikely to give rise to a tumor, since most mammalian genes exist in allelic pairs and the neoplastic phenotype is usually recessive to the normal phenotype. For example, when certain normal and transformed cells are injected into nude mice, tumor growth is suppressed; in cell growth fusion studies of certain normal and transformed cells, normal hybrids result. Somehow normal cells must exchange information with initiated cells. This communication could be blocked by the carcinogen or promoter. When blocking occurs, the initiated cell proliferates. As the mass of initiated cells grows, further genetic changes occur so that the cell itself cannot communicate and can metastasize.

Trosko has developed an *in vitro* assay to measure the degree to which chemicals interfere with the metabolic cooperation of cells and has tested at noncytotoxic doses many chemicals known to be promoters. He reports an apparent threshold in the dose–response relationships. Below the threshold the chemical does not appear to affect the ability of cells to communicate; above the threshold the effect increases with dose. However, the importance of this result is uncertain because the test is so new and is still being reviewed by the scientific community; on this matter, Yamasaki (63) claims that "there are no scientific data which indicate the existence of a threshold dose [by promoters]."

True and Secondary Carcinogens. It has been suggested that whereas true cancers are caused in healthy individuals by direct action on the cell, other, secondary cancers can occur when the body is severely damaged or weakened. Other authors have used the words "direct" and "indirect" instead of true and secondary. These secondary cancers would be expected to have a dose-response relationship similar to that of a toxic response; i.e., a threshold type, such as may be modeled by the probit formula. We mention here three (not mutually exclusive) mechanisms.

1. Toxicity can affect the rate of initiation by increasing the rate of fixation of DNA damage. Farber (1,2) and others suggest that mutation is a necessary but not sufficient step in the process of initiation. A cycle of cell proliferation may be necessary to fix the mutation. This is well established for the liver and appears to be true also for the bladder and pancreas. Regeneration rates in these organs increase with the degree of toxic insult. This has obvious importance for the types of assumptions made in modeling initiation rates. Farber emphasizes that, especially for quiescent organs (e.g., bladder, pancreas, liver, thyroid), the rate-limiting step for initiation may be cell damage and the regeneration that results; although many carcinogens can interact with DNA, only those that can induce reparative cell proliferation, due to their cytotoxicity, are likely to result in cancer. He emphasizes this relationship between cancer in these organs and chronic tissue damage.

We have found a statistical correlation between the finding of carcinogenic effect in the NCI/NTP bioassays and the chronic toxicity of the dose given in these bioassays (40,64–66), and a similar correlation was reported by Parodi et al. (67). However, there are many other

plausible mechanisms that would give the same correlation.

2. Bradley (68) has proposed that, in response to sublethal toxicity, the cell releases the contents of some lysosomes. DNA hydrolases contained in these lysosomes might enter the nucleus, causing single and double DNA strand breaks. In cell culture of rat hepatocytes, a relationship between cytotoxicity and single strand breaks has been found (69).

3. One of the toxic effects of a xenobiotic might be interference with metabolic processes in such a way as to alter the communications between cells. This could reduce the efficiency of the immune system and reduce the normal scavenging of abnormal cells, allowing their proliferation.

There are several carcinogens which might act through such mechanisms. Benzene is only known to cause leukemia in humans after the bone marrow has been damaged by toxic effects and pancytopenia has set in. Bladder cancer, produced by saccharin and by some industrial dyes, may be secondary to chronic irritation of the bladder. The same might be true for formaldehyde and nasal irritation. Sixty percent of the chemicals studied in the NCI/NTP Carcinogenesis Bioassay series have been found to be carcinogenic, but only 30% would have been so found if the maximally dosed group were omitted from the analysis (although this may simply be a result of lower statistical power of such an analysis). The observed correlation of measured carcinogenic potency and acute toxicity would be explained if a large fraction of these chemicals (of order, half) were secondary carcinogens, but the data are inadequate to make such a distinction.

Radiation Carcinogenesis vs. Chemical Carcinogenesis. "Human Studies" and "Animal Studies" present data on dose-response relations in animals and humans. Some of the discussion concerns the effects of radiation, since human exposures have been widespread and measurements of these exposures are available. The rest discusses the data on chemical carcinogenesis, usually from organic compounds. There are differences between radiation and chemical carcinogenesis, and these differences are reflected in the theories and mathematical formulations of dose-response relationships.

Radiation has the ability to penetrate cells and deposit energy within them randomly and is unaffected by the usual cellular barriers presented to chemical agents (70). Furthermore, while radiation carcinogenesis involves the formation of reactive oxygen species by the process of ionization, no metabolic activation step is required, so that the cellular machinery for such activation is not required. Cancer induction from external radiation exposures (e.g., X-rays) can be described simply, since carcinogen distribution, excretion, and bioaccumulation, which might contribute to the nonlinear structures of dose-response relationships, are absent. Any observed nonlinearities thus have fewer possible explanations, although the list is still fairly long, e.g., dose-dependent repair, multiple hits, cell killing and cell scavenging. Exposure to internally deposited radioactive compounds is complicated by distribution in the body, bioaccumulation, and radioactive half-life. However, dose is usually measured by the amount of energy per unit volume (rads) delivered to a particular target site; so, in contrast to chemical carcinogens, dose is already presumably in units of effective dose.

A distinction is generally made between high and low LET radiation. LET, or Linear Energy Transfer, is a measure of the average amount of radiation energy deposited per unit distance that the radiation travels (track length) where conventionally, energy is measured in units of 1000 electron volts (keV) and length in microns (μm). High LET radiation energy is typically dissipated in cells at surfaces (e.g., alpha radiation from radon daughters) and low LET radiation is deeply penetrating (e.g., X-rays). Physicists tend to emphasize the effect on the radiation and use the expression "energy loss" or "dE/dx" instead of LET.

For high LET radiation at high doses there are several examples where the dose-response relationship appears superlinear. This is usually attributed to cell killing (71); however, similar saturation effects are observed in cell culture in which only surviving cells are assayed. Thus, the superlinearity cannot be entirely due to sterilization, but perhaps to "some form of cell selection associated with cell killing" (72). In any event, the mechanisms are apparently quite different from those observed for chemical carcinogenesis.

For low LET radiation, it has been postulated that two nearly simultaneous hits on a cell are required to produce a single mutation (an event initiating a cancer). This would lead to a dose-response relationship that is quadratic in dose, and so sublinear. For high LET radiation, a single hit is supposed to be sufficient, leading to a linear dose-response relationship. Cohen (73) has reviewed the various lines of evidence supporting a sublinear dose response for exposure to radiation at low levels. In some experiments a given dose is less carcinogenic when spread out in time than when given acutely, suggesting that damage produced at low dose rates is more fully repaired than that produced at high dose rates. There have been various explanations for this phenomenon, but one is to postulate that single strand chromosome breaks are easily repaired, while repair of a double strand break, much more prevalent at high dose rates, is difficult and faulty. High LET radiation is found to be biologically more damaging than the same dose of low LET radiation. Since high LET radiation concentrates damage on a much smaller volume of tissue, it is more probable that multiple hits will occur within a small region. Assuming that two unrepaired hits within a small region are required for any initiation step, Cohen suggests two possibilities: double hits may be made either by the same or by separate particles. The number of double hits is proportional to the dose if the hits are by the same particle, whereas it is proportional to the square of the dose if two particles are required.

At low doses it is unlikely that two particles will strike the same sensitive volume and so a double hit occurs

mostly because a single particle acted twice within a small region. This leads to a linear dose-response relationship in the low dose region. At higher doses, double hits from two particles become likely, so here a quadratic term predominates. The precise point at which the quadratic effect begins to dominate depends on the type of radiation and what constitutes a sensitive volume. As a rule, the observation of an effect quadratic in dose should occur at much lower doses of low LET radiation, since it is much less likely that, at a given energy, a single low LET particle will deliver a multiple hit. For the very high LET alpha particles, which mostly deliver multiple hits, no dose-squared effect is expected. Animal studies are consistent with this view: Data on cancer caused by exposure to X-rays, gamma-rays, and beta-rays frequently indicate upward curvature for external radiation and internally absorbed radionuclides; alpha particles appear to give a linear relationship at low doses. In "Human Studies" we discuss how well human data agrees with this simplified treatment.

Human Studies

Introduction

Information on excess human cancer incidence and the exposures leading to it is rarely sufficient to characterize a dose-response curve, and never as complete as desirable. The best data available are for cases in which modulating factors have been identified as important (74). Aflatoxin B_1, for example, is known to consistently induce liver cancer in animals, but appears to be a cause of cancer in humans only when there is simultaneous exposure to the hepatitis B virus, or in the presence of cyles of famine and plenty. Human esophageal cancer can be related to alcohol intake, but is most prevalent in alcoholics who smoke heavily (75). There are no human data good enough, nor can any conceivably be good enough, to directly estimate the dose at which excess lifetime risk is of order 10^{-6} to 10^{-5}, which are values typically demanded in regulation.

The best set of human data is that obtained in a study of cigarette smoking in British physicians. Over the range of 4 to 40 cigarettes per day, the incidence of lung cancer is adequately described by a quadratic function in the number of cigarettes smoked daily. Lung cancers caused by occupational exposures to coke oven emissions can also be described by a nonlinear dose-response function with upward curvature. The similarity is perhaps not surprising since coke oven emissions contain many active constituents also present in cigarette smoke. Despite voluminous data on human cancers caused by radiation, conflicting hypotheses of sub-, super-, and purely linear dose-response relationships cannot be ruled out, especially for low-dose exposures. For low-LET radiation, the dose-response relationship appears sublinear in the low dose region, superlinear at high doses, and linear in between. For high-LET ra-

diation, dose fractionation may increase response above that for the same total dose given in a single exposure, corresponding to a superlinear relationship between response and dose rate.

For exposures to several different materials, the dose-response curve obtained in human studies appears to be sublinear. The nonlinearity is seldom strong enough to be statistically significant, however, and may be an artifact of the analysis. When cumulative dose is used as the indicator of exposure, there is a strong bias toward such nonlinearity if incomplete account is taken of the age of exposure and the age distribution of those exposed (e.g., see "Coke Ovens" and "Lung Cancer from Exposure to Radon Gas"). On the other hand, if the dose-response curve is truly sublinear (so that it rises more rapidly than linearly with dose), but the measurements of dose and response are uncertain, then such measurements would indicate a dose-response relation which is apparently more linear (76). An unbiased analysis requires complete and accurate exposure and outcome histories for each person at risk. Most studies fall short of this, but nevertheless provide useful information, especially when the size and direction of the bias can be estimated.

In reviewing the data, we have found it useful to fit various formulae to the experimentally observed dose-response curves in order to make consistent comparisons. Unless otherwise noted, such fits are our own, not those of the original authors.

Cigarettes

British Physicians. There are now many epidemiological studies that correlate increases in lung cancer risk with the number of cigarettes smoked and other smoking characteristics (77). The most informative dose-response relationship is obtained from the study of lung cancer incidence in British physicians begun by Doll and Hill (78), and continued by Doll and Peto (79). In this study, the subjects were from a well-defined, uniform population, with good health records and accurate statistics, and the analysis carefully took into account the age and time dependence of tumor development. The relationship between the average number of cigarettes smoked daily (dose rate) and the incidence of lung cancer, standardized for the age of the physicians, was characterized by a function approximately quadratic in dose rate (79).

$$\text{Lung Cancer Incidence} \propto (\text{cigarettes/day} + 6)^2$$

$$(35)$$

This fits better than the best linear fit ($p < 0.01$ for curvature), although a straight line fits reasonably well (Fig. 10) and was the initial preference (78).

The data on cigarette smokers are so extensive that the effects of different ages of starting and stopping smoking can be distinguished, and matched with a biological model. Apparently cigarette smoke acts both
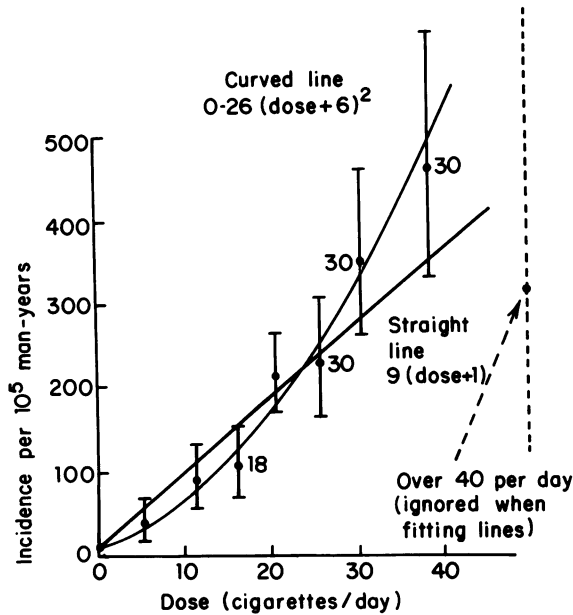
FIGURE 10. Age standardized tumor incidence associated with the smoking habits of British physicians (*79*). (98% confidence intervals shown about each point.)

as an early and a late stage agent (*76,80*). This might be expected, since cigarette smoke contains a mixture of initiators, promoters, and complete carcinogens (as determined in animals and *in vitro* experiments).

Tar Exposure vs. Incidence. An alternative measure of the dose of cigarette smoke, total tar exposure, has been used to study the effects of smoking (*81–84*). Lifetime tar exposure was estimated by counting lifetime cigarette consumption and weighting by the tar content of the cigarettes smoked. Relative risks were obtained by comparing smoking histories from Austrian lung cancer victims to those from hospital patients admitted for nonsmoking-related problems. Such relative risks are proportional to incidence, so that relative risk may be used as a surrogate for incidence.

Table 2 and Figure 11 illustrate the results obtained for two different types of lung cancer, squamous cell carcinoma (SCC) and Kreyberg I. The dose-response function appears to curve up in the region of low tar exposure and level off at high exposure. Fitting a linear function overestimates the relative risk at low doses, but grossly underestimates it at high dose. This behavior is especially pronounced for the squamous carcino-

mas. Kunze and Vutuc give the estimated relative risk for each dose group, but do not report sufficient information to assess the uncertainties of these estimates. Consequently, we cannot correctly weight each data point when fitting various formulae to these data. Nevertheless, in an attempt to discover whether there is any significant curvature in the dose-response curve at low doses, we fitted two alternative formulae using maximum likelihood techniques and assuming equal weighting for each point. The first formula was a simple quadratic curve, with the high dose points omitted from the fit. The second, using all the data, used the formula:

$$\text{Relative Risk} - 1 = (B \cdot D + C \cdot D^2) \exp(-A \cdot D))$$

$$(36)$$

(This formula has been used to analyze dose-response data, especially for radiation-induced cancers, in which the response levels off or falls at high doses. It is justified by an assumption that the high dose behavior is due to some such mechanism as cell killing, affecting all cells.) The estimated parameters for both formulae suggested a sublinearity at low dose, consistent with the findings of Doll and Peto, but this nonlinearity could well be an artifact of our procedure. The leveling off of response at high doses might have been due to overreporting of cigarette consumption by heavy smokers. Doll and Peto (*78*) found that those claiming to smoke more than 40 cigarettes per day were inaccurate in their estimates.

Coke Ovens

It has been suggested (*85,86*) that the dose-response relationship between coke oven emissions and lung cancer is nonlinear. Mazumdar et al. (*87*) estimated cumulative exposures for 8000 coke oven workers and compared these with their medical histories. If we fit polynomial formula to the raw Mazumdar et al. figures for age adjusted lung cancer incidence and cumulative exposure to coal tar pitch volatiles, a highly significant quadratic coefficient results, indicating strong upward curvature (Fig. 12). Such an exercise is misleading because cumulative exposures are not independent of age at entry into the cohort, length of exposure, or, most important, length of observation. The workers in the highest exposure groups were older, exposed longer,

Table 2. Relative lung cancer risk versus tar exposure at ages 51 to 60 years (*81*).

Tumor type	Tar exposure (arbitrary units)						
	<500	501–1000	1001–2000	2001–3000	3001–4000	4001–5000	>5000
SCC[a]	3.3	3.3**	30.1*	39.9*	48.1*	30.9*	56.9*
Kreyberg I	1.0	4.1**	15.7*	21.5*	20.8*	17.1*	21.5*
Total	4.3	7.4	45.8	61.4	68.9	48.0	78.4

[a]Squamous cell carcinoma.
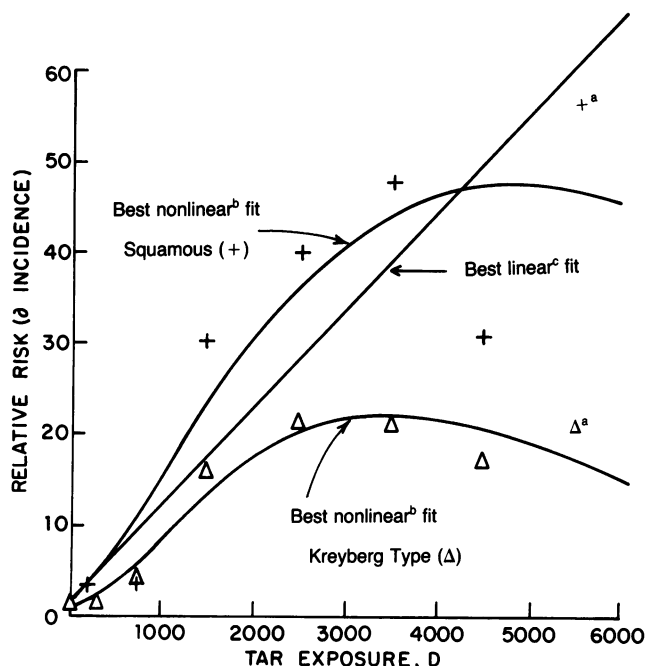*Significant at $p < 1\%$.
**Significant at $p < 5\%$.

