TOXICOLOGY AND APPLIED PHARMACOLOGY 21, 454–463 (1972)

Statistics vs Safety Factors and Scientific Judgment in the Evaluation of Safety for Man[1]

CARROL S. WEIL

Carnegie-Mellon University, Mellon Institute, Pittsburgh, Pennsylvania 15213

Received March 29, 1971

Statistics vs Safety Factors and Scientific Judgment in the Evaluation of Safety for Man. WEIL, CARROL S. (1972). *Toxicol. Appl. Pharmacol.* **21**, 454–463. The extension of the information obtained in animal toxicology experiments to evaluate hazard and predict safety in the use of chemicals for man is discussed. Illustrations are presented of the inapplicability of exaggerated or improperly designed animal tests. The many factors that influence the slope or shape of dose-response curves are illustrated, primarily using cancer as the response. These include duration and regimen of dosing, type of resulting neoplasm, strain, sex, nutritional status and age of the animal. Variation in slope of dose-response curves, even in 1 experimental design, single peroral dose, is extreme. Extrapolation to an LD35, an interval close to the LD50 midpoint, while theoretically applicable, was illustrated to be highly inaccurate. Therefore, the establishment of the shape of the low-response end of a dose-response curve by the use of large numbers of animals, and the statistical extrapolation of this information to predict a safe level for man, is deprecated. However, the applicability of a suitably large safety factor, e.g., 1/5000 of the lowest-effect dose level for cancer, is recommended by the author when the use of a material is deemed important. The size of the safety factor and the potential use of a chemical should be established by properly informed, scientific judgment.

Salac (1957) stated that failures are divided into 2 classes—those who thought and never did and those who did and never thought. The former type of failure might apply to several conferences and committees which have discussed dose-response relationships and the possibility of threshold levels for carcinogens, for example, without reaching a conclusion on a method or factor for setting a safe level for man. The latter type of failure might be applicable if incorrect conclusions, or extrapolations, result from improperly designed or analyzed experiments. The present paper involves the question of what is safety for man. Its estimation is discussed using either a statistical approach or scientific judgment, after the performance of animal experimentation. The safety issue is controversial, and the conclusions reached and thesis propounded by the author are in part, based on his interpretation of the references discussed.

The safe use of chemicals for man must include those of natural or synthetic origin, whether they be nutritive or nonnutritive in the case of food chemicals, or whether they be physiologically active or inert. The safety evaluation of such a chemical is largely

[1] This paper was presented as part of an invited symposium on "Dose Response in the Appraisal of Toxicologic Effects" at the Tenth Annual Meeting of the Society of Toxicology, held in Washington, D. C., March 9, 1971.

independent of the reasons for its presence in food or as a pharmaceutical or cosmetic product.

As recently described by Wogan (1969), many food constituents have been shown to be carcinogenic in one or more experimental animal species. These include contaminants introduced into foods by biological processes, such as metabolic products of microorganisms growing on food commodities, as well as compounds inherently present in commodities used as foods or arising as a result of metabolic processes of plants used as foods. Epidemiological, circumstantial evidence has been reported relating aflatoxins to primary liver cancer, but in most instances the mere presence of most of the animal-active, naturally produced agents has not yet been implicated as dangerous for man. Common sense indicates that, because of the very low concentrations of most of these in the diet of man, their role in carcinogenesis is probably low or nonexistent. It is the hypothesis of the author that the same common sense (alternately termed "scientific judgment") should be applied to all intentional or incidental additives. No matter what the biological effect, at some concentration under some sets of conditions, a dose level must exist below which no biological damage will occur during the life-span of the great majority of men. No matter how small the dose, however, 1, or a few, of millions of subjects may exhibit the critical response.

The definitions stated by the Food Protection Committee of the National Academy of Sciences (1970) will be used in the ensuing discussion, viz: (1) Toxicity is the capacity to produce injury. The term includes capacity to injure any of the mechanisms of the body, including for example the induction of carcinogenesis; (2) safety is the practical certainty that injury (to man) will not result from the substance when used in the quantity and in the manner proposed for its use; and (3) hazard is the probability that injury will result from the use of a substance in a proposed quantity or manner.

The evaluation of safety or hazard must take into account the conditions of use of the substance. Thus, the experimental design must be applicable. For example, when humans contact a chemical in their diets at insignificant levels, of what value is a sc injection study if the relevant route of contact for man is by ingestion; of what value is the use of a maximum tolerated level if only trace quantities are present in the environment; of what value is the use of an animal if it does not metabolize the chemical in a similar manner or rate as man?

