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RISK ASSESSMENT/ SAFETY EVALUATION OF FOOD CHEMICALS

Subcommittee on Food Toxicology
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PREFACE

The Committee on Food Protection has produced a series of documents recommending procedures for the safety evaluation of food chemicals. The concepts, methods, and philosophy embodied therein represented the best judgment of panels of experts as to the state of the art at the time each document was developed. Government, industry, academia, and the public have benefited therefrom. There are still many gaps in knowledge as to the best approaches to the safety evaluation of food chemicals, but significant progress in recent years justifies a revised edition of *Evaluating the Safety Food Chemicals* (FNB, 1970).

Increased public awareness, a concerned food industry, the expanding needs of regulatory agencies, and new and improved methods for safety evaluation have had impact on the guidelines and philosophy outlined in this revised edition. In the rapidly moving field of safety evaluation, guidelines and concepts require continuous updating as newer knowledge becomes available.

For these reasons, and in response to developments cited in the Introduction, the Committee deemed a revision of its earlier publication timely.

INTRODUCTION

Earlier reports from the Committee on Food Protection (FNB, 1954, 1959, 1960, 1965, 1967, 1969, 1970) described procedures that at the time were deemed appropriate to evaluating the safety of food chemicals. The reports included an initial consideration of physical and chemical properties of the food chemicals and some quantification of anticipated levels and patterns of consumption. These considerations were followed by toxicologic evaluations that included acute oral toxicity, subchronic oral toxicity, chronic toxicity, and reproduction studies in rodents and other species. The principal basis for the evaluation of safety was determination of what was then termed the "no-effect level" in a 2-year study with rats and dogs. In general, a no-effect level was regarded as one that produced no disturbance in growth, no observed clinical illness, no change in mortality rate or pattern, no adverse biochemical or physiological effect, no adverse effect on reproduction, and no gross or microscopic evidence of damage to body tissues or organs. An estimate of the "safe" level of intake for man was derived by applying a substantial safety factor to the no-effect level.

When the 1970 edition was written, these concepts of food safety appeared to be valid. Current knowledge, however, suggests that "safety" with regard to food chemicals is an elusive concept and cannot be established with certainty. With currently changing concepts, the acceptability of some degree of risk might be compatible with a general definition of safety.

While methodologies of testing now available seek to evaluate the adverse effects of food chemicals, potential "benefits" of a food chemical have proven more difficult to quantify (Darby and Hambraeus, 1978; Darby, 1980). The

latter may in the future constitute an important element in the regulatory decision-making process, and adequate methods for assessment of benefits are urgently needed. The Food Safety Council (1980) has recently proposed certain guidelines and a framework for the explicit evaluation of benefits of using a food chemical that may be compared with evaluation of risks. However, until such time as adequate assessment of benefits can be implemented, evaluation of risk is likely to remain the most important factor influencing regulatory decisions.

In the 10 years that have elapsed since 1970, significant advances have been made in toxicity testing and in the interpretation of the results of studies to predict hazards for man. New, revised, or proposed guidelines have been promulgated for carcinogenesis testing by the National Cancer Institute (Sontag *et al.*, 1976); for carcinogenesis, mutagenesis, and teratogenesis testing by the Canadian Department of Health and Welfare (Health and Welfare Canada, 1973); for testing the toxicity of household products by the National Research Council's Committee on Toxicology (1977) on behalf of the Consumer Product Safety Commission; for assessing food safety by the Food Safety Council (1978); for assessing the toxicity of pesticides by the Environmental Protection Agency (EPA, 1978); for assessing the safety of chemicals by the Environmental Studies Board and Committee on Toxicology (ESB/COT, 1975); and for evaluating toxic substances in drinking water by the National Research Council's Safe Drinking Water Committee (1977, 1980).