FIGURE 11. Relative lung cancer risk versus tar exposure in Austrian males aged 51–60 *(81)*. [a] Dose greater than 5000. [b] Nonlinear formula: relative risk = 1 + $(\beta D + \gamma D^2)e^{-wD}$. [c] Linear formula: relative risk = 1 + βD.
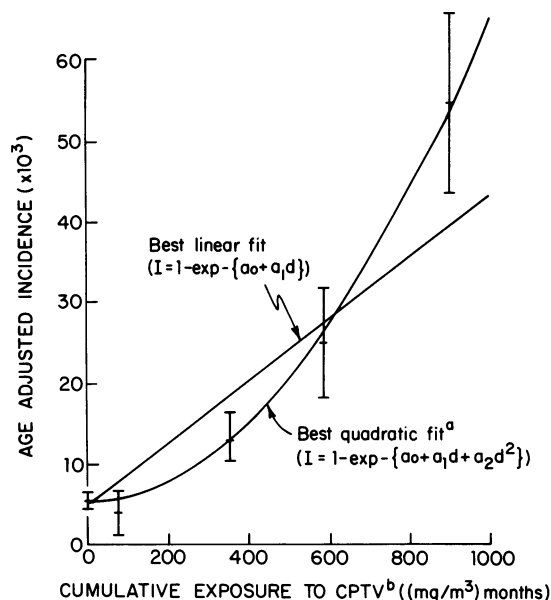
FIGURE 12. Age standardized incidence of lung cancer in coke oven workers versus cumulative exposure to coal tar pitch volatiles *(87)*. [a] Significantly nonlinear $(p < 0.01)$. [b] Coal tar pitch volatiles.

and cancers caused by earlier exposures to both coke oven emissions and cigarettes, which this study ignores, had more chance to develop.

An attempt has been made *(85)* to adjust approximately for the variable observation period by assuming a simple model which included a latency period. Exposures during one latency period prior to the time of observation were ignored, and exposures during the

next 10 years preceding were given less weight than the earliest exposures. With such assumptions, it was found that as the assumed latency period was increased, the dose-response relationship appeared more linear. With an assumed latency period of 15 years, a linear formula fits better than the nonlinear ones. Since lung cancer incidence increases roughly with the sixth power of age *(37)*, these observations are consistent with a multistage carcinogenesis model with just one stage linearly affected.

There are at least two ways to check whether the coke oven data truly demand a nonlinear dose-response relation. The best would use the original data on the 8000 workers studied to analyze how incidence varies with age, exposure rate and duration, and time from exposure to observation. An alternative, easier, but less satisfying check would be by simulation. Using assumptions about the age distributions and dose rates for the different cumulative exposures in the data given in Mazumdar et al. *(87)* and Land *(88)*, one could compute that the number of cancers expected were the true dose-response relation to be represented by Equation 26, with just one term linear in dose, as expected for a simple multistage model, and compare this with the observed number.

Radiation

Four large, widely reviewed reports discuss dose-response functions for radiation: the 1980 (BEIR III) Committee on the Biological Effects of Ionizing Radiation Report *(71)*, the 1972 BEIR I Committee report *(89)*, The National Council on Radiological Protection and Measurement (NCRP) report on radon *(90)*, and the report of the Working Group to Develop Radioepidemiological Tables *(91)*. The last report contains tables which assign probabilities that a particular cancer was caused by a given exposure to radiation (sometimes called probabilities of causation). A short review has been provided by Kohn and Fry *(92)*, and Upton *(72)* and Cohen *(73)* provide more extensive discussions of the effects of low doses. These reports contain a careful and extensive review of the literature. We concentrate on those cases where there is sufficient information to make some statement about dose-response functions and see what generalizations can be drawn from these cases. Unless otherwise indicated, the information used in this section can be found in the reports of the BEIR Commmittee and of the NCRP.

The effects of exposure of large populations to levels of ionizing radiation above background have been widely studied and documented (Table 3). When exposure has been used for medical purposes, exposure data are usually reliable, although follow-up is seldom perfect. Occupational exposures and doses received from atomic bomb explosions are not as well measured. Researchers at Lawrence Livermore Laboratory have recently recalculated the doses received in the Hiroshima and Nagasaki explosions, so that older reviews on dose-re-

Table 3. Some cases of human exposure to ionizing radiation.

Source of exposure	Exposure
Bombs:	
Hiroshima, Nagasaki	Single large exposure
Bikini Atoll, Nevada tests	Short exposure period
Medical treatments:	
thorotrast	Single large exposure
common X-ray exams	A few small exposures
X-ray treatments:	
scalp epilation	Single large exposure
ankylosing spondylitis	1–5 times week for 2 weeks–2 months
fluoroscopy (tuberculosis exam; postpartum mastitis treatment)	A few to hundreds of exposures
X-ray in pregnancy (childhood leukemia)	A few small exposures
Radium injections for ankylosing spondylitis and tuberculosis	Several injections over various periods up to 4 years
Occupational:	
radium dial painter	Continuous
underground miners (radon gas)	Continuous
nuclear workers: Hanford; Britain	Continuous
medical personnel	Continuous
Environmental:	
area of high natural background:	
Kerala, India; China; Brazil	Continuous
indoor air (radon gas)	Continuous

Table 4. Human cancers induced by ionizing radiation.

Relative importance	Sites
Major	Female breast, thyroid, lung, leukemia, alimentary tract
Minor	Pharynx, liver and biliary tract, pancreas, lymphomas, kidney and bladder, brain and nervous system, salivary glands, bones, skin
Magnitude of risk uncertain	Larynx, nasal sinuses, parathyroid (tumors), ovary, connective tissues, prostate[a]
Radiation-induced cancer not observed	Uterus[a] and cervix, testis,[a] mesentery and mesothelium, chronic lymphatic leukemia

[a]Cancers of reproductive tissue are not generally associated with radiation exposures (71); however, a recent study of British nuclear workers (albeit with only a small number of cases) found increased relative risk for testicular, uterine, and ovarian cancer, although none of the increases were statistically significant (207,208). Surprisingly, incidence of prostate cancer was significantly elevated ($p < 0.001$), with incidence increasing with exposure.

sponse relations must be considered in the light of the new dosimetry.

Quantity and Quality of Various Radiation Exposures. Radiation is known to cause a variety of human cancers, and is suspected of causing others (Table 4). Different tissues are affected to different extents, with responses depending on the type and qualities of the radiation and on the circumstances of exposure. Various other measures of biological damage similarly depend strongly on radiation quality (72). An example is the varying chromosomal damage (93) occurring at equal

absorbed radiation dose for radiations of different linear energy transfer (LET) (Fig. 13 and Table 5), and even acute lethal effects vary to a similar degree (94). As for chemical carcinogenesis, it is unlikely that the shape of the dose-response curve is the same for all tissue types and types and qualities of radiation. Human cancers have been shown to occur in several different tissues, but for only a few are the data good enough to attempt any dose-response modeling.

High Doses. BREAST CANCER. The relationships between absorbed dose and breast cancer seen in women exposed to radiation are linear at excess risks of order 1 to 5%. Figure 14 shows data on four groups of women: Japanese atomic bomb survivors, New York women given X-ray treatment for postpartum mastitis, and Massachusetts and Nova Scotia tuberculosis fluoroscopy patients. For the atomic bomb survivors, a lin-
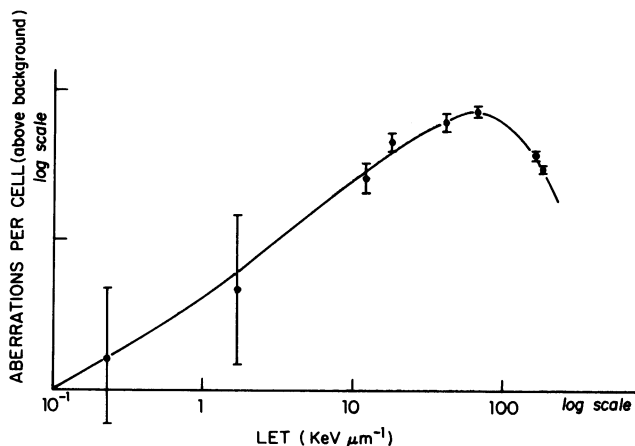

FIGURE 13. Variation in the predicted number of chromosome aberrations (above the background number) with average linear energy transfer (LET), at a constant absorbed dose of one rad (93).

Table 5. Chromosome aberrations per cell above background per rad of exposure.

Type of exposure	Yield/cell, × 10⁻⁴[b]	Approximate LET, keV/μm
15 MeV electrons	0.61 ± 1.12	NA
15 MeV electrons, pulsed	0.96 ± 1.92	NA
⁶⁰Co γ-rays	1.62 ± 0.29	0.2
250 kVp x-rays	4.82 ± 0.54	2
14.7 MeV neutrons	26.3 ± 4.0	12
Cyclotron neutrons (E = 7.6 MeV)	47.9 ± 3.3	19
²⁵²Cf neutrons (E = 2.13 MeV)	60.0 ± 1.9	40
Fission neutrons (E = 0.9 MeV)	72.8 ± 2.4	60
Fission neutrons (E = 0.7 MeV)	83.5 ± 1.0	73
²³⁹Pu 5.15 MeV Alpha particles	37.5 ± 2.4	170
²⁴²Cm 4.9 MeV	28.6 ± 1.5	190

[a]Calculated from the data of Edwards et al. (93).
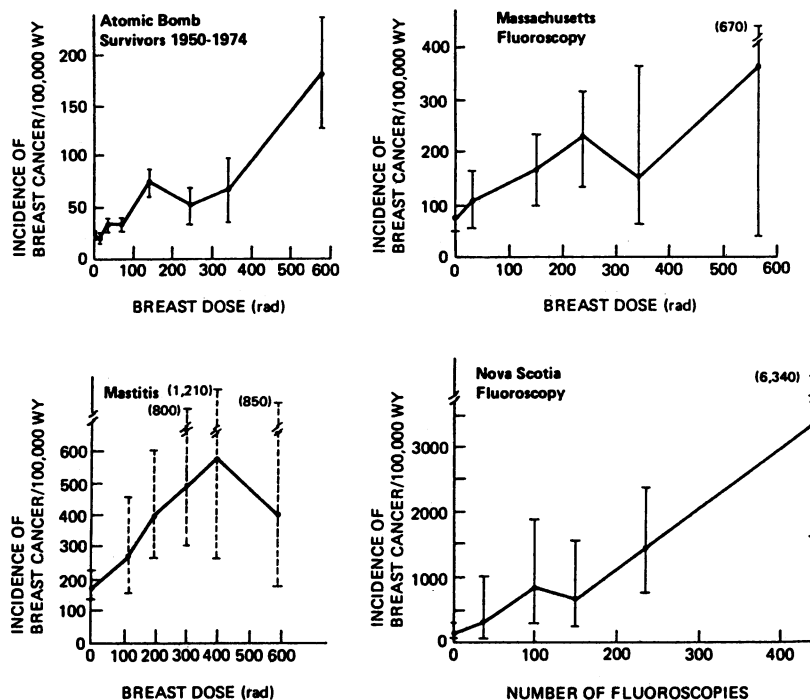[b]Plus or minus one SE.

FIGURE 14. Breast cancer incidence in women exposed to ionizing radiation (71).

ear dose-response relation was expected for the high-LET neutron dose component and nonlinear dose-response relation for the low-LET gamma dose component. This is not observed, for the best fitting model is linear in gamma dose. In Table 6 are the results of the BEIR Committee's attempts at formula fitting. They tried four different formulae: linear $[F_1(D) = a_0 + a_1 \cdot D]$; linear quadratic $[F_2(D) = a_0 + a_1 \cdot D + a_2 \cdot D^2]$;

Table 6. The shape of the dose-response curve for breast cancer.[a]

Series	Best fit
A-bomb survivors	No function fits significantly better than F_1. Where risks are statistically significant, data supports low dose linearity for those results corresponding to approximately 1% excess risk.
Massachusetts fluoroscopy	Linear model (F_1) fits well; upward curvature suggested $(F_2 : a_2 > 0)$, but not statistically significant $(p = 0.36)$.
Mastitis (dose to single breast)	Linear with significant cell killing component $(p = 0.01$ for b in $F_3)$.
Nova Scotia fluoroscopy	Linear fit (F_1) with no downward turn at high doses.

[a]F represents the risk, D the dose. $F_1 (D) = a_0 + a_1 \cdot D$, purely linear; $F_2 (D) = a_0 + a_1 \cdot D + a_2 \cdot D^2$, linear-quadratic; $F_3 (D) = F_1 e^{-bD^2}$, linear with cell killing.

linear with an assumed cell death term at high doses $[F_3 = F_1 \cdot \exp(-\beta \cdot D^2)$; and linear quadratic with cell death $[F_4 = F_2 \exp(-\beta \cdot D^2)]$. The quadratic terms were not significant in either of the cases tried, and the cell death term was significant only for the mastitis patients. The BEIR Committee also noted that the results are only dependent on cumulative dose; dose fractionation does not appear to matter.

THYROID. Thyroid cancers have been produced when X-rays were used medically in the treatment of pertussis, enlarged thymus, acne, hemangiomas, and ringworm, but none with the medical use of radioactive iodine. External X-radiation may be more efficient at inducing thyroid neoplasia than the internal exposure due to the iodine isotopes used medically, but the available data are also consistent with equal efficiency (95). The different dose rates and energy distribution within the thyroid may also contribute to this apparent difference between tumorigenic efficacy of X-rays and radio-iodine. In the cumulative dose range of 615 to 1500 rads, the dose-response relationship for X-ray induced thyroid cancers appears to be linear, but at lower doses the data are inadequate to distinguish between alternative hypotheses.

LUNG CANCER FROM EXPOSURE TO RADON GAS. Lung cancer has been associated with radiation exposures in three groups: patients given radiotherapy treatments for ankylosing spondylitis, Japanese atomic bomb survivors, and underground miners. In the first two groups radiation was distributed over much of the body, but the underground miners were irradiated pre-

dominantly in the lung by inhaled radon gas and radon daughters. Only for the last group are there sufficient data to obtain dose-response curves. These have suggestions of nonlinearity, but are mostly consistent with a linear relationship.

A lung disease peculiar to miners working the silver and copper mines of Central Europe was first described as early as 1500 A.D. and called male metallorum by Paracelsus. It was not until the early 1960s that the etiological role of radon was accepted for lung cancers found in underground miners (90). Radon daughters are the products of radioactive decay of radon gas, which escapes from the surfaces of fractured rock. The daughters adhere to dust and may then be inhaled and deposited in the lung. Here they decay further, giving off high LET alpha radiation which is absorbed by the cells just at the surface [within 10μm (96)] of the lung.

The data on occupational lung cancers in underground miners have been extensively reviewed (71,90). A summary of this data is shown in Table 7 and Figures 15 and 16. The unit of exposure used is the working level month, which for underground miners is equivalent to approximately 0. 5 rad (absorbed alpha dose to the bronchial mucosa). One working level corresponds to air containing short-lived radon daughters of any isotopic mix with a potential alpha radiation energy release of 1.3×10^5 MeV/L. Breathing such air for one working month (170 hr) results in an exposure of one working level month, abbreviated WLM.

Absolute excess risk of lung cancer is plotted (Fig. 15) against exposures for the six groups listed in Table 7. Figure 16 shows the data at higher doses for U.S. miners. Also shown are fitted curves (a polynomial formula), all of which give some evidence for positive quadratic or higher order terms in the dose-response relationship at the lower doses (except for the Newfoundland data, where there are only two data points). The differences from linearity are statistically significant for the Czech, Canadian group 1, and Swedish miners.

The observed excess lung cancer incidence at a given dose above 200 WLM differs substantially among the various groups of miners. This might be a result of differences in the age distribution of the study populations; of differences in techniques for measuring radiation exposures; of differences in exposure to cigarettes and other lung carcinogens; or of other differences between the populations. The exposure estimates for the U.S. Colorado miners were obtained from few measurements, with many estimates made indirectly from mines in other locations. Overestimation of exposure may have been systematic (97), leading to underestimates of risk per WLM. The dose-response data from U.S. mines is nevertheless consistent with observations of Chinese (predominately nonsmokers) living in areas of high background radiation who experience an excess exposure to radon gas of approximately 0.14 WLM/year (see "Areas of High Background Radiation"), although the age distributions of these two groups are substantially different.

Table 7. Excess risk of lung cancer in miners exposed to radon and radon daughters.

Exposure (WLM)	Excess risk/10⁶ person-year WLM	Excess risk/10⁴ person-years	Expected cancers/10⁴ person-years (background)
United States uranium miners			
Colorado plateau region			
60	− 3.1 (5.6)	− 1.9 (3.3)	7.6
180	8.0 (4.4)	14.4 (8.0)	6.8
300	7.8 (3.5)	23.4 (10.4)	7.7
480	7.8 (2.2)	37.4 (10.4)	8.0
720	2.7 (1.3)	19.4 (9.1)	8.0
1320	4.0 (0.7)	52.8 (9.6)	8.2
2760	2.8 (0.4)	77.3 (12.3)	8.0
7000	3.0 (0.6)	210.0 (44.9)	8.5
Czechoslovakian uranium miners			
72	4.6 (3.6)	3.3 (2.6)	3.3
124	11.2 (3.1)	13.9 (3.8)	—
174	13.8 (3.2)	24.0 (5.5)	10.6
242	11.9 (1.8)	28.8 (4.3)	—
343	15.6 (2.1)	53.5 (7.1)	11.7
488	22.6 (1.9)	110.3 (9.1)	—
716	17.2 (2.2)	123.2 (15.5)	17.1
Sweden			
Iron mines			
34	7 (3.2)	2.4 (1.1)	1.4
85 (smokers)	22.3 (7.8)	19.0 (6.6)	—
85 (nonsmokers)	16.3 (10.4)	13.9 (8.8)	—
Sweden			
Lead and iron mines			
240	28.4 (8.8)	68.2 (21)	7.7
390	35.0 (15.5)	136.5 (60.4)	23
Canadian uranium miners (Study 1)			
21	3.8 (3.6)	8.0 (0.7)	4
72	9.6 (4.2)	6.9 (3.0)	5
180	11.1 (6.6)	20 (11.9)	7
Canadian uranium miners (Study 2)			
15 (0–30)	− 7 (24)	− 1.0 (3.6)	3.3
46 (31–60)	4 (2.1)	1.8 (1.0)	1.8
76 (61–90)	12 (3.6)	9.1 (2.7)	3.0
106 (91–120)	5 (2.3)	5.3 (2.4)	3.1
150 (121–180)	12 (3.1)	18.0 (4.6)	3.2
Newfoundland fluorspar miners			
204	18 (2.4)	36.7 (4.9)	2.2
1600	6 (0.8)	96 (13.5)	3.5

ᵃNumbers in parentheses are approximate SE.