Recently testing has often involved the use of newborn rodents. As stated by Hanley *et al.* (1969), if the compound is given at such high doses that the mother is ill, it is not surprising that the fetus is affected, either directly by the compound crossing the placenta in conventionally toxic doses or indirectly by producing maternal malfunction. Furthermore, metabolism in the newborn often differs from that in the mature animal; the infant during lactation should receive its dose after potential metabolism of a material by the parent, rather than by injection or po intubation of the material itself. The metabolism of a material tested at high dose levels may be quite different from that of the same material at much smaller levels. While it has been demonstrated over several decades that properly designed animal studies are usually adequate for prediction of safety for man, inadequate or exaggerated designs should not be so used.

No method for establishing the safety of a chemical, natural or synthetic, with absolute certainty under all conditions of possible use is at hand, However, experience has shown that properly planned, conducted and interpreted animal experiments

provide the degree of practical assurance necessary for the evaluation of the safe use of chemicals for man. While it is common knowledge that many natural products can be harmful to animals when used improperly (in too large amounts, by an unusual route, to 1 or more specific strains), appropriate quantities of these natural products can, and are, being used safely.

Recently, discussion has centered on the current applicability of the methods used to determine toxicity, hazard and safety. These methods and principles have been used for many decades for chemicals known to produce injury to organs or to alter various functions in animals. However, the application of these same principles to reproduction, teratogenesis, mutagenesis or carcinogenesis has sometimes been questioned. It is a generally accepted principle that unless a physiological effect is dose-related, the alteration in function is often merely an artifact. At times measurements on control animals are the unusual finding (Weil and Carpenter, 1969). If chemicals are tested for any of the biological effects at only the maximum tolerated dose level, assessment of dose relationship to effect cannot be made. Properly designed and conducted animal experiments will show, at least in a limited range of doses, a real relationship of dose and effect in any of the parameters measured, for example, in liver weight or cancer induction.

The crux of the matter is the extension of this dose-response information to the prediction of the safe level for man: shall it be by statistical extrapolation from the dose-response data obtained; shall it be by experiments with large numbers of animals at very low dose levels; or shall it be by the application of a suitably large, arbitrary, safety factor or factors? Some of the discussion that follows will be centered around carcinogenesis as determined in animals, but the principles involved apply to any altered physiological function.

As stated by Weisburger and Weisburger (1968), "In recent years there have been impressive advances in analytical techniques by which smaller and smaller amounts of chemicals can be reliably identified and estimated. By means of such refined technology, it has occasionally been found that foodstuffs, prior to any treatment, already contained recognized carcinogens . . . . Also, as more knowledge on dose-response relationships with chemical carcinogens has been obtained, these agents have been found to behave like most other drugs, showing decreasing activity with declining doses and sometimes even reaching a no-effect level . . . while there probably is a no-effect level with chemical carcinogens, this level is valid only for a specific experimental protocol."

Bock (1968) also stated that there may be a region at very low dose levels where no response is observed, i.e., a threshold. In any experiment with limited numbers of animals, an apparent threshold is possible. However, this can vary from 1 experiment to another unless conditions are rigidly standardized. The shape or slope of dose-response curves often changes with different experimental conditions. For example, Fig. 2 in Bock's paper illustrated that the dose-response curve may be either linear or curvilinear using the same chemical by the same route to the same strain of mice, depending on the number of weeks that the mice received their skin painting exposure to benzo[*a*]pyrene.

The dose-response relation in radiation-induced cancer, as denoted by Upton (1961), depends on the type of neoplasm, constitution of the host, conditions of irradiation and other variables. Developing from a single exposure early in life, leukemia

(lymphosarcomas in the thymus) followed a sigmoid dose-response curve, the slope and intercept of which varied with the strain and sex used (Upton, 1961, chart 5). When the dose was administered in several successive exposures, the incidence varied not only with the total dose, but with the number of exposures, dose per exposure, interval between exposures, nutritional status of the animal, etc.