Numerous conferences and workshops have been held to consider conventional toxicity testing protocols (acute, subchronic, chronic, inhalation, dermal, etc.), as well as such newer areas as mutagenesis or genetic toxicology, behavioral or neurotoxicology, immunotoxicology, and various types of target-organ toxicity. The U.S. Senate Committee on Agriculture, Nutrition, and Forestry has examined the topic of food safety (U.S. Congress, 1979). In recent years all levels of government, industry, academia, and society as a whole have become increasingly involved in issues related to the safety of various chemicals, to the Delaney clause, and to food safety policies. There has been extensive debate on many related matters, including the concepts of maximum tolerated dose (MTD),* threshold

*An operational definition to facilitate the chronic testing of chemicals in the National Cancer Institute bioassay program.

dose effect, and the use of safety factors versus risk estimate projections. Related developments during this period include new requirements for good laboratory practices (GLP) (DHEW, 1978); the Occupational Safety and Health Administration guidelines on carcinogenesis (OSHA, 1980); and the passage of toxicology related legislation, e.g., the Federal Environmental Pesticide Control Act, a revision of the 1947 Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (U.S. Congress, 1972); the Toxic Substances Control Act (TOSCA) (EPA, 1979); the Safe Drinking Water Act (U.S. Congress, 1974); and a report on food safety policy (IOM/NRC, 1979).

Because valid risk assessments cannot be made without adequate data, toxicologists have also taken a closer look at various aspects of testing methodologies. Modifications and improvements of existing methodologies are being made. Additions to existing protocols or proposals for new protocols are being developed. Testing methodologies are being examined in order to improve assessment of risk while reducing expenditures of time, money, and scientific resources. If a new protocol does not improve assessments and reduce expenditures, it will not provide any real benefits to the consumer. Rather, it will merely supplement the traditional approach, lengthen the current list of procedures, and ultimately increase costs. It should also be pointed out that authenticated cases of human injury resulting from the approved use of chemicals in the production, processing, packaging, or storage of food are rare. Therefore, established, effective methodologies should not be abandoned in favor of new approaches that may be attractive only because they are new, different, fast, or relatively inexpensive.

These many developments have made the 1970's an exciting, albeit somewhat unsettling, decade in the history of toxicology and have had a significant impact on food safety evaluation.

RISK ASSESSMENT

RECENT DEVELOPMENTS AND CURRENT NEEDS

The 1970 edition of this report took the position that no short-term studies could be relied upon to predict such long-term effects as the induction of tumors. That edition also noted that, although it would be desirable to have short, simple, and safe methods for evaluating the safety of food chemicals directly in the human species, such methods were not then in sight. Despite the considerable progress that has been made in toxicity testing during the last decade, both of the above positions, taken in 1970, still appear to be valid.

The procedures and concepts deemed most applicable to the evaluation of the safety of food chemicals in the past, however, have been found in need of expansion in three major areas: metabolism, interaction, and reproduction. Currently, neither the metabolic pathways followed by a compound nor the physiological response induced by metabolic overloading can be ascertained unless the tests are accompanied by specific collateral studies. No suitable methodologies are currently available for predicting adverse effects of exposures to a mixture of chemicals on the basis of data from tests performed on single chemicals. Detection of any but the most severe damage to reproductive function and embryonic development is not possible with current standard tests. Furthermore, the process of extrapolation from one species to another, the applicability of data derived from rodents to man, and particularly the apparent extreme sensitivity of the mouse to chemicals require serious consideration and study.

Guidelines for estimating toxicologically insignificant levels of chemicals in foods were proposed in an Appendix to the 1970 edition of *Evaluating the Safety of Food*

Chemicals. In an effort to avoid misapplication of experimental effort, the guidelines covered chemicals with a substantial history of commercial production, degradation products of pesticides with established safe levels, and other organic chemicals meeting special structural restrictions. Their criteria involved exclusion of chemicals of known biological activity, consideration of the relationship of the chemical in question to substances of established low toxicity, and assumptions concerning metabolic fate.

These guidelines were an administrative tool by which a judgment of safety could be made for substances at the levels found in foods. They were intended to aid in establishing a reasonable system of priorities for selecting food chemicals for testing as to risk. Although these guidelines have some utility in setting priorities, they are no longer considered adequate for estimating acceptable risk, as that risk is defined in this report. They are being replaced by more sophisticated methods, such as those proposed by the Food Safety Council (1978) and Cramer *et al*. (1978).

Partial solutions to some of the problems of 1970 have been achieved. Studies of the metabolism of food chemicals are required (or at least highly recommended) in several of the current versions of new safety guidelines (EPA, 1978; Food Safety Council, 1978; IOM/NRC, 1979) and are frequently included in industry protocols.