One analysis of the data on U.S. miners (98) shows that excess risks from cigarette smoking and radiation can be represented by a formula which is the product of two terms, one linear in radiation exposure, the other linear in cigarette consumption, so that much of the difference in excess risks for different groups of miners may be due to differences in smoking habits. This is confirmed by a reanalysis of grouped data for the Colorado miners (97). However, the risks from radiation and smoking seem to be additive in the data from Hiroshima and Nagasaki (91), and some studies suggest a protective effect of smoking at high radon exposure. This last effect is attributed to the abnormally large discharge of mucus in the lungs of heavy smokers: this mucus absorbs the alpha radiation and so protects the
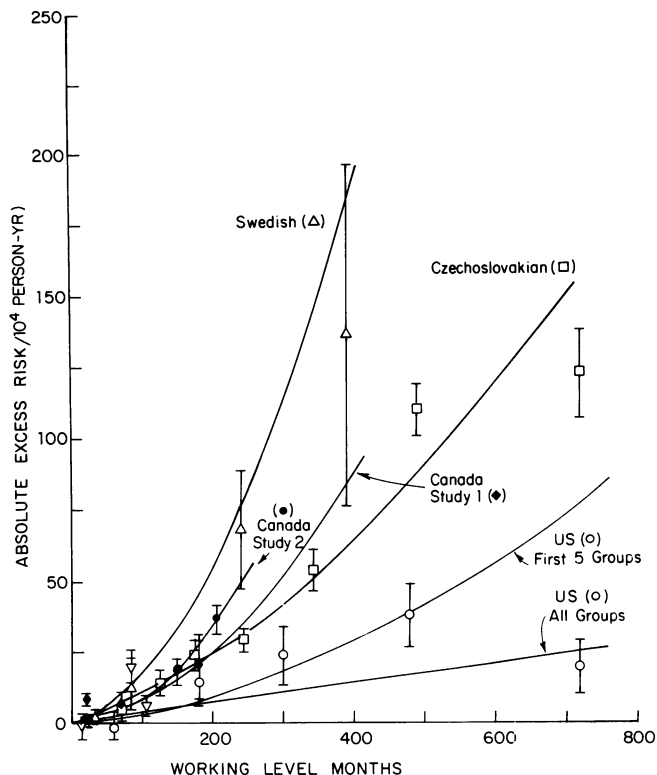
FIGURE 15. Lung cancer in men occupationally exposed to radon gas in underground mines. Absolute excess risk (per 10^4 person years) versus exposure (in working level months—see text).
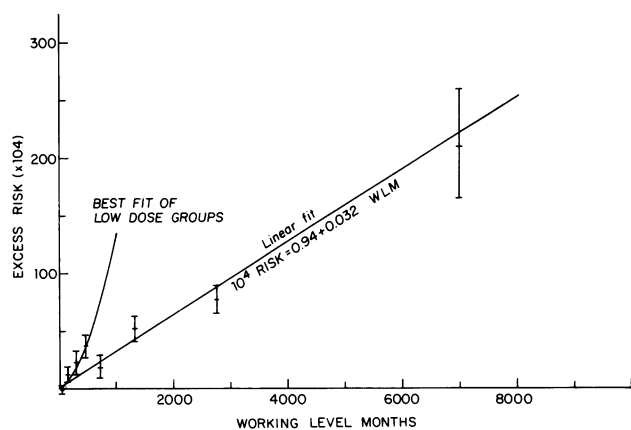

FIGURE 16. Lung cancer in U.S. uranium mines of the Colorado Plateau Region. Excess risk (per 10^4 person years) versus exposure (in working level months—see text).

mucus absorbs the alpha radiation and so protects the surface cells. To complicate the interpretation of any of the data, there may be other factors contributing to lung cancer in miners. Fibrous materials akin to asbestos, such as scarn and eummingtonite, are known to be present in Swedish Zinkgruvan mines; and other probable lung carcinogens, arsenic and nickel, are present in the Czech mines (99). In the above analyses, no other such factors have been incorporated.

An alternative way of examining the nonlinearity (90) is shown in Figure 17, in which the excess risk divided by the exposure is plotted against exposure (we have added our estimates of statistical uncertainties). The data on U.S. miners alone indicate that the excess cancer risk per cumulative WLM may be lower at high doses than at intermediate doses. Excess lung cancer risk per WLM is nearly the same for all U.S. miners exposed to more than 500 WLM, but is significantly less ($p < 0.05$) than excess risk per WLM for U.S. miners exposed to 100 to 500 WLM. A decrease in excess risk per WLM at high doses is also evident for two other groups with exposures greater than 500 WLM: Newfoundland fluorspar miners and Czechoslovakian uranium miners (Table 7).

In all groups, the lowest risk per WLM is that measured at lowest dose (below 100 WLM), and in most cases the value at the lowest doses is significantly lower than the mean of the values at higher doses, corresponding to upward curvature of the dose-response curves. This curvature, though consistent, may be an artifact. In most groups, cumulative exposure increased with age, (as is evident on examining the last column of Table 7), so that those exposed to higher doses are observed later in life, when cancer has had a greater chance to develop.

LEUKEMIA. *Atomic Bomb Survivors.* The Nagasaki and Hiroshima atomic bomb explosions emitted both low-LET gamma radiation and high-LET fast neutron radiation. According to radiometric theory, the leukemia incidence in the irradiated survivors should be described by a linear-quadratic dose-response formula (with the quadratic term coming from the gamma component of the radiation). It was originally thought that the neutron component of the dose was substantially higher at Hiroshima than at Nagasaki, in which case tumor incidence data from Nagasaki should have been more nonlinear with dose than similar data from Hiroshima. Originally this appeared to be the case (100), but later analysis using recalculated doses (with a much reduced difference in neutron component) shows the dose-response curves for the Hiroshima and Nagasaki data to be nearly identical (101).

The BEIR III Committee (71) tried fitting three formulae—the "LQ-L" formula, which contained both linear and quadratic terms for gamma dose and only a linear term for the neutron dose; the "L-L" formula, with only linear terms for the two types of radiation; and the "Q-L" formula, which has a purely quadratic term for gamma dose and a purely linear term for neutron dose. Although the nonlinear formulae describe the Japanese leukemia experience adequately (L-Q: $\chi_{11}^2 = 10.4$, $p = 0.49$; Q-L: $\chi_{12}^2 = 12.3$, $p = 0.42$), they provide no better fit than the linear model (L-L: $\chi_{12}^2 = 11.5$, $p = 0.49$). The subsequent dose recalculations at Livermore National Laboratory (101) also result in dose-response curves consistent with linearity (Fig. 18). The most recent cumulative statistics in the Nagasaki Tumor Registry, together with the new dose calculations, lead to dose-response curves which are fit best by a pure quadratic formula (102), although the
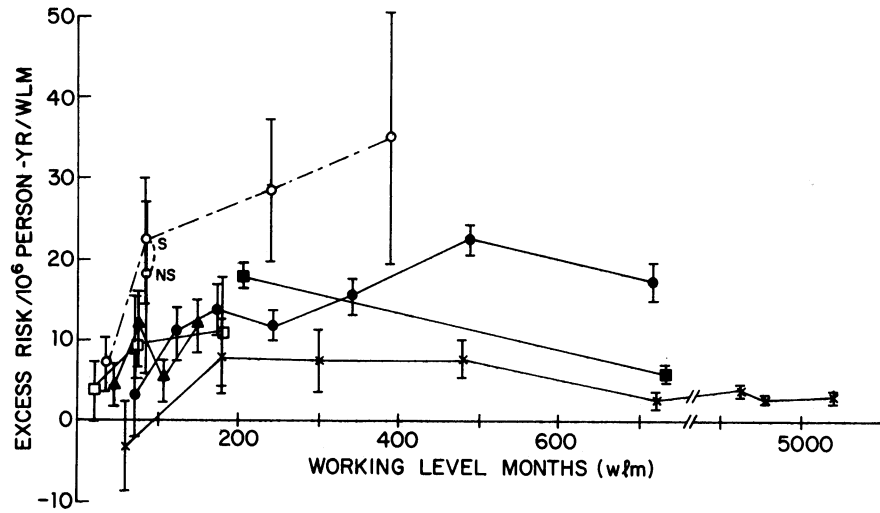
FIGURE 17.   Excess lung cancer risk per 10^6 person years per working level month (WLM) in underground miners (71,90). (X) U.S. miners (CO Plateau Region); (●) Czechoslovakian miners; (○) Swedish miners, NS = nonsmokers, S = smokers; (□) Canadian 1 miners; (▲) Canadian 2 miners; (■) Newfoundland miners.

may have substantially different dose-response characteristics at low doses.

Childhood Leukemia.   Childhood leukemias have been associated with *in utero* X-ray exposures (103). A dose-response curve with estimated uncertainties for each data point (104) is shown in Figure 19. A linear fit to this is as good as any could be, and better than a quadratic curve, although that also is consistent.

BONE.   Significant increases in osteosarcomas have been induced by exposure to high doses of bone-seeking
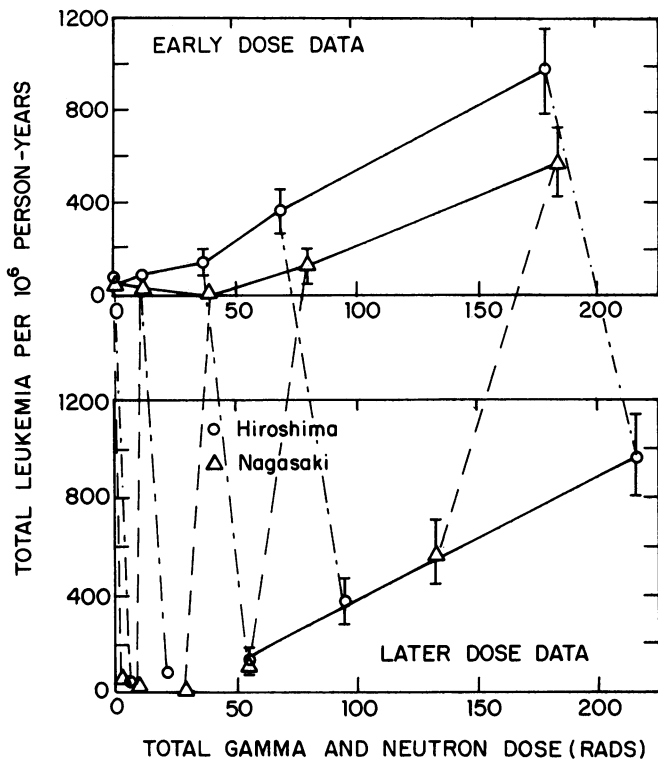
FIGURE 18.   Leukemia incidence in atomic bomb survivors versus old (top) and more recent (bottom) dose estimates (101). Error bars represent one SD.

quadratic term in a linear-quadratic fit is not statistically significant.

The use of total leukemias as a measure of response in such dose-response curves, expecially for use in risk assessments, has been criticized (72). Since the diverse types of leukemias represent different diseases, each
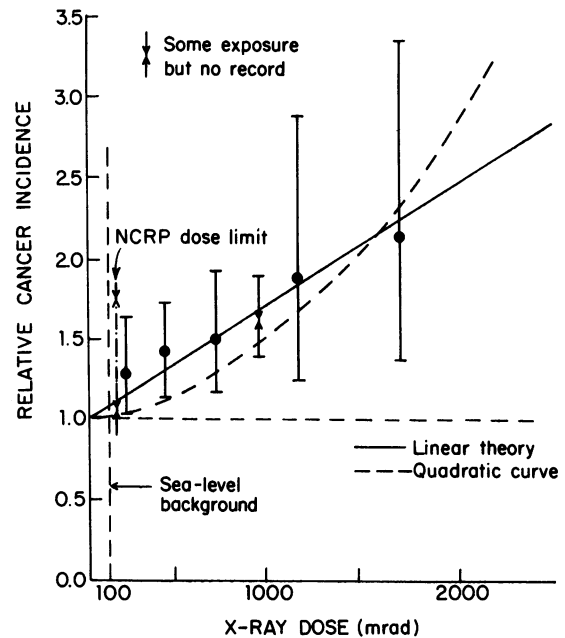
FIGURE 19.   Childhood leukemias resulting from *in utero* X-ray exposures (103).

radium isotopes (Ra-224, -226, and -228) deposited internally, and by high doses of therapeutic X-rays. No increase in bone cancers was seen among Japanese survivors of the atomic bomb: five cases have been observed in those within 1400 meters of ground zero, where 4.67 cases would be expected. The incidence of osteosarcoma at high doses of high-LET radiation appears to be a superlinear function of the dose rate.

Intravenous Injection of Radium 224. The most precise information is for Germans intravenously treated with radium 224 (half-life 3.64 days) for ankylosing spondylitis or tuberculosis. The dose rate may have been important: a single injection leading to 1 rad average skeletal dose corresponded to a lifetime risk of osteosarcoma of 4×10^{-5}, whereas injections over several years leading to the same 1 rad average skeletal dose corresponded to a lifetime risk of 2×10^{-4} (105).

When dose rates are constant over a long period, a comparison of cumulative dose with incidence can provide some insight on the differential effectiveness (incidence/rad) of the radiation at low versus high doses, but such a comparison for discontinuous and short exposures can be misleading. As an example, Figure 20 shows a comparison of cumulative average skeletal dose with osteosarcoma incidence for the German patients treated with radium 224.

Fitting an exponential polynomial formula to this data suggests a strong upward curvature (the quadratic term is highly significant, $p \leq 0.001$). A naive interpretation of this nonlinearity—that low dose rates are less tu-
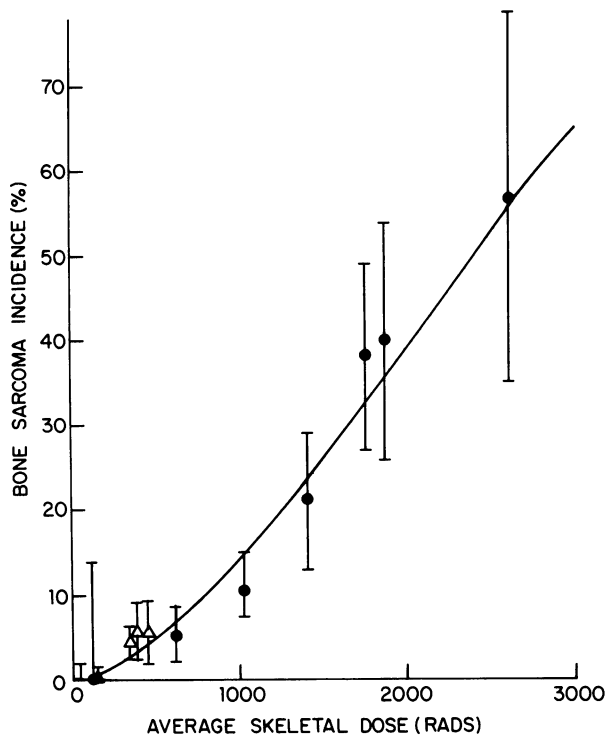
morigenic than high—depends on an assumption that the exposure periods were roughly equivalent. Here, the highest cumulative doses were the result of the lowest dose rates, and so the interpretation could be precisely the opposite. This is indicated on Figure 21 (104), which relates incidence per rad to the treatment period to suggest that a given amount of radium was most carcinogenic if given over a long period of time (low dose rate). We also show in Figure 21 the dose rates (rad/month) over the treatment period of the various exposed groups. However, a complete analysis would require that the effects of cumulative dose and period of exposure be simultaneously modeled, and it is unclear what the outcome of such an analysis would be (E. Pochin, personal communication).

Poisoning of Dial Painters with ^{226}Ra and ^{228}Ra. Luminous paint containing radium was used for watch dials. The painters of these dials licked the tips of their brushes to form a good point, ingesting some of the paint in the process. Most exposure was to radium 226 (half-life 1600 years), with possible additional exposure in some cases to radium 228 (half-life 5.8 years). Some of the painters later developed bone sarcomas. The dose-response relationship appears very nonlinear (Fig. 22). At high doses, above 1000 rads, induction of sarcomas can be represented by a nonlinear dose-response curve (106)

$$\text{Risk} = 3.7 \times 10^{-8} \cdot D^2 \exp(-1.4 \times 10^{-4} \cdot D)$$

$$(37)$$

where D is the dose in rads. Information on the shape of the dose-response curve at low doses is equivocal.

Low Doses. DISAGREEMENTS ABOUT SUB- AND SUPERLINEARITY. It is generally considered conservative to estimate the risk of exposure to low doses of radiation by a linear extrapolation from the Japanese bomb experience, but some challenge this view, claiming that such risk estimates are grossly underestimated. This controversy has been extensively reviewed (71,73), and we merely summarize some of it here.