In RF mice (Upton, 1961, Chart 6) the incidence of granulocytic leukemia was increased many times by a single exposure to 150 r; the dose-response curve leveled off and declined between 300 and 400 r. The shape of the curve in the dose region below 150 r was not precisely known, but the available data were more consistent with a quadratic than with a linear function. The probability of bone tumors varied with the injected dose of radioactivity (Upton, 1961, Chart 7). None of the curves in this figure suggested a linear relation between the probability of neoplasia and the dose of radioactivity injected. On the contrary, the curves appeared sigmoid and highly variable in slope.

With mammary tumors an essentially linear relationship appeared, using a limited dose-span and a cutoff of observation at 11 mo of age; after this age the incidence of spontaneous breast tumors in the controls increased progressively with time, thus greatly changing the slope, if not the shape, of the dose-response curve. These data, stated Upton, are not representative of the final tumor incidence even at high-dose levels. Furthermore, the dose-response curve was based on the pooled frequencies of breast tumors of all histologic types, some of which had higher incidence than others.

The tendency for the carinogenic dose-response curve eventually to reach a saturation point and then to decline at high levels appeared to be a consistent phenomenon with many types of neoplasms, e.g., the induction of thyroid tumors by ^{131}I in rats (Upton, 1961, Chart 11).

Thus, the data suggest that the dose-response curve may vary in slope and intercept, depending on the type of neoplasm in question, the constitution of the animal, the way in which the dose is distributed in space and in time and on various other influences. In no instance to date has a linear relationship between neoplasia and radiation dose been adequately demonstrated. Consequently, attempts to estimate the carcinogenic hazards of exposure to small amounts of radiation are necessarily speculative.

Upton stated that from the practical standpoint the establishment of permissible exposure levels commensurate with public safety requires that the toxicity of small amounts of radiation be tentatively estimated, even by extrapolation from animal data when human statistics are lacking. "It is not yet possible," he stated, "to define the kinetics of carcinogenesis or to prove the existence or absence of a threshold level for carcinogenesis by extrapolation of the dose-response curve from regions of detectably significant dosage."

Brues (1958) summarized the situation as follows: (1) present data on human leukemogenesis fail to indicate a linear relation between dose and effect. A critical analysis of the data failed to establish any human leukemogenic response below about 100 r; (2) other instances in which carcinogenic agents have been examined from the standpoint of dose and dose-rate relations show many clear examples where the relation is nonlinear, and none in which linearity is unquestionably demonstrated; (3) theoretical consideration of the probability that a single critical molecular event, such as a mutation, will give rise to cancer indicate that a malignant change must be an

extraordinarily improbable result of such a perturbation. It is also very difficult to reconcile this mechanism with the rather comparable spontaneous and induced cancer incidences in species with greatly different numbers of cells; and (4) any scheme in which carcinogen-related multiple events are required to produce a tumor is incompatible with a linear relation; while, if a disordered state of tissue is an important factor, a true threshold may occur. There is much evidence from cancer research indicating that one or both of these conditions (multiple events and a true threshold) are involved in the carcinogenic process.

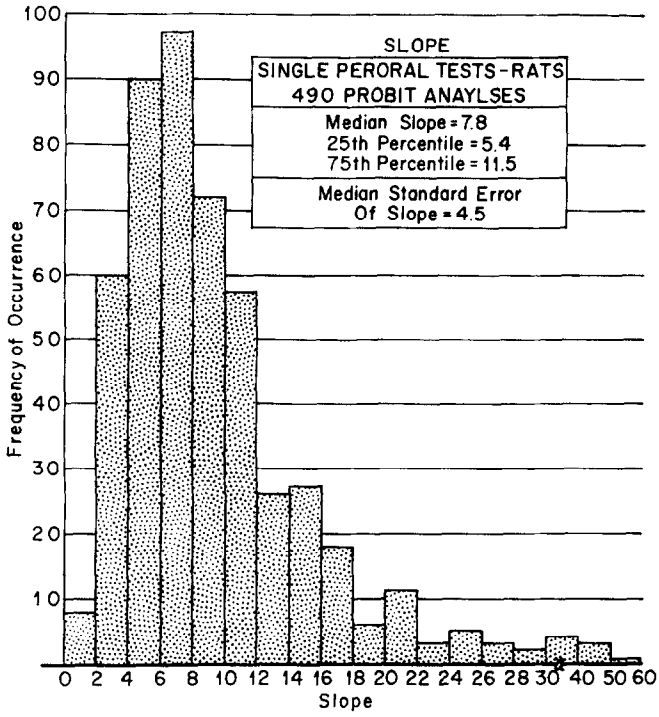

FIG. 1. Slopes calculated from 490 single po dose tests in rats.