It is likely that metabolic studies at use levels in man and at use levels as well as toxic levels in laboratory species used for toxicity testing will eliminate many of the shortcomings of testing methodology and make possible a more accurate prediction of human response. More accurate predictions depend upon a clearer understanding of the mechanism of action of the test chemical and its interaction with other internal and external factors. Metabolic studies will improve capabilities for overall assessment based on elucidating mechanisms of action.

Little progress has been made in our capacity to predict the effects of exposure to multiple agents on the basis of tests carried out with single chemicals. This difficult and complex problem will require further research, as it is not reasonable to expect each possible interaction to be investigated in the course of a risk assessment. Currently, the only practical approach is to carry out tests with the specific mixtures of compounds for which information is needed.

Additional research is needed on the role of placental

transfer and fetal storage of food chemicals in reproductive and embryonal tissue damage. The current testing protocols for reproductive damage and teratogenic effects provide information about the end-stage effects, but the scientific basis for these effects is not clear. Thus the predictive validity of the tests is limited.

None of the genetic toxicity testing protocols, either singly or in combination, as yet provides a satisfactory basis for eliminating the usual 2-year chronic tests for predicting the carcinogenicity of food chemicals in man. Short-term tests may, however, provide important new tools for investigating the ability of food chemicals to induce genetic effects in some *in vitro* and *in vivo* systems. Moreover, because of the rather good correlation between mutagenicity and carcinogenicity for some classes of compounds, genetic toxicity tests offer promising methods for the preliminary screening of some potential carcinogens.

Chronic exposure tests on selected animals continue to be a mainstay of toxicological test procedures, largely because most of the early predictors of delayed injury have not been adequately validated. It should be pointed out that certain studies on human subjects can be done under controlled conditions and, in those situations where the benefit in terms of knowledge to be gained clearly outweighs the anticipated risk to the subjects, the results of such studies may contribute uniquely to the overall assessment of risk.

INFORMATION RELEVANT TO ASSESSMENT

Before risk of injury from ingestion of added or indigenous chemicals in foods can be assessed, it is essential to have adequate qualitative and quantitative data on the physical and chemical properties, the extent of use, and the biological effects of the chemicals. An adequate data base concerning the nature of biological effects of a chemical and the dosage or dosage rates that can be expected to produce each of the effects is necessary for estimating risk associated with its use. The following section summarizes the information that can be used for risk assessment of a food chemical. It is not proposed, however, that all the information be collected for each assessment.

Information for Complete Assessment of Human Risk

(A) Identification and characterization

1. Chemical name, common name, and synonyms

2. Trade names
3. Source and purity, including identification of impurities
4. Empirical and structural formulas
5. Chemical and physical properties, including storage stability
6. Analytical procedures, including sensitivity, accuracy, and precision
7. Manufacturing processes and techniques
8. Commercial specifications

(B) Manner and extent of use by humans
1. History of use in foods
2. Natural occurrence in foods
3. Quantity produced (manufactured and imported) for use as a food chemical
4. Patterns of use as a food chemical
5. Per capita intake (range and average in terms of milligrams per kilogram of body weight per year)
6. Intake by age and other subgroups in population, if applicable

(C) Animal tests involving pathological examinations (two mammalian species: one rodent, one nonrodent)
1. Acute
2. Genetic toxicology
3. Kinetics (including metabolism)
4. Subchronic (short-term feeding designed to provide comprehensive data on biochemical, functional, and morphological effects)
5. Reproduction and teratology
6. Special studies: target organ toxicity, interaction
7. Chronic (long-term feeding designed to provide comprehensive biochemical, functional, and morphological data on cancer and chronic degenerative changes)
8. Extrapolation of animal data to humans

(D) Monitored effects in man
1. Clinical observations
   -- Kinetics (absorption, distribution, metabolism, and excretion)
   -- Interactions with drugs, nutrients, and other food chemicals
   -- Special studies
2. Epidemiological and other human data
   -- Inadvertent exposures
   -- Clinical reports
   -- Anecdotal reports

Identification and Characterization

The chemical structure and physical properties of a substance determine its intrinsic reactivity with endogenous tissue molecules as well as with food constituents. Acquisition of data on these properties of a food chemical is the first step in its toxicity evaluation. As experience is gained in characterizing the nature and mechanisms of biological effects produced by chemicals, it may become possible to base some assessments of toxicity on structure-activity relationships of analogues. Although toxicity cannot now be adequately predicted solely from data on the chemical structure and physical properties of a substance, these data are important to an overall evaluation of the chemical. The purity of the chemical and the identity and level of impurities are important factors.