Mancuso, Stewart, and Kneale. Mancuso et al. (107,108) reported that the experience of employees at

FIGURE 20. Bone sarcoma incidence (105) in German patients treated with ^{224}Ra versus cumulative skeletal dose (in rads). The shape of this curve is misleading—see text.
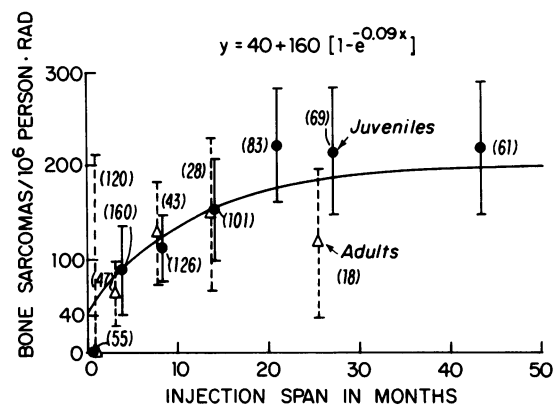

FIGURE 21. Bone sarcoma incidence per 10^6 person-rad. The same data as Fig. 20—see text.
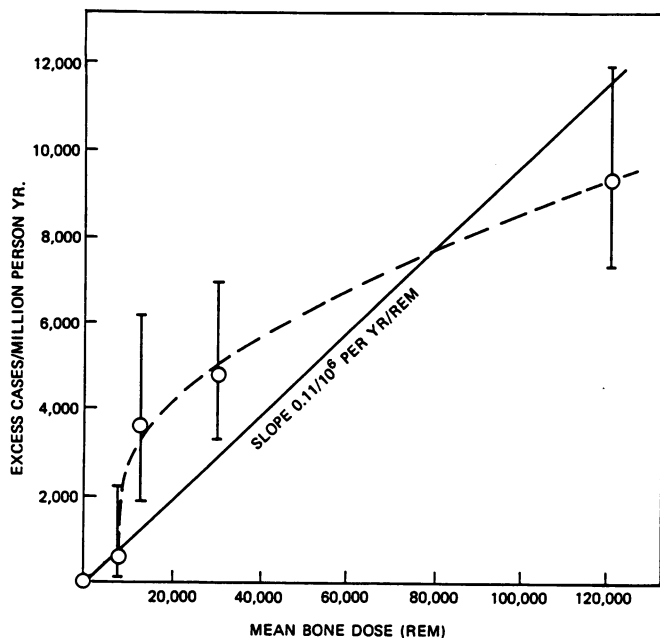
FIGURE 22. Bone sarcomas in radium-exposed watch-dial painters (88).

the Hanford Works in Richland, Washington, who were exposed to highly fractionated low doses of radiation, has been far different from that of the atomic bomb survivors, who were exposed to an acute and relatively large dose. They present evidence that the incidence of some cancers at Hanford is an order of magnitude greater than expected using conventional extrapolations from the atomic bomb experience. Others (109–111) have confirmed the findings of increased multiple myelomas and pancreatic cancers, but the absence of myeloid and lymphocytic leukemia, the cancers most expected, and the low statistical power of the study are good reasons for viewing the claims of greater than expected effects with skepticism. A second follow-up study (112) reported that the excess of pancreatic cancers was no longer statistically significant, but that there was an excess of stomach cancers that approached significance; as an aside it is noted that one of the pancreatic cancers is likely to have been a stomach cancer. The number of independent statistical tests of different cancer categories in these studies makes it quite likely that, simply by chance, one such category will appear to have a statistically significant excess of tumors.

Bross et al. Unconventional statistical techniques were applied to the Tri-state Leukemia Survey to show that exposures in the 1-rad range from diagnostic X-rays resulted in risks significantly larger than would be "expected from the linear extrapolation procedures used in the BEIR report," which "disregards subgroups in the general population that are particularly vulnerable to x-rays" (113). These conclusions have been strongly criticized (71,114) on the grounds that they rely on highly biased and arbitrary statistical techniques,

and also because of the casual treatment of exposure data.

Cohen–Sublinearity at Low Doses. Cohen has compared measured rates of cancer incidence with those predicted for environmental radiation exposures using the dose-response relationships developed by the BEIR committees.

The BEIR I estimates (89) imply that environmental radon causes at least 80% of all lung cancers in female nonsmokers, while for the age group 24 to 44, at least 44% more lung cancers should be observed (115). If the histology of the tumors observed is taken into account, the BEIR I estimates appear more at variance with observation. Sixty-nine percent of lung cancers in miners exposed to radon gas are of the small cell undifferentiated type, while only 4% of lung cancers in the general nonsmoking population are of this type, so that overpredictions could be at least as large a factor of 20. Cancer rates in underdeveloped countries and early in this century in the U.S. are used to further strengthen this case. The estimates given in the later BEIR Committee report (71) are claimed to overpredict lung cancer risk in nonsmokers from background radiation by at least a factor of 40 (116).

Some members of the 1980 BEIR Committee argued that the dose-response relation for radiation-induced leukemia was superlinear; specifically, that risk at low doses is proportional to the square root of the dose. However, such a dose-response relationship would predict higher leukemia rates than those observed in the various U.S. states between 1925 and 1933 for ages 25 to 34 (115).

AREAS OF HIGH BACKGROUND RADIATION. Most evidence for nonlinearity of radiation dose-response relationships has been produced in occupational settings, where exposures and risks are large and uncertain, or from therapeutic exposures which are also large but of short duration. The observed dose-response data correspond to lifetime risks of 1% or more, so that any nonlinearity observed in these studies must be considered high dose nonlinearity. For protection of the public, it is the behavior of the dose-response relationship at low doses which is of greatest interest. In practice, small excess lifetime risks (below 1%), which may be caused by small exposures, cannot be determined unless the background rates of the cancer under study are also very low, and a comparable control population is available.

Of special interest, therefore, are populations living in areas of naturally high radiation levels but where background exposures to man-made carcinogens are low. The primary sources of elevated background radiation are radon (radon-222) and thoron (radon-220) and their decay products, so that elevations in lung cancer rates would be expected in high background areas. The two gases mentioned are themselves members of the decay chains of uranium and thorium, respectively.

Populations satisfying the required criteria can still be found in rural areas of less developed countries. Two

have been carefully studied: those exposed to the monazite deposits in Kerala, India, where most of the radioactivity (95%) arises from thorium decay products; (117) and residents in the Dong-anling and Tongyou regions of Yangjing County, Guangdong Province, China, where both uranium and thorium decay products yield relatively high radiation rates. Neither of these populations has been shown to be at any increased risk for lung cancer.

Pochin (118) has discussed the statistical and technical difficulties in studying these ideal populations. Even if the highly exposed and control populations are well defined and stable, observations on extraordinarily large populations are required to discern small differences in malignancy rates. These studies are extremely valuable even if significant excess risks are not observed, for they provide upper bounds on such excess risks at low doses. Such upper bounds can be compared with the effects observed at higher doses, for example, to test whether there must be some nonlinearity in the dose-response relationship.

In Yangjing, smoking among women is unusual and among men it is low, and exposure to industrial pollution is negligible. The background of lung cancers should therefore be low, and if radon and thoron are causing lung cancer here, their effect may be detectable.

The exposed and control population, also from Guangdong Province, have been carefully followed by the Chinese High Background Radiation Research Group (119,120), which keeps health statistics and makes radiation measurements. Unfortunately, the control and exposed populations are not exactly comparable. The age distributions appear to be changing (De-chang Wu, personal communication), and while 90% of the families of the highly exposed have lived in the area for over six generations, the control population appears to be more mobile.

A summary of radiation backgrounds in shown in Table 8 (121), and from these it is estimated that exposure rates from radon daughters alone are 0.26 and 0.12 WLM/year, and total exposure rates are 0.38 and 0.16 WLM/year, in the high and low background areas, respectively.

In the high background area there were 23 lung cancer deaths in 764,696 person years of observation compared to 27 deaths in 777,482 person years in the control area. Adjustments for age and sex lead to lung cancer mortality rates of 2.7 per 10^5 in the high background

Table 8. Background radiation in Guangdong Province, China (121).

Radiation type/source	High background area (mWL)	Control area (mWL)
Radon progeny		
Indoor	5.2	2.2
Outdoor	4.7	2.5
Thoron progeny		
Indoor	11.7	3.9
Outdoor	2.8	1.3

area and 2.9 per 10^5 in the control area (121). An excess exposure to 0.14 WLM/year of radon daughters thus has not produced a detectable increase in lung cancer mortality.

The expected increase in lung cancer mortality for such an increase in exposure can be computed using the BEIR III (71) analysis. This calculation yields an expected 2.9 cases/year excess (the study period was 14 years) in the high exposure population, significantly higher than the observed result. However, such a calculation requires the assumption that the effects of cigarette smoking and radiation exposure on cancer mortality are additive. Making the alternative assumption that the effects are multiplicative, the expected excess risk per unit radiation exposure would be increased by 50% for smokers, and decreased by a factor of six for nonsmokers. With this assumption, the expected excess cancer mortality would be 0.48 cases/year, not significantly different from that observed.

Whittemore and McMillan (98) have reanalyzed the data on lung cancer in U.S. uranium miners in order to elucidate the interaction between cigarette consumption and radiation exposures. They find that multiplicative effects dominate; all the additive risk formulae tried fit the data significantly worse ($p < 0.01$) than the multiplicative formulae. The formula

Relative Risk =

$$(1 + 0.0031 \cdot \text{WLM})(1 + 0.00051 \cdot \text{PACKS}) \tag{38}$$

described the data well, where WLM is the total cumulative radiation exposure in working level months and PACKS is the total cigarette consumption 10 years prior to the time an individual was observed. The more recent analysis of a more limited set of this data (97) supports such a mixed (additive and multiplicative) formula.

This multiplicative formula indicates that excess risks for nonsmokers are much smaller than would be obtained from the BEIR II estimates. We may use it to estimate the excess cancer mortality expected in the Guangdong Province study described previously. Since smoking habits in control and highly exposed populations are said to be the same, we ignore the second factor in the above equation. To obtain a worse case estimate, assume an exposure of 0.22 WLM/year for 65 years, which yields a relative risk of 1.042. Thus, an excess of 0.08 cases/year, or one case over the 14 years of the study, would be expected. Since the standard error is approximately 5 cases, the lung cancers observed for control and highly exposed populations are consistent with the U.S. uranium miner data as analyzed by Whittemore and McMillan.

ENVIRONMENTAL RADON AND LUNG CANCER IN THE UNITED STATES. Using radon exposure calculated from levels in U.S. buildings, Cohen (116) predicts lung cancers due to radon using the BEIR III formula, and finds them a factor of 40 too high. He attributes most

of this difference (a factor of 10) to deficiencies in the BEIR III analysis, but the rest (a factor of 4) to possible nonlinearity in the dose-response relation.

CHROMOSOME DAMAGE AT LOW DOSES. Chromosome damage in blood lymphocytes of nuclear dockyard workers have been studied by Evans et al. (122). Exposure was to mixed gamma and neutron radiation, usually below 5 rems/year. There was a significant increase in certain types of chromosome damage. After adjusting for worker's age and time of sampling, this damage was shown to be consistent with a linear relationship of effect with cumulative dose.

Radiation—Summary. Table 9 is a summary of the foregoing information. Simple theories of radiation carcinogenesis suggest that the dose-response relationship for radiation should depend on target site and radiation quality. High doses of high LET radiation should give a superlinear dose-response curve, and low doses of low LET a sublinear curve. Human data are mostly consistent with these expectations, but they do not rule out other possibilities. Most of the low LET data appear consistent with linearity (breast, thyroid, and leukemia from X-rays), although a recent reanalysis of the leukemia incidence arising from the atomic bomb radiation suggests a purely quadratic curve (102). For high LET radiation, the apparent shape of the curve may depend on the quality of the data. At high doses the dose-response curve is superlinear for bone cancers in the dial painters and the ankylosing spondylitis victims exposed to ^{224}Ra, but at low doses the data is equivocal. There is some evidence of sublinearity in the dose-response curve for miners exposed to high LET radiation from radon daughters, but here it is unclear whether age, time, and smoking effects are completely taken into account.

Exposure to Ambient Vinyl Chloride

Human liver angiosarcoma is a very rare tumor which has been associated with exposure to just a few agents: arsenic, vinyl chloride, and thorium dioxide (Thorotrast). The rarity of the disease is such that when even only a few cases occur in the same location, exposure to one of these agents is strongly suspected.

Over the past 30 years, 125 known angiosarcomas cases have been produced among those occupationally exposed in plants using vinyl chloride monomer. There is substantial clustering in these data, with up to six cases occurring in some plants and none or only one in others. This suggests that a detailed study of the past working conditions in these plants could allow estimation of exposures, and hence give information on the dose-response relationship. However, problems of legal liability make individual companies unwilling to release such data voluntarily (R. Adams, personal communication).

The geographical distribution of angiosarcoma cases in nonoccupationally exposed populations can be used to infer some information about the dose-response relationship. Brady et al. (123) report on all residents in

Table 9. Dose-response curves for radiation induced human cancers.[a,b]

Cancer site	Curve
Breast (A-bomb survivors, fluoroscopy, X-ray)	Linear to high doses; at high doses some evidence of a downward turn, possibly due to cell killing
Thyroid (bomb fallout, medical treatments)	Possibly linear; further study required
Lung (underground miners, radon gas, for α particles expect low dose linearity and superlinearity at high doses)	Individual studies consistent with linearity assumption; quadratic term in some studies statistically significant; some cases of significant downward curvature at high doses
Lung (atomic bomb)	Linear and quadratic models fit equally well
Lung (Guangdong, China, an area with high radon background)	Equivocal when compared with high dose data on miners
Bone (radium, expect low dose linearity, high dose sublinearity)	Superlinear at high doses; equivocal results for low doses
Childhood leukemia (*in utero* X-ray)	Linear
All leukemias (atomic bomb)	Analyses differ over whether linear or quadratic. Most recent gives quadratic
Leukemia, Tri-state study (NY, MD, and MN, mostly exposure to X-rays)	A claim (113) that risks at low doses are higher than would be expected from the Japanese experience is not generally accepted
Esophagus, stomach, intestine and rectum, liver, pancreas, lymphatic system, kidney, bladder, brain, skin	Data on dose-response very limited or nonexistent
All cancers except leukemia (atomic bomb survivors)	Cannot discriminate between linear and quadratic, although linear fits best
Major sites except leukemia and bone (atomic bomb survivors)	Cannot discriminate between linear and quadratic
All cancers (Nagasaki)	Upward curvature (92)[c], linear model best (102) when leukemias are excluded from the analysis
All cancers (Hanford plant: occupational exposures)	Superlinearity claimed (107), but the claim strongly criticized, and refuted by follow-up (112)

[a]Unless otherwise noted, information from BEIR Committee Reports I and III (70,88).
[b]Linear implies that cancer risk, R, is proportional to the dose D: $R \propto D$; quadratic implies that $R \propto D + c \cdot D^2$, where c is constant.
[c]At a median dose of 120 rads, Kohn and Fry calculate a risk per rad of 4×10^{-4}/rad; at a median dose of 35 rads they estimate a risk per rad of 1.3×10^{-4}/rad.

New York State (excluding New York City) diagnosed as having liver angiosarcoma from 1970 through 1975 and reported to the state's tumor registry. Each case's proximity to a vinyl chloride or polyvinyl chloride manufacturing facility was ascertained, as shown in Table 10.

From the New York State statistics, we calculate an incidence of 16 cases/6 years/10.68 million people = 0.25 cases per million per year, nearly twice the national annual incidence of 0.14 cases/million. However, excluding those with known exposure to As, ThO_2, or vinyl

Table 10. Cases of angiosarcoma related to proximity of victims to a vinyl chloride or polyvinylchloride plant.

Proximity to plant	No. cases of angiosarcoma
In plant (worker)	1 (male)
Near plant (< 1 mile)	4 (females)
Away from plant: exposed to As and ThO_2	2 (1 male and 1 female)
No knowledge of exposure to As, ThO_2 or VC	9 (3 females and 6 males)

Table 11. Predicted number of angiosarcomas cases caused by 20 years of exposure to 0.5 ppm ambient vinyl chloride.

Model type	Risk formula (dose in ppm)	Reference	Cases expected
Linear	0.007 × dose	(124)	4.3
Linear	0.0002 × dose	(125)	0.1
Gamma multhihit:			
Human data	0.038 × $dose^{0.41}$	(8,124)	72.9
Human data	0.0008 × $dose^{0.41}$	(8,125)	1.5
Animal data	0.007 × $dose^{0.41}$	(8)	13.4

chloride (including those living near plants using vinyl chloride) gives a rate equal to the national average.

There were four cases near vinyl chloride plants, where the air concentrations of vinyl chloride were quite low. This incidence can be compared with what would be expected on the basis of dose-response relationships obtained by fitting various formulae to animal and occupational data. Kuzmack and McGaughy (124) assumed a linear dose-response relationship and used human data to estimate that a lifetime risk of 0.13 corresponds to 20 ppm lifetime exposure to vinyl chloride, implying a potency of 0.007/ppm. The National Academy of Sciences (125) also assumed linearity and used human data, but obtained a potency of 0.0002/ppm. This analysis, however, used earlier data on angiosarcomas, and there is also reason to believe that the average exposure was overestimated. The Food Safety Council (8) used Maltoni's data for angiosarcoma induction in rats to estimate risks at low doses of vinyl chloride by assuming that the dose-response relationship could be represented by a gamma multihit formula, specifically (see also Fig. 5):

$$Risk = 7.2 \times 10^{-3} \times dose^{0.41} \qquad (39)$$

Population distributions near vinyl chloride plants and the distribution of exposure to vinyl chloride have been estimated (124). Approximately 50,000 New York residents live within a 1 mile radius of vinyl chloride plants and were exposed to approximately 0.5 ppm of vinyl chloride, on average, before emissions were controlled in about 1975. Before the effect of vinyl chloride on angiosarcoma incidence was identified in about 1973, few attempts were made to have a tight system, and typically, 5% of all vinyl chloride processed was released as fugitive emissions. After control, the emissions were negligible for our calculation purposes. The plants did not begin operating until about 1955, so that lifetime average exposure is approximately 0.5 ppm × 20/70, or 0.14 ppm. The number of cases expected is shown in Table 11.

As may be seen, either of the formulae proposed for the dose-response relationship could agree with the four cases of angiosarcoma seen in the population near vinyl chloride plants. If Kuzmack and McGaughy's interpretation of the vinyl chloride occupational exposure data is the most accurate, their linear formula is the better predictor; on the other hand, if the NAS interpretation is correct, the gamma multihit formula is the better predictor, in spite of the peculiarities of its biological interpretation.

Table 12. Bladder cancers in 78 men occupationally exposed to β-napthylamine and benzidine at least 30 years after the time of first exposure (127).

Length of exposure, years	Number of men with tumors	% with tumors (life table corrected)
< 1	3	9
1-< 2	1	17
2-< 3	3	48
3-< 4	3	70
4-< 5	8	80
≥ 5	17	94

An additional epidemiological study was performed by Mason (126) who compared cancer mortalities in counties with plastics fabrication and manufacturing facilities with those counties without such facilities. He was unable to distinguish the rare liver angiosarcomas from other liver cancers, so his study has little sensitivity and cannot be used in the same way.

Bladder Cancers Induced by β-Napthylamine

The primary aromatic amines β-napthylamine and benzidine have long been known to induce bladder cancers in occupationally exposed men. Despite the many case reports, there is little information on the dose-response relationship. The best available information is still the study by Williams (127), who traced the history of 78 men exposed to β-napthylamine and benzidine under poor working conditions. He cautioned that a "dirty worker may absorb 20 times the dose of a clean one under identical working conditions," but considered length of exposure the only practical estimate of dose, and gave statistics on exposure and tumor incidence for the 78 men. These are given in Table 12, and plotted in Figure 23 (127–129). In Figure 23 we have added approximate uncertainty estimates, which were not given in the original. Fitting polynomial formulae to this dose-response curve indicates that there is some departure from linearity; a quadratic formula is a better fit than a linear formula, for example ($p = 0.02$).