Much of the preceding discussion has involved various types of tumors formed after radiation. While chemical carcinogens may or may not act similarly, the principles of the response to different experimental conditions and the resultant animal tumor-incidence data are the same.

It has been demonstrated that the shape of the curve in the actual dose-response area varies with changes in experimental conditions. It must be remembered that in using different chemicals, even with the same set of conditions, there is a wide range of slopes. A histogram is presented in Fig. 1 of 490 probit-estimated slopes, each with its own uncertainty, resulting from single po dose assays in rats. These slopes varied from 1.38 to 64.89. In all likelihood, similar variation in slope would be found with different chemicals in the area of lower response to decreased doses. The slope of any biological curve varies from point to point (even so with any transformation thereof). The slope is usually a maximum at the midpoint.

Even if dosing thousands of animals at low dose levels could establish the most probable shape or slope of a dose-response curve, this slope would also have its large uncertainty and would hold only for specific experimental conditions, e.g., the strain of animal used, the route of administration, the diet, and especially the chemical tested. A different chemical (as well as other experimental conditions) would result in different slopes.

The change in dose-response curves by alteration in experimental conditions has been discussed. As stated by Weisburger and Weisburger (1968), there probably is a no-effect level for chemical carcinogens, although this level is valid only for a specific experimental protocol. They discussed evidence that administration of carcinogens resulted in irreversible alterations, but stated that under experimental conditions dose levels can be found for which no tumors are seen over the life-span of the animals. They and others have discussed how experimentally produced "no-effect" levels may become active when diets are varied in certain ways, or when the agent used is tested in combination with other selected agents. It is the hypothesis of the author, however, that under any conceivable set of practical combination of conditions there is a dose level that will result in no harm during the life-span of animals or man.

Should one attempt to determine this low-risk level for man by extrapolation of animal experimentation data? It is common knowledge that the extrapolation of values beyond the region covered by the data is very dangerous. The uncertainty of the validity of the approximation increases with the remoteness of the estimated point from the midpoint of the curve (if a definite curve exists).

The data in Table 1 indicate the extreme caution one must use in even a slight extension of data from their midpoint. In 1952 a project was started to determine joint toxic action following acute po intubation. LD35 values were calculated for 10 chemicals. It may be seen that they are very close numerically to the LD50 values for each material. When groups of rats were given this estimated LD35 po, the actual mortalities were never near 35%. A median of 6% kill resulted rather than the predicted 35%.

Many cancer investigators and statisticians have discussed the applicability or nonapplicability of various curve-fitting procedures to estimate a noncarcinogenic level

TABLE 1

COMPARISON OF CALCULATED LD35 TO MORTALITY OBSERVED AT THAT DOSE LEVEL

Material	Calculated		Observed mortality at LD35	
	LD50	LD35	Ratio	%
Acetonitrile	5.75	4.24	3/20	15
Aniline	0.476	0.424	2/20	10
Diethylene glycol, monoethyl ether	6.31	5.62	1/20	5
Ethanol	6.06	5.40	3/40	8
Ethylene chlorhydrin	0.058	0.051	0/20	0
Ethylenediamine	0.076	0.064	1/20	5
Ethylene glycol	5.22	4.46	1/18	6
Methanol	9.54	8.45	4/20	20
Phenol	0.32	0.28	0/20	0
Propylene glycol	15.9	12.4	1/39	3

for the test animal. Some, such as Mantel *et al.* (1961), used linear regression with an arbitrarily assigned slope. Zweifel (1966) preferred application of the likelihood principle, dismissing *a priori* any preconceptions about which model is "best." Statisticians have shown that 3 distribution forms, probit, logit and 1-hit models, estimate the midpoints, and even some value such as the ED16, quite similarly for cancer or other dose-response data. However, they also point out the wide difference between the tails of these distribution forms, despite their similarities in the center (Mantel, 1963). It has been stated that the estimation of 1 cancer per hundred-million subjects, termed the virtual safe dose by Mantel and Bryan (1961), would be one-hundredth of the TD1 (1 % tumor dose) by the probit curve, one hundred-thousandth of the TD1 by the logistic curve, and one-millionth of this using the 1-particle curve. Thus, even if the TD1 could be experimentally well determined by the use of thousands of animals (and this is doubtful), mathematical extension of this to a virtually safe dose would be beset with great uncertainty. Therefore, megamouse experiments or arbitrary distribution-involved extrapolation of dose-response curves offer little practical hope of prediction of safety for man.