Aflatoxin

The dose-response relationship for aflatoxin provides an example of how a simple use of mathematical formulae can be misleading. A frequently used procedure
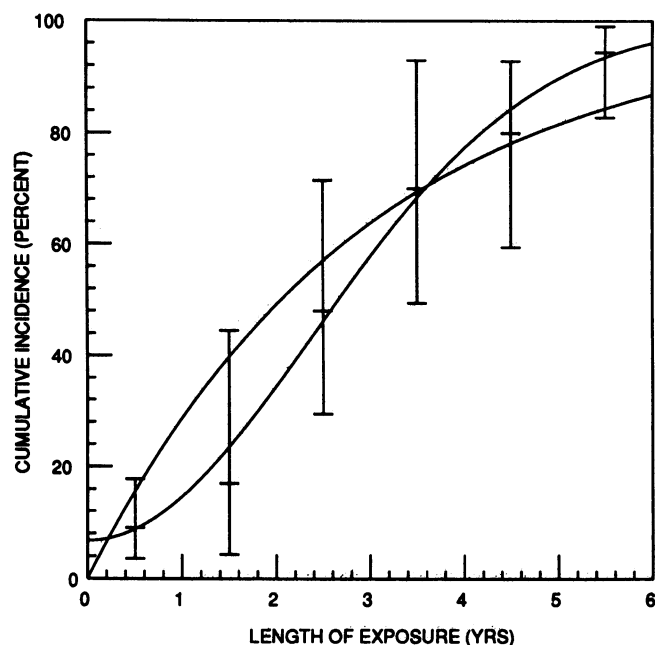
FIGURE 23. Incidence of urinary bladder cancers in 78 distillers of β-naphthylamine and benzidine (127). Error bars represent approximately one SD. The solid lines are (exponential) linear and quadratic best fits.
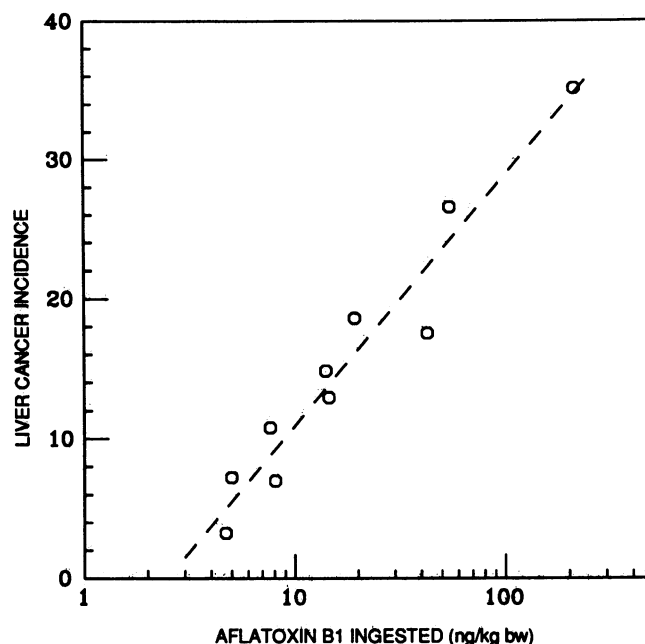

FIGURE 24. Incidence of primary liver cancer versus aflatoxin exposure (133). Incidence is per 10^5 per year. Note the logarithmic dose scale. No other factors are taken into account—see text.

for estimating this dose-response relationship has been to compare the incidence of primary liver cancer in various geographical regions with the logarithm of the average daily aflatoxin consumption in the same regions (130–133). A straight line on this logarithmic plot fits the data well (Fig. 24), suggesting that incidence is linear in the logarithm of dose of aflatoxin. A Mantel-Bryan formula also fits this data well.

However, the role played by aflatoxin in the causation of human cancers is unclear. The incidence of primary liver cancer (PLC) varies substantially from place to place, from as low as 0.4% of all cancers in some Western countries to as high as 65% of all cancers in some areas in underdeveloped countries. It had been thought that exposure to aflatoxin B_1, a potent liver carcinogen in animals, could explain these geographical differences (130,131); but it now seems that other factors have some role (134). Indeed, hepatitis B virus (HBV) appears to have a causal connection with most liver cancers (135–138). Aflatoxin exposure results in depression of cellular immunity (139), allowing persistent hepatitis infection; further, aflatoxin more readily accumulates in livers damaged by HBV infection. The effects of aflatoxin and HBV might therefore be synergistic.

Enwonwu (140) argues that schistosomiasis is important in the etiology of PLC, although this can be questioned (141) by the strength of the association between HBV and PLC where schistosomiasis is absent, and the strength of the association between schistosomiasis infection and previous infection with HBV. It is possible that the effects of aflatoxin exposure are enhanced by protein malnutrition (140), for such malnutrition impairs

the activity of the liver's mixed-function oxidase system and so might allow accumulation of aflatoxin in the liver. Subsequent liver regeneration when high protein foods become available would result in DNA replication, in which process aflatoxin-induced mutations could become fixed in daughter cells. Alcohol consumption is associated with PLC in Western countries, where most primary liver cancers are found in alcoholics with a history of alcoholic disease (135,136), but this association may arise because of the presence of HBV in those with alcoholic liver disease (136,137).

It is unlikely that HBV is the sole cause of liver cancer. Such a hypothesis fails to explain why incidence of PLC is always substantially higher among males than among females in a given area or why rural and urban areas of Greece (with the same prevalence of HBV) have substantial differences in the incidence of PLC (142). In the Murang district of Kenya, PLC incidence varies substantially between communities at high and low altitudes, yet HBV prevalence remains roughly constant (143). In this case differences in aflatoxin exposure may account for much of the geographical variation in PLC incidence.

Data from this region (144) may thus provide a dose-response relation between aflatoxin exposure and PLC (Fig. 25), with less chance of being affected by the effects of HBV. For males, the dose-response relationship is consistent with a linear hypothesis; the best fit for the female groups includes a quadratic term, although it is not statistically significant ($p = 0.14$).

There is information on aflatoxin intake and liver cancer incidence for other countries, but the variation in prevalence of HBV infection is generally not known.
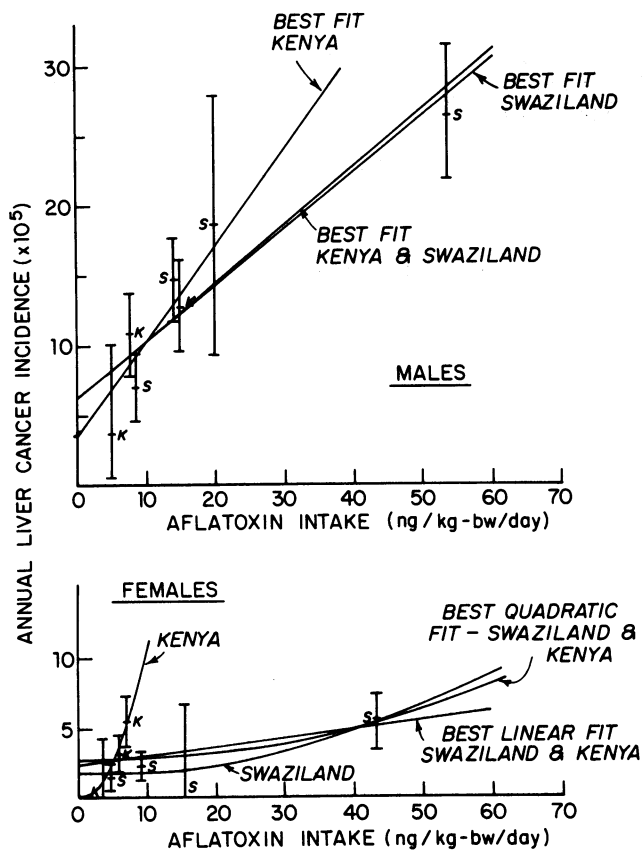
FIGURE 25. Dose-response curves for aflatoxin intake. K Populations in Kenya with similar hepatitis B prevalence (143,144). S Populations in Swaziland (145).

Dose-response modeling in such cases would be expected to yield variable and inconsistent results, and this is what is observed. Data from some countries appears consistent with that from Kenya, while data from other countries yield contradictory results.

Peers et al. (145) have expanded on earlier work (146,147) to obtain aflatoxin exposure and liver cancer incidence data for different regions in Swaziland. This is in qualitative agreement with the Kenya data (Fig. 25): the dose-response relationship for males is linear; for females, nonlinear, but not significantly so ($p = 0.375$). Alpert et al. (148) studied Uganda; their data is somewhat nonlinear (downward curvature), but exposure information is uncertain. Bulatao-Jayme et al. (149,150) report on primary liver cancer incidence in the Philippines as a function of aflatoxin intake and alcohol consumption, which both appear to be highly correlated with PLC. Unfortunately, their presentation of data does not allow for dose-response modeling. Data from Songkhla and Ratburi Thailand support the hypothesis that aflatoxin and PLC are related (151-153), but is too limited for dose-response modeling.

Stoloff (133) claims that aflatoxin consumption has little correlation with primary liver cancer in the U.S. He compared liver cancer incidence for different areas of the United States with aflatoxin consumption. High-

est past consumption of aflatoxin occurred in the Southeast because of large concentrations in corn, the staple starch there. However, there was no significant elevation in incidence of liver cancers in that region. The study did not take into account alcohol consumption or HBV infection prevalence. Stoloff compares the North and West to the Southeast, which has a substantially lower rate of alcoholism (154). It is interesting to note that application of the dose-response relation found in Kenya to aflatoxin consumption in the U.S. predicts many more liver cancers than are observed. The difference is presumably due to differences in HBV infection prevalence, in alcoholism, in other (unidentified) environmental factors, or to some difference in population susceptibility.

Animal Studies

High Dose Experiments of Relatively Small Size

There have been many animal experiments performed in order to evaluate the carcinogenicity of various materials. Most of these have used relatively few animals and few doses. Plotting the outcomes (for example, the fraction of animals with tumors) against the doses should give some information about the dose-response relationship, but this information is generally very limited by the statistical uncertainty inherent in the small experiment sizes. Further information may be gleaned by examining a large set of experiments simultaneously, or by examining several relatively small experiments performed on a single material. Naturally, the best way to obtain information on the dose-response relation is to perform large experiments designed for this purpose.

NTP/NCI Experiments. The Carcinogenesis Bioassay Database System (155) is a computerized database containing details of carcinogenesis bioassays performed for the National Cancer Institute and National Toxicology Program. Bailar et al. (156) have analyzed consistently all the bioassays recorded in this database (by July 1985, approximately 300 chemicals) for consistency with linear dose-response curves at the high doses used. Each experiment is too small to individually provide reliable evidence of consistency, but when all outcomes (different tumor sites and types) are taken together it is possible to discern whether the whole set is consistent. They find that the distribution of outcomes differs significantly from what would be expected if all materials acted linearly at each site for every tumor type, with an excess of both sublinear and superlinear behavior which persists even after correction for competing risks. This analysis can only show that for some combinations of material, animal, and site and type of tumor, sub- and superlinear responses occur, but cannot identify in which such combinations they occur. Furthermore, the analysis does not deny the possibility of linearity at lower doses than were used in the experiments.

Aflatoxin: Variable Results in Different Experiments. Aflatoxin B_1 is a potent carcinogen in animal bioassays. At least one of its metabolites binds directly to DNA (157,158) (Fig. 8). This metabolite is thought to be responsible for the strong biological activity of aflatoxin (159), but mechanisms are not well enough understood for the prediction of the shape of a dose-response relationship. A linear relationship has been experimentally found between applied dose and DNA adduct formation. However, as discussed previously, it does not necessarily follow that the dose-response relationship between initiation and applied dose is linear. Variation with dose of the site of action and the extent of repair, either of which would lead to a nonlinear dose-response relation, are currently under investigation (160,161), (Irvin, personal communication).

Figure 26 and Table 13 present some of the best data available for estimating the dose-response relation. In one experiment (162) the results were significantly non-linear ($p < 0.01$), while in the remaining three (163–165) linear relationships fit well. The potency of aflatoxin apparently differs substantially between different strains of rat, although some of this variation may be

due to the strong influence of diet on aflatoxin liver carcinogenesis (166).

In two of the experiments shown, the dosing schedules for different animals were varied, requiring a more complicated treatment. We used Equation 22 to model the age dependence of tumor induction and simultaneously account for the dosing schedules, allowing a test as to whether the observed results were consistent with a linear relation between dose and tumor induction rate. With this assumption, the maximum likelihood estimate for r and s in Equation 22 can be obtained. r may be interpreted as the total number of stages in the carcinogenesis process and s the stage affected by the carcinogen, although this is an oversimplification. We prefer to think in terms of fitting formulae that give some information on the time dependence.

For the Barnes and Butler experiment the maximum likelihood estimate for r is 4, and for s is 2. This is consistent with the Wogan and Newberne intubation experiment. However, in their feed experiment the best estimate for n was 7, and for r it was 3. Although these results are not entirely consistent with one another, they all indicate that aflatoxin B_1 most strongly affects the middle of the process and that incidence is proportional to age to some fairly large power, i.e., $p(t) \propto t^k$, where $k \sim 4$ or more. This could partly explain the apparent large variation in the carcinogenic potency of aflatoxin between the experiments in Table 13, for the experiment in which aflatoxin appeared the weakest (163) lasted only 1 year while that by Wogan et al. (162) lasted 2 years, and the Butler and Barnes experiment (164) 86 weeks.

Although the formula fitting described here is suggestive of certain hypotheses (such as those mentioned in the previous paragraph), there are others which are just as consistent with these experiments. For example, one can use the same sort of model as the one that led to Equation 22, but allow two stages to be affected by the carcinogen. Then there are several sets of parameter estimates which are consistent with all these experiments, but which might be interpreted differently.

Benzo[a]pyrene. There have been many carcinogenesis bioassays on benzo[a]pyrene using different species, strains, dose rates, and other experimental details. In an effort to determine how much of the variation in observed effects could be simply explained by variations in a few experimental protocols (such as age and period of dosing) Zeise and Crouch (38) analyzed those experiments in which benzo[a]pyrene was administered orally to mice. The approach was to use an extended multistage formula (Eq. 22) to analyze each experiment, and compare the parameter estimates, interpreted as a potency, a number of stages, and the stage affected. They found that forestomach cancer is likely to involve a small number of stages (less than 4) and that the dose rate versus response function may be nonlinear. The potencies measured in the experiments varied substantially, their distribution being lognormal with a standard deviation of ~0.6 (base 10 logarithms). Some of the variance could be explained by the variation with
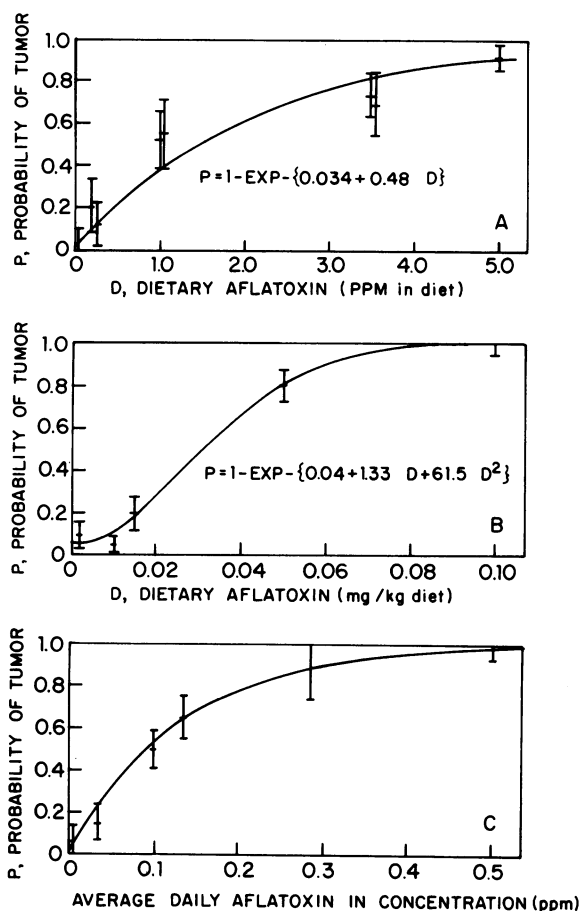

FIGURE 26. Dose-response curves for aflatoxin in three rat studies. (A) data from (163); (B) data from (162); (C) data from (164). Average dose computed using Eq. 22—see text.

Table 13. Aflatoxin dose-response in rats: multiple dose group studies.