A similar conclusion was reached by the Panel on Carcinogenesis of the Advisory Committee on Protocols for Safety Evaluation of the Food and Drug Administration in a 1969 draft of a report probably to be issued. They stated, "It would be imprudent to place excessive reliance on mathematical sleight of hand, particularly when the dose-response curves used are largely empirical descriptions, lacking any theoretical, physical or chemical basis."

It is obvious that even if a low risk, no-effect level was found for a chemical under a particular set of experimental conditions, the need to extrapolate, for example, from mouse to man, still remains. If tumors in mice were used as the end point, the application of statistics in any form could only predict probable safety for mice. As stated by the Food Protection Committee, Food and Nutrition Board, National Research Council (1970), there is no generally accepted way of quantitatively extrapolating dose-response data in predicting a noncarcinogenic level for man. This fact is the principal basis for urging that such compounds be permitted for use in foods only if an explicit judgment has been made that demonstrable benefit greatly exceeds the risk. Safety of each substance must be evaluated on results of adequate tests and a knowledge of the projected uses.

Should any of these doubts on the shape of the cancer dose-response curve as affected by chemical and host factors, sex, strain, diet, nutritional status, size of dose, age of animal, and dose-regimen (single dose, chronic dose, intermittent administration), mitigate against the setting of a probable no ill-effect level for man? It is obvious that the same factors influence many other phases of the toxicity of chemicals.

Even if there is a low threshold dose for some carcinogens, some biologically insignificant level must exist. Below this negligible level (or combination of doses resulting in this), practically no cancers or other effects would result during the life-span of man. The infeasibility of setting this by experimentation and then by extrapolation has been discussed. The ensuing discussion will illustrate its estimation by the application of common sense.

One might question whether or not a negligible level should be set if cancer or any other biological effect has been shown to result in some species by some route. This

depends on the applicability or practicality of the experimental design and on the necessity of use of the material. To say that all carcinogens (in the unscientific sense of tumor by any route in any animal) should be banned is ridiculous. It is the old poison per se doctrine which most toxicologists thought was laid to rest by the 1958 amendment to the Food and Drug Act. Radiation is not banned; limits are set for its safe use. Charcoal-broiled steaks are not banned, although they have been shown to produce carcinogenic hydrocarbons. The eating of eggs is not forbidden, though under ridiculous experimental design they have been shown to be carcinogenic. The author is a firm believer in the use of proper statistics to compare quantitative results of treated and control animals. But good toxicologists must realize that the matter of most importance is proper experimental design. One must keep in mind the statistical approach to be followed from the results of these designs, but no statistics for prediction of safety should be applied to grossly exaggerated designs.

As Golberg (1970) stated: "The best we can do experimentally is to create an arbitrary set of conditions of administration of a test compound which we consider to be as relevant as possible to the conditions of intended human or animal exposure . . . . The best we can hope to achieve is the assessment of reasonable men who are fully conversant with the present state of the art." Experience and scientific judgment must be relied upon, in the final analysis, in applying experimental results to conditions of use for man.

Even if cancers such as those induced by radiation or by the chemicals discussed by Druckrey (1967) are irreversible, some total dose will be inconsequential during the life-span of man or his progeny. If this cannot be set by extrapolation using statistical means, it can and must be set by the application of scientific judgment of the experimental data if the need for use of the material is deemed essential.

It is a fact that analytically detectable levels of naturally occurring or synthetic materials, shown experimentally to be carcinogenic under a particular set of conditions, will often be present in the diet of man. The various proposed statistical procedures for setting a "safe" level have been shown to result in variable and unreliable estimates even for the particular test animal used. Therefore, an arbitrary factor must and can be applied to experimentally positive data if the chemical in question is deemed necessary for use. The first requirement, as previously stated, is that the design of the experiment is applicable to predict the degree of safety of a material for man (Weil, 1970). Then, using the minimum measured cancer-producing dose-level (MiE), a factor of 5000 is proposed for the maximum amount of this material in the diet of man. This factor is derived as follows: for many years a factor of 100 has been used; it has been stated that animal to animal variation is seldom greater than 10-fold, and that another 10-fold factor is probably adequate for most materials to translate the results from animal to man. An additional 10-fold factor is now being proposed for the criterion of carcinogenesis on the theory that this type of action may be less reversible than some others and, multiplied by the previous factor of 100, may approximate a level which would likely be inactive if cocarcinogenesis or initiation-promotion is present with materials in the diet.