Study	Aflatoxin content in diet		Liver tumor incidence
Newberne (163): linear dose response $[p(d) = 1 - e^{-bd}]$ fits very well. Strain is CD unless otherwise specified. Length of study: ~ 1 year.	ppm		
	5.0		14/15
	3.5		11/15
	3.5		7/10[a]
	1.0		5/9
	1.0		8/15
	0.2		2/10
	0.2		1/10[b]
	0.005		0/10[c]
Wogan et al. (162): (Male Fischer rats) quadratic term statistically significant $(p) = 0.004$	μg/kg		Liver cell carcinomas
	0		0/18
	1		2/22
	5		1/22
	15		4/21
	50		20/25
	100		28/28

Study	ppm	Duration of exposure, weeks	Hepatocellular carcinomas
Butler and Barnes (164): (Male Porton Rats) consistent with a linear model, but also with nonlinear models. Length of entire study: 86 weeks.	5.0 (0.005)[d]	1	0/13
	5.0 (0.04)	3	3/20
	5.0 (0.14)	6	12/19
	5.0 (0.29)	9	6/6
	0.5 (0.50)	86	25/25
	0.1 (0.10)	86	17/34
	—	—	0/46

Study	ppm	mg/kg/day	Time of sacrifice, weeks	Male	Female
Wogan and Newberne (165)	1.0 (for 2 weeks)	0.15	18	0/3	0/3
	1.0 (for 2 weeks)	0.15	35	0/5	0/7
	1.0	0.15	82	0/16	1/13
	1.0	0.15	18	0/3	0/3
	1.0	0.15	35	4/5	0/5
	1.0	0.15	41	16/17	0/7
	1.0	0.15	52	—	1/3
	1.0	0.15	64	—	4/4
	0.3	0.05	18	0/3	0/3
	0.3	0.05	35	2/7	0/5
	0.3	0.05	52	8/13	0/5
	0.3	0.05	60-70	—	11/11
	0.015	0.0025	18	0/3	0/3
	0.015	0.0025	35	0/3	0/5
	0.015	0.0025	52	3/5	0/3
	0.015	0.0025	68	12/12	—
	0.015	0.0025	80	—	13/13

Intubation amount per treatment, mg/kg body weight	Number of treatments[f]		Time of sacrifice, weeks	Male	Female
	5	1	16	0/3	—
	5	1	25	0/5	—
	5	1	38	0/5	—
	5	1	55	0/4	—
	5	1	69	1/5	—
	1.0	5	35	—	0/4
	1.0	5	82	—	2/16
	0.5	10	18	1/3	0/3
	0.5	10	35	1/5	0/3
	0.5	10	82	4/19	6/17

[a]Holzman rat.
[b]Originally given (209) as 0/10.
[c]Strain not specified.
[d]In parentheses, average daily dose, in ppm, calculated using Eq. 22 (38) assuming the second of four total stages was affected by the carcinogen.
[e]Feeding over 1 day with meal contaminated with 1.0, 0.3, and 0.015 ppm aflatoxin corresponds to an exposure of approximately 0.15, 0.05 and 0.0025 mg/kg body weight.
[f]Given on consecutive days.

dose rate, for on average, potencies were largest when benzo[a]pyrene was given at high dose rates. The results of the large experiment by Neal and Rigdon (167) are consistent with this explanation and indicate a sharp discontinuity in the dose rate versus response curve. Both above and below (but not across) the discontinuity the results are consistent with a linear formula (Fig. 27). The slope corresponding to the higher dose rate is an order of magnitude greater than the slope for the low dose rate group. Thus, the dose rate versus response relationship may be described by a hockey-stick shaped function.

This relationship is consistent with some results for DNA adduct formation (55), but not with others (168) which show a linear relationship between benzo-[a]pyrene dose and DNA adduct formation in the mouse forestomach. However, the results are also consistent with the alternative hypothesis that benzo[a]pyrene affects two stages in the process of tumor formation, an early stage and a late stage, both in a linear fashion.

Large Experiments

Saccharin. When male rats are exposed to sodium saccharin *in utero* and then dosed throughout their lives, the dose response relationship for bladder tumors is strongly nonlinear (Fig. 28 and Table 14). In most, but not all, experiments in which rats were dosed continuously throughout their life but not *in utero*, no bladder tumors were seen (169,170). The results of two generation bioassays are shown in Table 14. They give consistent results for the two strains, with the Sprague-Dawley rat more sensitive than the Charles River rat.

Many differrent mechanisms have been proposed to explain the carcinogenic activity and dose-response characteristics of saccharin, although some proposed

mechanisms have been later ruled out. It was initially thought that the tumors might be due to saccharin-induced bladder stones, which would probably not be formed at the low levels of human exposure. This would make saccharin a secondary carcinogen. However, the presence of microcalculi did not appear to be correlated with the formation of bladder tumors in any of the two generation bioassays. Saccharin does not appear to be metabolized in rats (169), even with concurrent exposure to phenobarbitol or 3-methyl-cholanthrene. It also does not bind to bladder or liver DNA *in vivo* and so is not usually considered to be a complete carcinogen (171). It may act as a promoter or cocarcinogen, although the mechanism for any such action is not understood (169,172). However, recent work shows that at very high doses it can produce structural disturbances in eukaryotic chromosomes *in vitro* and can be a very weakly active germ-cell and somatic-cell mutagen *in vivo* (173). In addition, its administration following freeze ulceration, which induces regenerative hyperplasia, resulted in bladder tumors in the absence of a chemical initiator (174).

The concentration of saccharin in various tissues of male Charles River CD rats was studied by Sweatman and Renwick (172). Saccharin was fed to the rats at 1 to 10% dietary concentration, at or below concentrations found to produce bladder tumors in earlier studies (169,170,175–179). Table 15 and Figure 29 show their results. The relationship between dietary concentration and plasma, kidney, or bladder concentration is nonlinear. An increase in the slopes of both the tumor response curve and the plasma saccharin concentration curve occurs at roughly the same place (compare Figs. 28 and 29).

Concentrations in the urinary bladder and kidneys appear to be greater than in the plasma, but these dif-
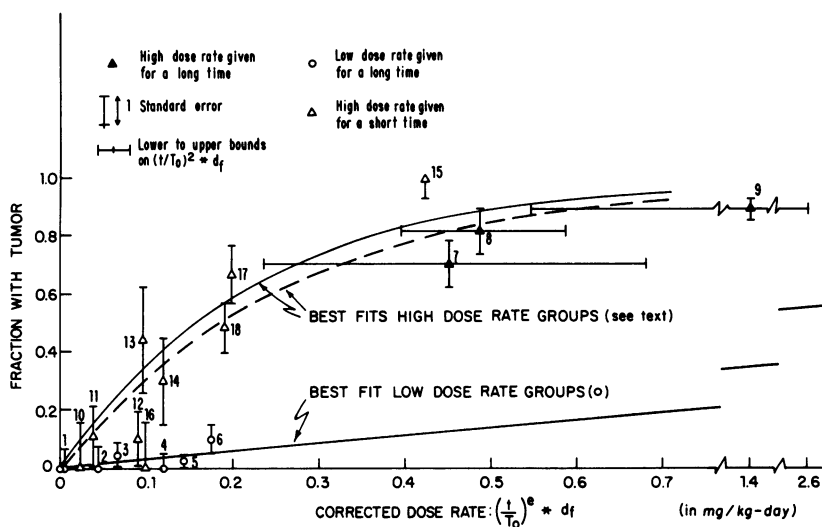

FIGURE 27.   Dose-response curves for benzo[a]pyrene-induced forestomach tumors in mice (38). The dose-response curves appear to be different for high dose rate and low dose rate groups. Eq. 22 was used on the data from a single experiment (167). Each number indicates a group of mice with differing exposure-time profiles. e and f are the same as r and s, respectively, in Eq. 22, and d_f is the multiplier of b in Eq. 22. For $e = 2, f = 1$.
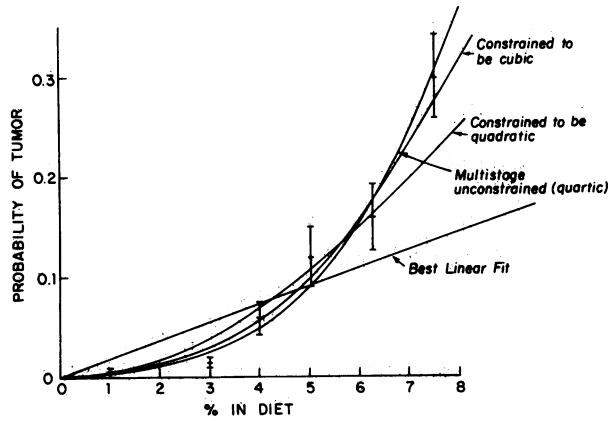
FIGURE 28. Probability of bladder tumor in rats exposed to saccharin in their diet (175).

Table 14. Sodium saccharin: bladder cancer in male rats exposed in utero and throughout life.

Study	Dose, (% in diet)[a]	Animals with tumor/total number tested
1973 FDA Study	0.0	1/25[b]
(176,179),	0.01	0/16
Charles River CD	0.10	0/27
rats	1.0	0/22
	5.0	1/21[b]
	7.5	7/23[b]
Canadian Health	0	0/42
Protection Branch	5	12/45
(169), Sprague		
Dawley rats		
Wisconsin Alumni	0	0/12
Research	0.05	0/10
Foundation (178,179)	0.5	1/12
Sprague-Dawley	5	8/15
Rats		
Calorie Control	0	0/324
Council (175),	1	5/658
Charles River CD	3	8/472
rats	4	12/189
	5	15/120
	6.25	20/120
	7.5	37/118

[a]% in diet of animals and their mothers.
[b]Numbers of animals with tumors may differ in other citations of this study due to disagreements in pathological diagnosis of lesions.

Table 15. Tissue saccharin concentrations in male rats fed 1-10% saccharin in their diet (172).

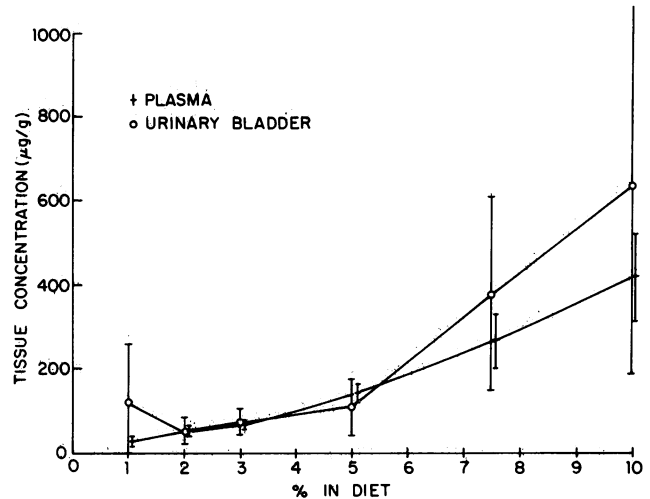| Dietary level, %	Saccharin concentration in μg/g or μg/mL[a]		
	Plasma	Bladder	Kidneys
1	29.6 ± 9.7	120.7 ± 129.3	101.6 ± 79.7
2	53.2 ± 10.7	51.6 ± 35.3	129.3 ± 36.1
3	69.9 ± 12.7	72.9 ± 29.3	201.1 ± 51.1
5	146.4 ± 42.8	118.8 ± 64.9	465.9 ± 108.8
7.5	266.6 ± 62.0	377.3 ± 235.4	1008.8 ± 367.8
10	418.0 ± 103.0	632.6 ± 441.6	1014.2 ± 392.6

[a]Plus or minus SE.

FIGURE 29. The concentration of saccharin in the tissues of male rats which were fed saccharin in their diet (172).

ferences are not statistically significant. Concentrations of saccharin in the bladder remain relatively constant between 1 and 5% dietary intake, but markedly increase at higher dietary levels (7.5% and 10%). However, these differences alone cannot explain the nonlinear dose-response relationship for bladder tumors, since F_1 males are not unique in accumulating saccharin at high dose levels. The same happens in females and F_0 males and neither of those experience bladder tumor rates as high as F_1 males (180).

One piece of evidence suggesting that saccharin is a promoter in bladder tumorigenesis was its nonlinear dose-response curve. If this nonlinearity is explained simply as a result of pharmacokinetic mechanisms, this argument is valueless. It is possible that saccharin is a promoter with a linear effective dose (e.g., bladder concentration) versus response relationship, but there is evidence which suggests otherwise. Trosko (62) applied his in vitro assay for interference with metabolic cooperation to saccharin, and found a strongly nonlinear response.

An alternative promoter mechanism has been proposed (181,182). High dietary concentrations result in enlargement of the cecum, producing an accumulation of protein there, and changes in the composition of the intestinal microflora. These changes have been associated with the production of three to four times the normal quantities of substances known to have promoting or carcinogenic properties: indole, phenol, and p-cresol. Indole is known to be a cocarcinogen for the urinary bladder; furthermore, its renal clearance as indican is inhibited by saccharin (183). Both p-cresol and phenol have been used as promoters in skin carcinogenesis experiments.

Greenfield, Ellwein, and Cohen (59) have compared a two-stage model with the results of experiments on bladder carcinogenesis in Fischer 344 rats exposed to sodium saccharin (with or without prior exposure to

FANFT, an initiator). They suggest its action is due to increases in mitotic rates, and hence stem cell populations, but that it does not affect the rate of initiation or transformation of cells.

There are thus several proposed mechanisms to explain the action and nonlinear dose-response relationship of saccharin: that it acts as a promoter by interfering with metabolic cooperation among cells; that it acts as a promoter by increasing mitotic rates; that it is weakly mutagenic and may cause initiation if applied at a time when the bladder epithelium is rapidly proliferating; that it exhibits nonlinear pharmacokinetics; that it acts indirectly by inducing the production of the promoters and cocarcinogens indole, phenol, and p-cresol; and that it induces calculi which are somehow related to the formation of bladder tumors. Which, if any, of these finally prove to be the major mechanism(s) of action remains to be seen, but that so many different mechanisms have fallen in and out of favor serves as an indicator that we should proceed cautiously when assuming particular mechanisms.

Radiation. Various shapes of dose-response relationships are seen in experiments on animals in which tumors are induced by radiation exposure. There are upward curving (sublinear) dose-response relationships for low LET radiation [e.g., mammary adenocarcinomas and adenofibromas in rats (184), skin cancer in mice and rats (185,186)]. In several studies the incidence of mammary cancer reaches a constant value, less than 100%, at high dose (89). The dose-response curve for mammary cancer in some strains of mice exposed to whole body radiation is complicated, since the radiation also induces ovarian tumors which secrete estrogen and stimulate growth of mammary tumors. There are also several experiments in which tumor incidence decreases at high doses, an observation usually explained by supposing that at high doses the radiation is killing cells which would otherwise have given rise to tumors. Examples include leukemia (187) and ovarian tumors (71) in mice.

In addition to total dose, the dose rate is of importance in controlling the shape of the dose-response curve for radiation induced tumors. These effects have been reviewed by Upton (72). The effectiveness of low LET radiation in inducing leukemias appears to decrease as the dose rate is reduced, possibly because small amounts of damage can be repaired (71). The opposite effect has been observed for (high LET) alpha radiation. At a fixed, relatively high cumulative dose of intraperitoneally injected Ra-224, reducing the dose rate can significantly increase osteosarcoma incidence (Table 16 and Fig. 30).

Muller et al. (188) suggest that this effect may be due to a reduction in cell loss rate at lower dose rate, to cell proliferation (normal or stimulated by the radiation) increasing the number of cells exposed, or to promotional effects of radiation being enhanced by the increased time of action at low dose rate. They argue against the second possibility because the risk of osteosarcoma does not unduly increase when fractured tibiae are irradiated.

Vinyl chloride. Vinyl chloride is known to cause liver angiosarcoma in rats, mice, and humans. In Sprague-Dawley rats, the incidence of angiosarcoma increases with exposure until a plateau is reached (Fig. 31); higher exposures have no additional effect (189). A straight line connecting the point at high dose to the origin underestimates the observed low dose incidence, while use of a gamma multihit formula for fitting the dose-response relationship results in overestimates of low dose risk. The gamma multihit formula suggests a tumor risk proportional to the square root of dose (8).

As discussed in "The Pharmacokinetic Models," the shape of this dose-response curve has been explained by the pharmacokinetics of vinyl chloride and its metabolites (47-52,190). Gehring et al. (51,52) used animal data to estimate the relationship between the amount of vinyl chloride entering the body and the amount of active (carcinogenic) metabolite formed, that is an ef-

Table 16. Osteosarcomas in female mice after ID injections of ^{224}Ra (188).

|	No. of mice		Injected activity, μCi/kg-body weight	Total dose, rad	Osteosarcoma incidence, fraction of animals	SD
Single injection	92		50	1500	0.14	0.036
	50		36	1080	0.08	0.038
	93		25	750	0.11	0.032
	49		12	360	0.22	0.059
	92		10	300	0.15	0.037
	91		5	150	0.15	0.038
	200		2.5	75	0.10	0.022
	200		1.0	3	0.07	0.018
	460		0	0	0.01	0.005
Protracted injection				μCi/ injection	Weeks of treatment	
	49	1.5	12	4	360	0.39	0.07
	50	0.5	12	12	360	0.46	0.07
	50	4.5	36	4	1080	0.22	0.059
	50	1.5	36	12	1080	0.62	0.069
	100	0.5	36	36	1080	0.92	0.027
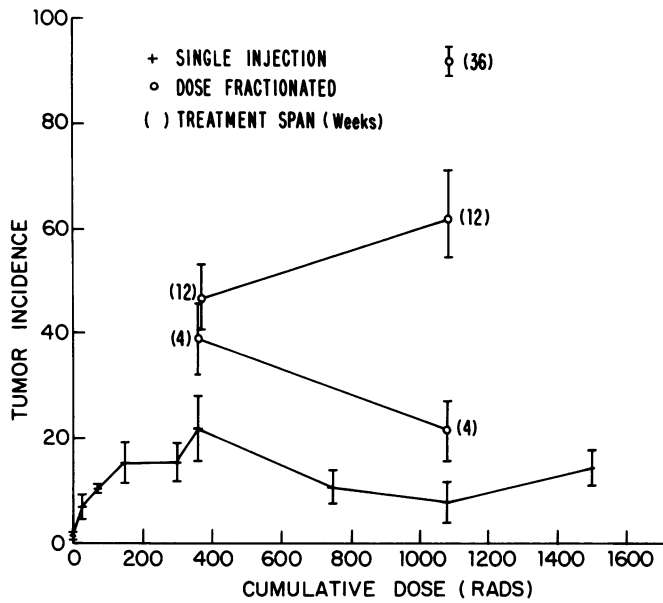
FIGURE 30. Osteosarcomas induced in female mice injected IP with ^{224}Ra.
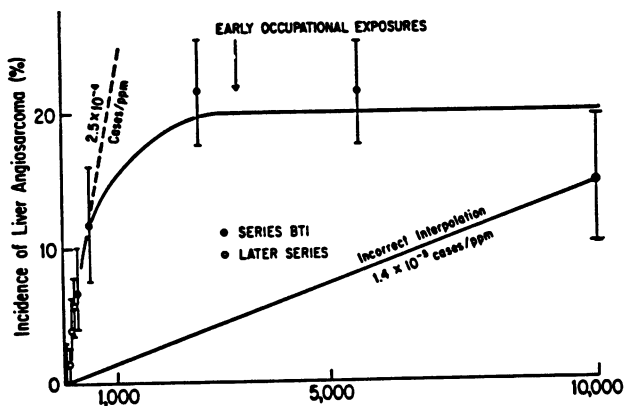
FIGURE 31. Dose-response function for vinyl chloride exposure of rats (189).

detailed multistage model (Eq. 23, with 3 stages). The compartment model was used to determine the quantity of DNA adducts (taken to be the effective dose) for a given applied dose. Figure 32 shows the average amount of DNA (nm DNA adducts/g DNA) bound after 30 min exposure as a function of vinyl chloride concentration. Ranking the organs by the amount of binding gives an order similar to that obtained if they are ranked by tumor response to vinyl chloride. Figure 33 shows the observed response versus a measure of effective dose in the liver, together with the shape of curve expected from a multistage model with three stages.