Cocarcinogenesis with more than 1 constituent in the diet has not been proved conclusively. The study of Sugai *et al.* (1962) could not be properly controlled, as feeding heated oils or their adducts was stated to be impossible because of toxicity. No groups were fed only the basic diets plus the heated oils or their adducts; therefore,

the increase in tumor incidence by a combination of the basic diet, 2-acetylaminofluorene and heated oil adduct might be simply an additive effect. It is notable, however, that a factor of 1000 would cover even the cocarcinogenicity reported on skin application tests by Bingham and Falk (1969).

A final factor of 5 is suggested because the level used from the animal experiment for division by the factor of 5000 is the minimum-effect level, and not the maximum experimentally produced no cancer-effect dose level. The MiE level in a proper experimental design should be no more than 5 times that of the no-effect level; it should be selected because it is more repeatable than the latter.

Thus, if 100 mg/kg were the MiE for cancer in an animal experiment, a dose level of 0.02 mg/kg is proposed as a probably safe level for man. In other words, for a 70-kg man eating 2000 g of diet per day, a MiE of 100 mg/kg would be equivalent to 0.35% in the diet. If 3500 ppm is the MiE, then 0.7 ppm would be allowed in the diet of man. Therefore, if any experiments are designed using thousands of animals at low levels, the author proposes that properly controlled studies should involve the testing of response at a predicted, biologically insignificant level. Combinations of such levels using different chemicals or different conditions are more appropriate than attempts to find the probably nonexistent shape of the end of the dose-response curve for any 1 chemical or set of experimental conditions. It is difficult to imagine hazard for man from a level 1/5000 of a minimum-effect level resulting from a properly designed and conducted animal experiment.

It is notable that Druckrey (1967), who on the basis of many experiments concluded that clear dose-effect and time relationships exist for carcinogenesis, and who could find no indication of a subthreshold dose for primary effects on the cellular level, still proposed that "1% of the lowest dosage, which given daily over the whole life-span to susceptible experimental animals, producing cancer only at the end of the life-span, can be considered as the maximum tolerable dose for human beings. This, however, only in such cases in which a complete exclusion from human environment is not feasible." The proposed factor of one five-thousandth of a minimum effect level is 50 times more conservative than that suggested by Druckrey.

The criteria for insignificance and the size of the safety factor derived by scientific judgment will, therefore, depend on the biological alteration seen in properly designed animal experimentation. While a safety factor of 100 applied to the no ill-effect level has proved adequate for most food chemicals, a factor of 1 or 10 might be sufficient for some biological effect deemed to be of little or no hazard when applied to the health of man. Another of 1000 or higher can, and should be used for other, perhaps less reversible, injury effects if the chemical in question is essential for human use. Thus complete banning of the use of an essential material is contrary to the application of common-toxicological-sense and is not necessary to ensure safety for man.

Because of the practical certainty of a dose-response relationship between any biological stimulus and its subsequent effect, and because of the demonstrated uncertainty of mathematical extrapolation of data to a low-risk level, it is believed that a negligible hazard level of a material in the environment of man can best be set by the judgment of competent scientists.

The use of a factor of safety based upon informed scientific judgment is the only practical method of determining a safe level of intake for man from the results of tests

upon animals. No other method has been used, nor in fact is any other method feasible. The recommendation of the Mrak Commission and many competent scientists to seek modification of the Delaney clause to permit the Secretary of Health, Education, and Welfare to use his judgment to determine when evidence of carcinogenesis or any other biological effect justifies restrictive action concerning analytically detectable traces of chemicals in food is reasonable.

In summary, for the evaluation of safety for man, it is necessary to: (1) design and conduct appropriate toxicologic tests, (2) statistically compare the data from treated and control animals, (3) delineate the minimum effect and maximum no ill-effect levels (Niel) for these animals, and (4) if the material is to be used, apply an appropriate safety factor, e.g., (a) 1/100 (Niel) or 1/500 (MiE) for some effects or (b) 1/5000 (MiE), if the effect was a significant increase in cancer in an appropriate test.

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