Experiments with 2-AAF. In most animal experiments the aim is to dose the animals at maximum tolerated doses (MTD), so that experiments are as sensitive as possible to carcinogens. In such experiments,
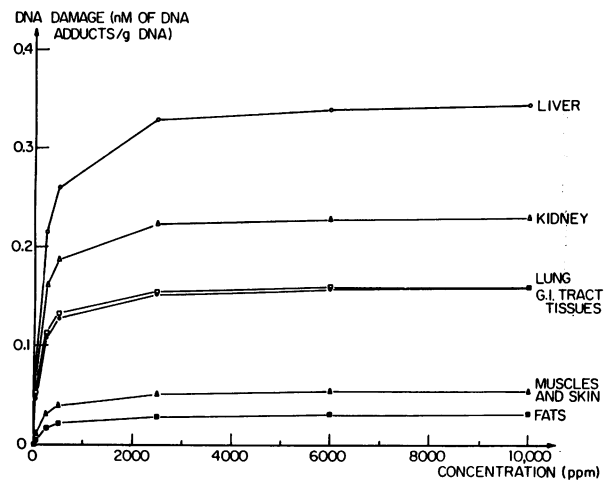
FIGURE 32. Effective dose to the liver (DNA adducts, in nM/g DNA) versus vinyl chloride exposure, predicted by a pharmacokinetic compartment model (57).

tabolites (47–52,190). Gehring et al. (51,52) used animal data to estimate the relationship between the amount of vinyl chloride entering the body and the amount of active (carcinogenic) metabolite formed, that is an effective dose, and so obtain a dose-response relationship using the effective dose. They then applied this dose-response relationship to humans, using a scaling law based on body surface area, to estimate effective doses in humans.

The transport of exogeneous materials round the body, and its metabolic activation, can be described by physiologic compartment models which include a kinetic description of the metabolic transformations occurring in each body compartment. This has been attempted in the case of vinyl chloride (58,191) with the additional attempt at coupling this compartment model to a fairly

FIGURE 33. Risk of liver angiosarcoma in rats exposed to vinyl chloride versus the effective dose to the liver (57).

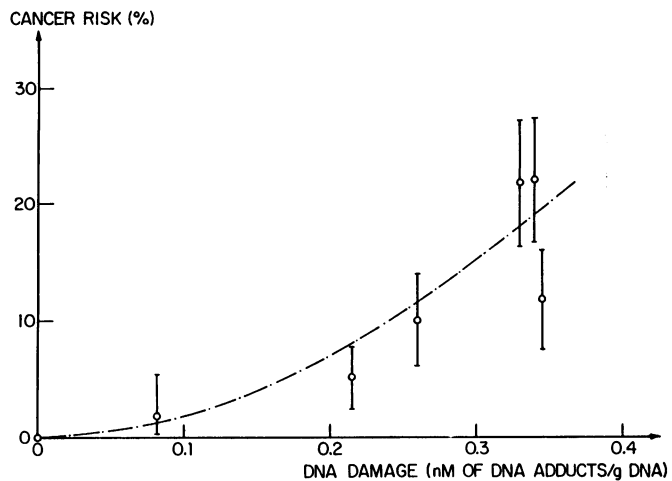effects that occur only at high doses may be observed. Usually we are interested in human effects at much smaller doses, far below those used in animal tests. It is tempting to believe that a series of very large animal experiments might help answer the question of how to perform high to low dose extrapolations. The ED01 study is such an experiment in which large groups of mice were fed the carcinogen 2-acetylaminofluorene (2-AAF) at several doses far below the MTD. A total of 24,193 BALB/c female mice were used. Several analyses of the data have been performed (192–194).

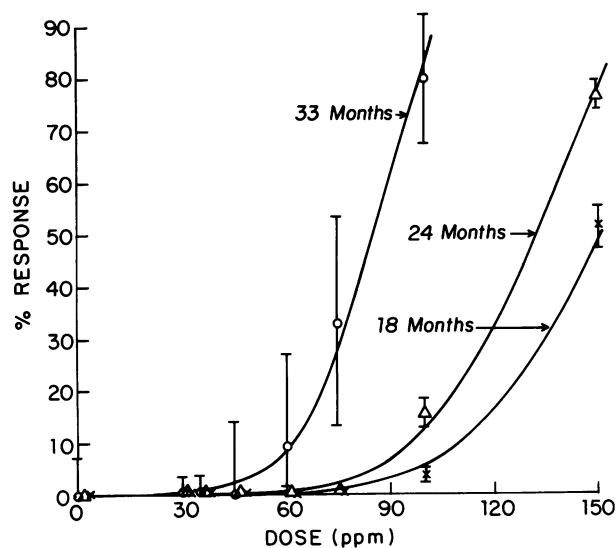In Figures 34 and 35 we show the probabilities of

FIGURE 34.   Bladder tumor incidence versus dose of 2-AAF (193) in female BALB/c mice.
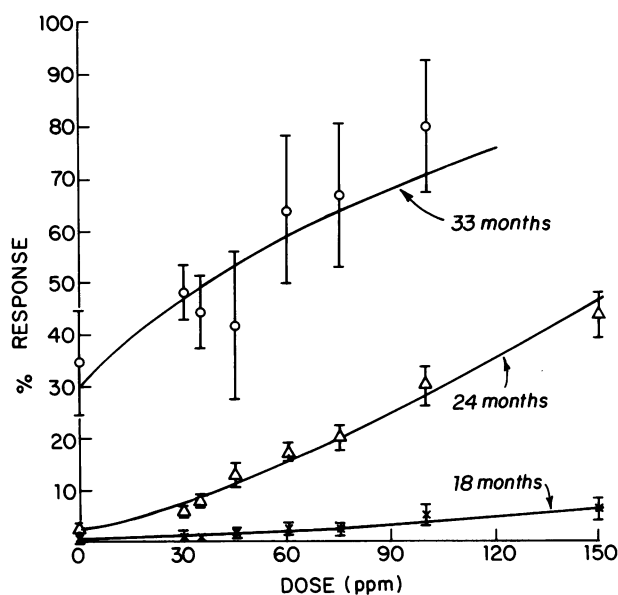

FIGURE 35.   Liver tumor incidence versus dose of 2-AAF (193) in female BALB/c mice.

tumor for bladder and liver tumors observed in the sacrificed animals, together with fitted lines corresponding to polynomial formulae. The dose-response relationship for the normally rare bladder tumor is highly nonlinear, while that for the normally common liver tumor is much more linear. However, there is a small, statistically significant, nonlinear component to the dose-response curve for liver cancers in animals sacrificed at 24 months ($p < 0.00004$).

Brown and Hoel (195,196) find fault with those analyses that assume factorable hazard function (e.g., like Eq. 26). They find liver cancer incidence to be a nonlinear function of the dose applied, but that the nature of the nonlinearity can be explained in at least two ways. First, with a multistage formulation with a total of 4 stages, in which two stages are affected by the carcinogen; second, again using a multistage formulation, with 2-AAF affecting an early stage (e.g., possibly behaving as an initiator) but with nonlinearity introduced through pharmacokinetic reactions at low doses.

N-diethyl- and N-dimethyl-nitrosamines.   The British Ministry of Agriculture, Fisheries and Food commissioned a large animal bioassay, using over 4000 Colworth rats to characterize the dose-response relationship for cancers caused by N-diethylnitrosamine (DEN) (esophagus and liver) and N-dimethylnitrosamine (DMN) (liver). The data was meticulously analyzed by Peto et al. (197,198), who compared observed numbers of animals with tumors to numbers expected under the hypothesis that the treatment had no effect on tumor incidence (Tables 17 and 18).

They compactly summarized the data using a formula of the form

$$-\log(1 - p(t,d)) = b \cdot (d + a)^k t^m \tag{40}$$

where $p(d,t)$ is one minus the probability of an animal being alive and free of a particular tumor after t years of treatment at an average daily dose d, in the absence of other causes of death and other tumors. That is, $p(d,t)$ is a measure of the cumulative incidence in the absence of competing risks. The integer exponents k and m were not formally estimated, but were chosen to give good agreement with the data. The results of this detailed analysis are shown in Table 19.

One can show that $p(d,t)$ is approximately proportional to the ratio of the observed to expected numbers of animals with tumors. This ratio is plotted against the concentration of DEN or DMN in the feed (which we use as the measure of dose) in Figures 36, 37, and 38. Superimposed upon the plots are polynomial curves of the form

OBSERVED/EXPECTED =

$$x_0 + x_1 \cdot d + x_2 \cdot d^2 + \cdots + x_7 \cdot d^7 \tag{41}$$

where each coefficient is forced to be non-negative. We

fitted such curves to the data from differing numbers of dose groups, and label them on Figures 36–38.

The strong nonlinearity seen for esophageal cancer induced by DEN is reflected in the summaries in Table 19 where the estimates for "a," the background parameter, are zero. For both males and females, the curves fitted to low dose data diverge strongly at low dose from those fitted to all data points (curves A and B, Fig. 36).

Response to liver tumors differs in males and females, despite the similarity of the summary statistics in Table 19. For males, all the fitted curves are similar at low doses. For females, however, the increase in tumor response as dose increases appears to be initially less rapid. This is emphasized by the divergence of the curves fitted to low dose data only, from those fitted to all data points. Thus, although the dose response relationship for DEN- and DMN-induced liver cancer appears to be linear at low doses in females as well as males, the slope of the curve at low doses is not as well predicted in the former case by high dose data.

That the response to rare esophageal cancers was nonlinear at low doses (Fig. 36 and Table 17) came as no surprise (197,198). The theoretical arguments of "Time and Age" suggest exactly such a result for tumors with low background incidence, although it is possible that a still larger experiment, in which background incidence was just observable, one would find dose-response characteristics more similar to that observed for liver tumors in the female rats. At sufficiently low dose rates, Peto et al. found the more common liver cancers simply proportional to the dose rate; in addition, other nonneoplastic lesions of the liver, such as hyperplastic nodules, were present at low doses with an incidence roughly proportional to the dose rate.

The Use of Dose-Response Relationships

Uncertainty

The shape of dose-response relationships for cancer at low doses is largely unknown because there are few or no data and because there is no generally accepted theory. Nonetheless, legitimate questions are continually asked by the public about the probable response (the risk) at these doses. Any risk assessor who writes down a value for risk is making a judgment based upon belief and upon constraints imposed by position, by statute, or otherwise.

Most discussions of uncertainty in carcinogen risk assessment avoid this uncertainty due to lack of understanding and concentrate on the statistical uncertainty of a particular epidemiological study or bioassay. Crouch and Wilson (199,200), and Gaylor and Chen (201), emphasize the importance of including also the uncertainty in interspecies comparisons, and Wilson et al. (202) emphasize including the uncertainty about whether or not a chemical is a human carcinogen. The essence of risk is uncertainty. To ignore uncertainty, therefore, is to incorrectly describe the risk. However, the uncertainty about dose-response relationships is so large that it is difficult to handle. Rather than being swept under the rug, it should therefore be emphasized.

One way of doing this is to perform all the mechanics, the arithmetic of risk assessment, first, and leave the uncertainty in dose-response relationship for last by qualifying all results with an explanation of this uncertainty. Performing such mechanics usually requires the use of some formula to represent the dose-response relationship, and so one should be chosen which is consistent with available data and appropriate for the task at hand. We mention below two such choices, which include also an appropriate parameterization of the formulae. This approach is valuable, for it may turn out that this uncertainty is almost irrelevant, for example, in trading off one risk against another. It may also turn out to be more expensive to argue over the shape of the dose-response relationship than to reduce exposure so that the risk is *de minimis* even with pessimistic assumptions (e.g., assuming that a linear formula adequately reflects the shape of the dose-response relationship). However, such a course of action must be explicit, and only taken if it does not prejudice subsequent actions.

Best Estimate of Risk. The National Cancer Institute was requested by Congress to produce a set of tables estimating the probability that an observed cancer was caused by a specified radiation dose. It was decided (91) to use a linear-quadratic formula (a linear formula was used for breast and thyroid) to represent the dose-response relationship. The best estimate of risk from the use of such a formula, and therefore the best estimate for the probability of causation, is lower than that which would have been obtained had a linear formula been used.

It is too early to tell how this, or similar studies for chemicals, might be used, for example, in legal proceedings. The tables, representing a consensus of the opinions of members of an expert committee, might be used for assigning liability. An expert witness would then have the onus of proof to explain why the risk is more or less than given by the tables. In some legal situations, however, a best estimate of risk may not be the parameter of concern; although the practice is changing, the law has tended to accept only demonstrated physical harm as a basis for action, and not a risk estimate. Based on this past practice, therefore, courts may regard these tables as too speculative. Contrariwise, the tables might be considered insufficiently conservative for setting safety standards.

Upper Bound on Risk. Most risk assessments are performed to assess whether a chemical can be widely used or whether further precautions must be taken in its use. This particularly applies to risk assessments by the FDA, CPSC, and EPA. It is appropriate for this application to chose a conservative representation for the dose-response relationship (i.e., one that gives pessimistically large estimates of risk at the doses of interest).

With this in mind, the FDA chose the Mantel-Bryan

Table 17. Dose-response relationship for tumors causing death in DEN-treated rats (197).

DEN concentration, ppm	Male			Female		
	Expected	Observed[a,b]	Ratio	Expected	Observed[a,b]	Ratio
Esophagus		$M^c + B^d$			$NK^e + M^c + B^d$	
0	58.0	0 + 0	0.0	16.7	0 + 0	0.0
0.033	15.0	0 + 0	0.0	4.1	0 + 0	0.0
0.066	15.8	0 + 0	0.0	4.3	0 + 0	0.0
0.132	15.4	0 + 0	0.0	4.2	0 + 0	0.0
0.264	13.6	0 + 0	0.0	4.4	0 + 0	0.0
0.528	15.5	0 + 0	0.0	4.1	0 + 1	0.2
1.056	14.5	3 + 5	0.6	4.1	1 + 3	1.0
1.584	14.0	9 + 4	0.9	3.6	3 + 0	0.8
2.112	10.5	21 + 5	2.5	3.4	1 + 10 + 1[e]	3.5
2.640	10.0	21 + 6	2.7	2.6	1 + 0	0.4
3.168	8.8	24 + 5	3.3	2.1	4 + 2	2.9
4.224	6.4	12 + 6	2.8	1.5	5 + 1	3.9
5.280	4.9	16 + 7	4.7	1.3	9 + 2	8.5
6.336	3.8	15 + 7	5.9	0.9	5 + 3	8.5
8.448	2.4	20 + 8	11.7	0.6	2 + 1	4.8
16.896	0.6	9 + 7	25.7	0.1	3 + 0	21.2
Total (all doses)	210.0	150 + 60	1.0	58.0	1 + 43 + 14[e]	1.0
Liver						
0	61.4	1	0.02	123.0	1	0.01
0.033	15.6	1	0.06	30.2	0	0.00
0.066	16.4	0	0.00	31.1	0	0.00
0.132	16.1	5	0.31	30.3	1	0.03
0.264	14.5	2	0.14	32.1	1	0.03
0.528	16.2	4	0.25	29.6	3	0.10
1.056	15.0	8	0.53	29.6	23	0.78
1.584	14.1	14	0.99	26.3	37	1.4
2.112	11.2	7	0.62	24.6	38	1.5
2.640	11.1	17	1.5	19.1	47	2.5
3.168	10.4	17	1.6	16.4	42	2.6
4.224	8.1	26	3.2	12.3	42	3.4
5.280	6.4	26	4.1	10.5	43	4.1
6.336	5.1	30	5.8	7.4	47	6.4
8.448	3.9	25	6.4	5.1	55	0.8
16.896	1.4	44	32.0	1.4	49	4.7
Total	227.0	227	1.0	429.0	429	1.0

[a]Each control group initially consisted of 240 animals.
[b]Each treatment group initially consisted of 60 animals, except females given 2.112 ppm, which began with 66 animals, and those given 16.896 ppm, which began with 54 animals.
[c]Malignant.
[d]Benign, no malignant present.
[e]Not known due to tissue loss.

Table 18. Dose-response relationship for liver tumors causing death in DMN-treated rats (197).

DMN concentration, ppm	Male			Female		
	Expected	Observed[a,b]	Ratio	Expected	Observed[a,b]	Ratio
0	86.3	1	0.01	120.3	1	0.01
0.033	23.0	1	0.04	31.0	1	0.03
0.066	22.4	3	0.13	30.3	0	0.00
0.132	22.0	3	0.14	28.8	2	0.07
0.264	21.9	3	0.14	31.3	3	0.10
0.528	23.9	3	0.13	29.2	5	0.17
1.056	21.0	5	0.24	30.6	5	0.18
1.584	21.1	3	0.14	25.5	27	1.1
2.112	21.7	13	0.60	25.4	23	1.3
2.640	17.2	27	1.6	21.2	44	2.1
3.168	17.1	33	1.9	19.4	48	2.5
4.224	14.6	36	2.5	16.0	53	3.3
5.280	10.4	46	4.4	11.2	52	4.6
6.336	9.4	49	5.2	10.5	51	4.9
8.448	6.4	55	8.6	5.5	55	10.0
16.896	1.7	59	34.5	1.9	58	30.8
Total (all doses)	340.0	340	1.0	438.0	438	1.0

[a]Each control group initially consisted of 240 animals.
[b]Each treatment group initially consisted of 60 animals.

Table 19. Summary dose-response formulae for the effects of DEN and DMN.

Chemical/site	Sex	$1 - p(d,t)$[a]	at t = 2 years
DEN/esophagus	Female	$11.16 \cdot d^3 \cdot t^7$	$1430 \cdot d^3$
DEN/esophagus	Male	$21.17 \cdot d^3 \cdot t^7$	$2170 \cdot d^3$
DEN/liver	Female	$32.09 \cdot (d + 0.4)^4 \cdot t^7$	$4110 \cdot (d + 0.04)^4$
DEN/liver	Male	$18.70 \cdot (d + 0.04)^4 \cdot t^7$	$2390 \cdot (d + 0.04)^4$
DMN/liver	Female	$51.45 \cdot (d + 0.1)^6 \cdot t^7$	$6570 \cdot (d + 0.1)^6$
DMN/liver	Male	$37.43 \cdot (d + 0.1)^6 \cdot t^7$	$4790 \cdot (d + 0.1)^6$

[a]Approximate cumulative incidence from exposure to dose d, given in mg/kg-body weight/day, after t years.

procedure (28,29) in 1965; this uses the probit formula, with the parameters chosen conservatively. At the time, this choice was considered conservative. Since 1975, it has been realized that the dose-response relationship could be linear at low doses. In 1977 the FDA changed over to using representations of dose-response relationships which are linear at low doses (203–205), and the same approach was adopted by the EPA (44).

Risk Comparisons. In some cases, being too conservative is also too expensive, or can lead to unacceptable hazards in other ways. This is well recognized, for example, in the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Toxic chemicals are often deliberately introduced into the environment for good and sufficient purposes of eliminating insects, fungi, and rodents. According to FIFRA, the risk of the chemical must be compared with the risk of alternatives, which might include the insects, fungi, or rodents themselves,

and the cost must be considered also.

It may be argued (206) that all risks are considered comparatively by everybody in their everyday decisions. Such a view is not universally accepted, and is certainly not currently incorporated in regulatory policy. However, in risk-risk comparisons it is necessary to estimate risks on a comparable basis. It is not appropriate to estimate one risk conservatively, while producing a best estimate for another. One particularly common example of such failure to calculate risks comparably is in comparison of a chemical which is a known carcinogen with another which has not been adequately tested, with the latter often being assumed to have zero risk. In the cases where a comparison is mandated, or even desirable, great care should be taken to ensure that similar assumptions are made in both cases, so that uncertainties tend to cancel in any comparison.

Conclusions

In this review we have shown that there are no data and few reliable theories to tell us the shape of dose-response relationships at doses corresponding to a lifetime risk of one in a million, a value which is sufficient to interest regulatory agencies. There are some data on humans at incidences corresponding to lifetime risks of 1% and above; the two largest animal bioassays provided data at lifetime risks of a few tenths of a percent.

Biological reasoning suggests the presence of nonlinearities of all sorts in dose-response relationships (e.g., some theories on promotion allow for threshold effects,
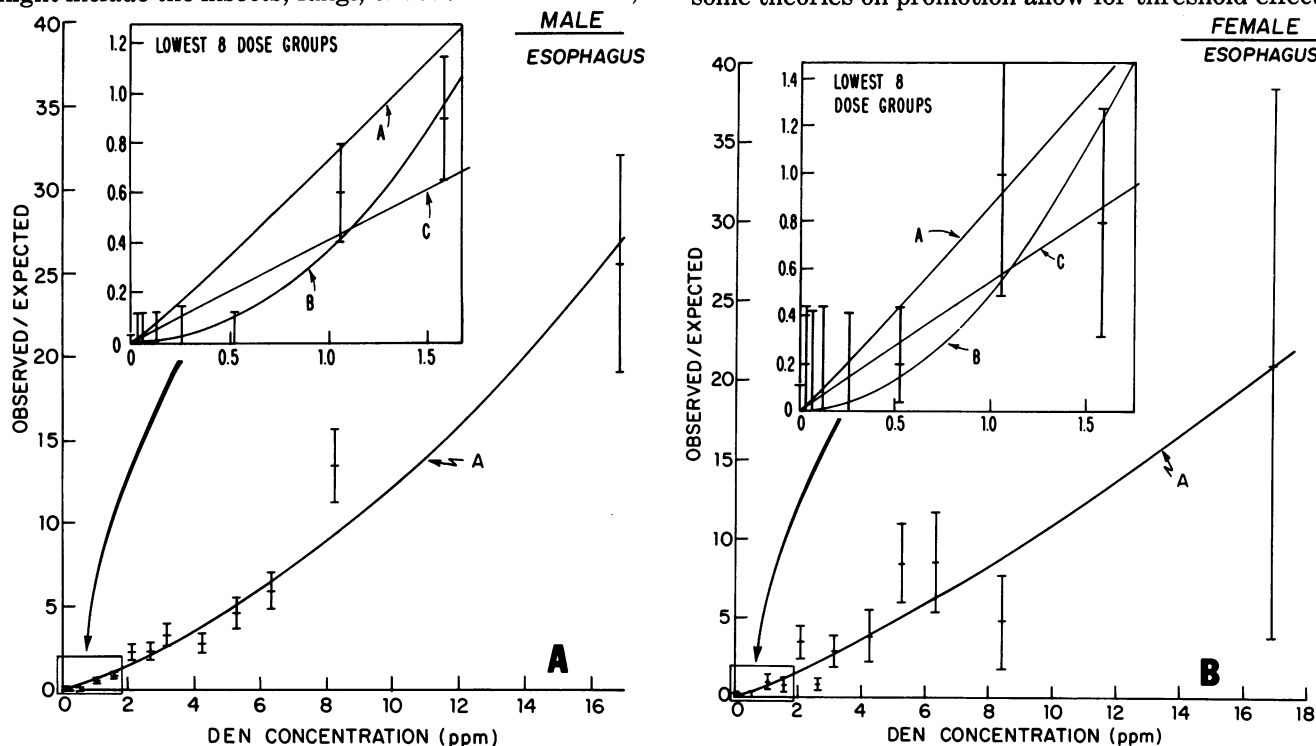

FIGURE 36. Observed/expected ratio of lethal cancers of the esophagus in rats versus dose of diethylnitrosamine (197,198). (A) Male rats; (B) female rats. Curve A: a polynomial formula fitted to all the dose groups; curve B: a polynomial formula fitted to the lowest eight dose groups; curve C: a linear formula fitted to the lowest eight dose groups.
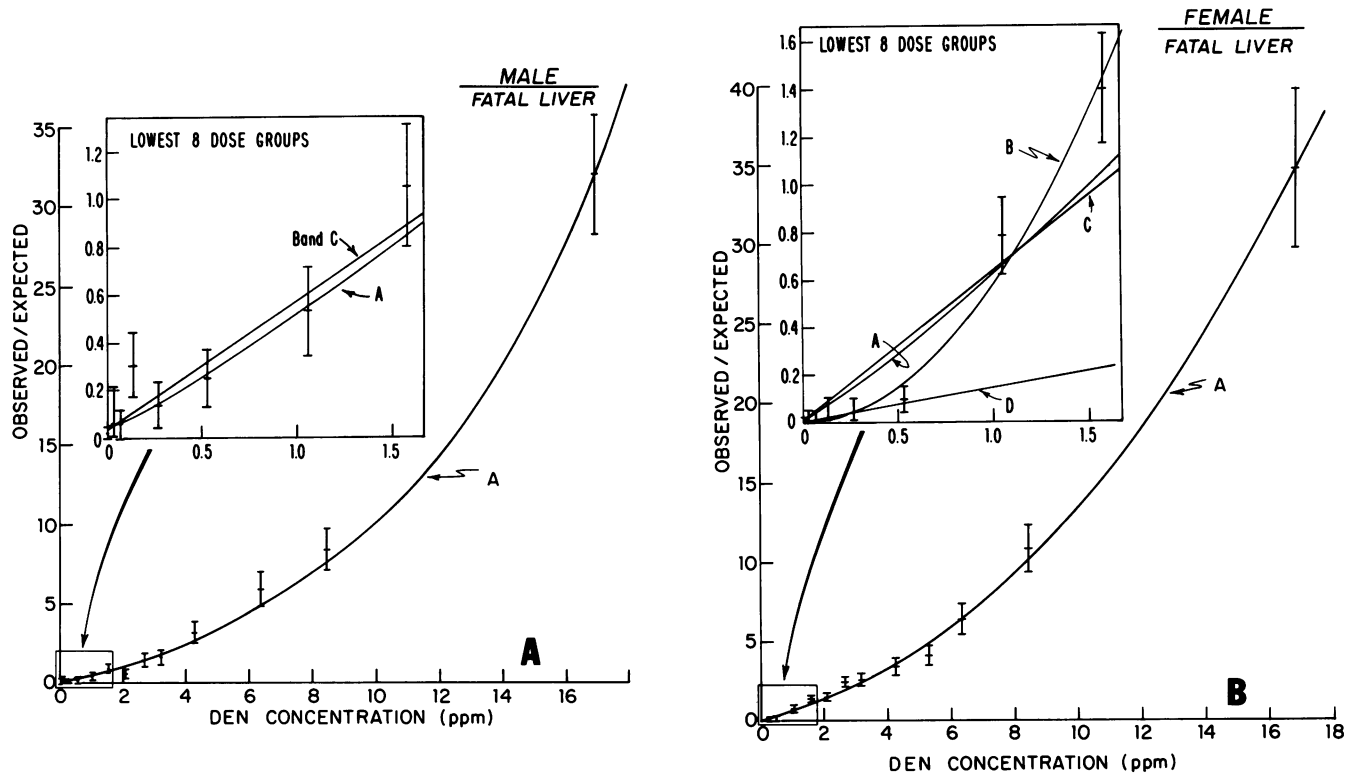
FIGURE 37.   Observed/expected ratio of lethal cancers of the liver in rats versus dose of diethylnitrosamine (197,198). (A) Male rats; (B) female rats. Curve A: a polynomial formula fitted to all the dose groups; Curve B: a polynomial formula fitted to the lowest eight dose groups; Curve C: a linear formula fitted to the lowest eight dose groups; Curve D: a linear formula fitted to the lowest four dose groups.

a pure initiator can have a linear dose-response relationship, saturation of a metabolic pathway or population effects can lead to a superlinear relationship). Superlinearities are usually attributed to only a few phenomena that should be distinguishable by experimentation. All known examples are high-dose effects, and it is not clear whether superlinearities occur in the unobservable low-dose range. The observed linear and sublinear dose-response relationships can be explained variously, with models of each explanation suggesting substantially different estimates of risk at low doses. An assumption of linearity should then give a reasonable upper bound for low dose risk estimates, but this may not be helpful if a threshold is strongly indicated and control costs are relatively high.

Data from animal experiments are often used to indicate the presence of nonlinearities in dose-response relationships, and the precision of such experiments can be high enough to clearly delineate such nonlinearities. However, there is no guarantee that the dose-response relationship in humans will be similar to that found in experimental animals. There may be species differences, and the interindividual variability in humans is likely to be larger than that between inbred experimental animals. Furthermore, humans are simultaneously exposed to more variable amounts of other agents that can affect carcinogenesis than are experimental animals kept in controlled conditions. Data from human

studies suggest that nonlinearities in dose-response relationships are prevalent, but in many cases the apparent nonlinearities may be artifacts due to limitations in data collection and analysis. Many published data may be misinterpreted by failure to take account of age effects and the effects of dose timing. The usual effect of such omissions has been to cause a dose-response relationship to appear more sublinear.

The best data from a human study, that on cigarette smoking habits of British physicians, shows sublinearity (risk approximately quadratic in the number of cigarettes smoked). The data on Austrian lung cancer victims' exposure to cigarette tar are also consistent with this sublinearity. The data on radiation exposures are, in general, better than that for other human carcinogens, though usually not precise enough to identify nonlinearities. High LET radiation is expected to give a linear response at low doses and superlinearity if doses are high enough, while for low LET radiation one anticipates sublinearity at low doses. Observations on the dose-response relationship for low LET radiation (breast, thyroid, and leukemias from medical or X-ray treatments or exposure to the atomic bomb) are all consistent with linearity. The most recent reanalysis of leukemia incidence in atomic bomb victims suggests a slight upward curvature. For high LET radiation there are indications of superlinearity and dose-rate dependence at high doses (bone cancer in ankylosing spon-
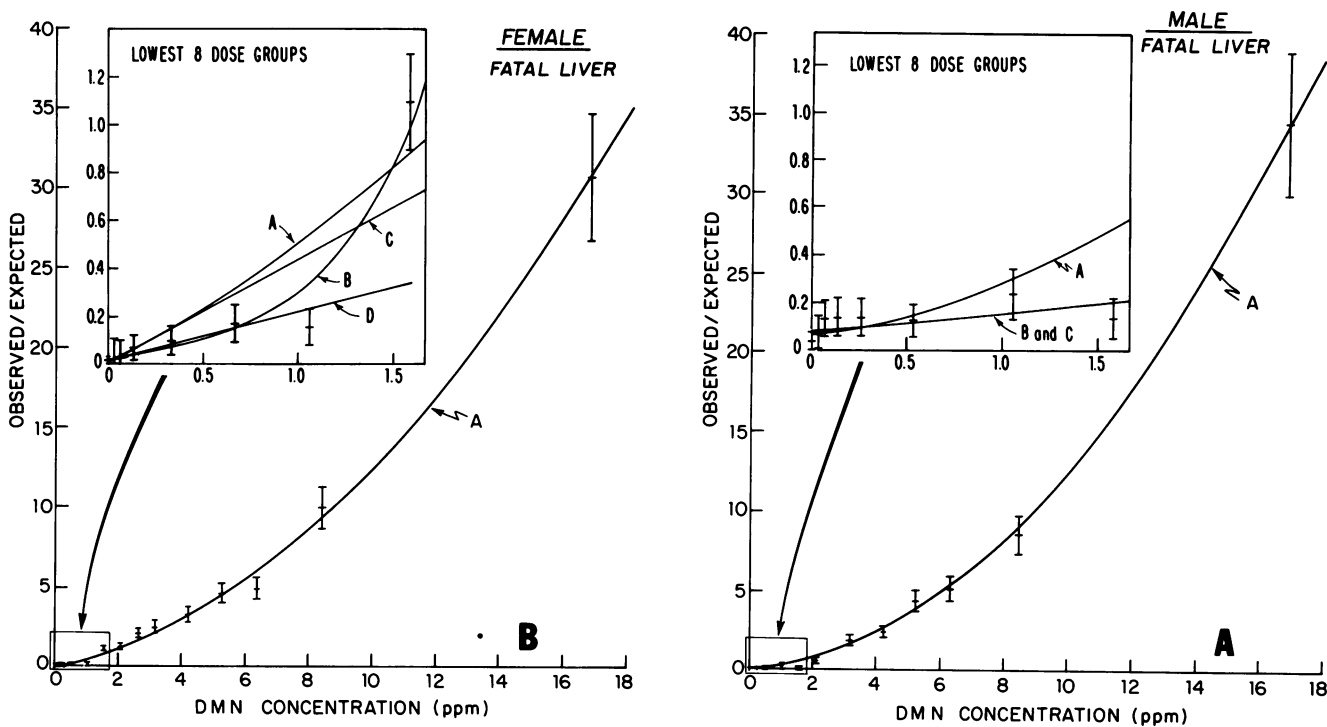
FIGURE 38. Observed/expected ratio of lethal cancers of the liver in rats versus dose of dimethylnitrosamine (*197,198*). (A) Male rats; (B) female rats. Curve A: a polynomial formula fitted to all the dose groups; Curve B: a polynomial formula fitted to the lowest eight dose groups; Curve C: a linear formula fitted to the lowest eight dose groups; Curve D: a linear formula fitted to the lowest six dose groups.

dylitis victims and dial painters exposed to radium), but at low doses the relationship is equivocal, with some evidence of sublinearity in the case of underground miners exposed to radon gas. Although there has been some debate over whether effects have been superlinear at low doses, it seems unlikely that these data will support this hypothesis, but it will require further follow-up to be sure. The effects measured in geographical areas with high levels of background high LET radiation also do not appear to be consistent with an assumption of superlinearity, while the data of high-dose occupational exposures are not precise enough to determine whether they are consistent with a sublinearity assumption. One major difficulty in drawing any conclusion is that the result depends on how much synergism one assumes between cigarette smoke and radon gas. In summary, reliable high dose data from human studies contains examples of superlinearity (radium injections and bone cancer), linearity (various radiation exposures), and sublinearity (smoking).

The variety of shapes of dose-response curves observed for humans is also seen for animals. Statistical analysis of a large number of small experiments indicates the presence of both sublinear and superlinear curves at high doses. In relatively small experiments on aflatoxin B_1, one finds some experiments significantly nonlinear at low doses, others linear. The dose-response curve for saccharin has been studied in several ways. All bioassay results appear to be sublinear, if they were sensitive enough to detect any effect. Several biological

mechanisms suggested for the action of saccharin have been tested in a variety of animal experiments. There is some evidence for each mechanism, suggesting that one should be cautious in accepting any one of them as the correct mechanism.

Radiation carcinogenesis in rodents appears to be consistent with theoretical ideas: upward curvature for low LET radiation, superlinearity at high doses for high LET radiation. The well-known case of superlinearity for Maltoni's vinyl chloride experiments has been explained by a pharmacokinetic mechanism,. Taking into account pharmacokinetics by using the effective dose gives a dose-response curve that appears sublinear. The large set of small experiments on benzo[a]pyrene shows it to be more potent if given at a high dose rate, and a single, larger experiment is consistent with this interpretation.

The largest bioassay reported to date is that on 2-AAF fed to BALB/c mice. The dose-response relationship found for liver cancers in this experiment is consistent with a nonlinear pharmacokinetic model, with a multistage model in which an early stage is affected, or with a multistage model in which two stages are affected. The dose-response curve for bladder cancer, a much rarer tumor, was strongly sublinear.

Finally, the large experiments on *N*-diethyl- and *N*-dimethylnitrosamine show substantial nonlinearities over the entire range of doses tested. Of special interest were the comparisons of the low dose responses with those at higher doses in both the rare esophageal tumors

and the more common liver tumors. The more common tumors might be expected to give a much more linear dose-response curve in the low dose regime, as discussed in "Dose Response Formulae." The dose-response curve for liver tumors actually appeared to be as nonlinear at low dose as that for esophageal tumors, at least in female rats.

For many cases where nonlinearity is found in animal bioassy data, auxiliary data (e.g., on mutagenesis and pharmacokinetics) should be capable of providing additional information on the cancer dose-response relationship outside the range observable in animal carcinogenesis bioassays. However, careful study of those chemicals with a well-characterized dose-response relationship for carcinogenesis, including accumulation of many more data on pharmacokinetics and cell dynamics, together with careful mathematical modeling, is required to distinguish between plausible biological explanations of such data. Currently, such additional data are too few and the explanations too inadequately verified for them to be used for regulatory purposes.

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