There are still many substances (including natural flavoring and coloring agents) commonly added to foods that are not chemically characterized. Some mixtures are identified only on the basis of accepted methods of preparation or extraction. However, variations in a production process or equipment may change the chemical constituents of an incompletely characterized material in a manner that results in unexpected toxicity. Complete chemical characterization is essential if toxicological data are to have predictive value for samples other than the particular sample being tested. The *Food Chemicals Codex* (FNB, 1972a) provides quality and purity specifications for more than 650 food chemicals that are amenable to chemical and physical characterization.

Determining toxicity and predicting rates and pathways of degradation or other chemical changes during food processing, storage, and preparation can become very complex when various physical and chemical forms of the substance in question are present. If indirect additives such as pesticide and fumigant residues, packaging migrants, and processing aids are present, they add to the complexity.

Evaluation of health risk can be more easily accomplished when the food chemicals under consideration are characterized and appropriate standard analytical techniques are available. Degradation or reaction products formed by the reaction of additives with food components should be identified and suitable assay systems provided. Data to be used in evaluations of health risk should be obtained under exaggerated as well as traditional conditions of processing, storage, and preparation.

Extent of Use

Determination of the risk from a food chemical requires knowledge of its toxicity and a reasonable estimate of the frequency and level of human exposure (FNB, 1979). An assessment of varying amounts of the food chemical likely to be consumed by segments of the population is desirable. However, improved methodology and more extensive and complete quantitative food consumption data are needed before reasonably accurate assessments can be made for intakes of many of the food chemicals. Therefore, for the time being, the toxicity of the proposed food chemical must serve as the primary data base for subsequent scientific or judgmental decisions concerning assessment of acceptable risk, with estimates of consumption serving as supplementary data.

Biological Effects

Well-designed clinical and epidemiological studies in humans utilizing controlled and quantified exposure dosages should, presumably, provide the most meaningful data base for the estimation of risk. Unfortunately, there are numerous difficulties. First, medical ethics and legal considerations may preclude controlled clinical studies of a chemical that has no (or only indirect) beneficial health effects. Such studies would be permitted and acceptable only if human exposures were already known to occur, or, in the case of a new substance, if fairly extensive data on biological effects in test animals were available in advance and did not suggest irreversible toxic effects. Second, according to current FDA regulations for testing new drugs and cosmetics, the selection of experimental subjects for a preliminary clinical study must be restricted to a limited number of healthy individuals. This restriction may lead to a failure to detect an effect that occurs at low frequency (i.e., only in the most susceptible individuals) in a large population sample. Third, although well-controlled human studies might make it possible to test the full range of expected exposure levels, it would be impractical to conduct such studies for a sufficient duration to approximate the expected human exposure period. Thus, while effects from acute exposure might be assessed by direct clinical investigation, effects unique to chronic exposures are usually determined only through rather imprecise epidemiological studies. Such studies are more precise in those situations where specific toxic effects are strongly indicated in prior tests on appropriate animal species.

Acquisition of adequate toxicological data for estimating risk associated with food chemicals, therefore, necessarily requires controlled experimental studies in animals, supplemented whenever possible by data from humans. Estimates of risk can be made by quantitative consideration of toxicity data in relation to patterns of food chemical use, physical-chemical characteristics, and distribution.

Toxicological Studies

A number of national and international agencies have developed guidelines for obtaining data essential to determination of the toxicological characteristics of food chemicals. The increased sophistication of the science of toxicology, as well as increased concern about food safety, is reflected in the complexity of current protocols compared with requirements accepted a decade or more ago.

Nevertheless, the basic principles underlying toxicity testing for evaluating health risks of chemicals remain essentially unchanged. The principle that toxic effects in animals are applicable to man underlies toxicological testing just as it underlies the knowledge base of experimental biology and comparative medicine. Appropriate extrapolation of data from animal tests to man requires an understanding of inherent species differences with regard to metabolism and disposition, target tissue sensitivity, and rates of injury and repair. The principle that the intensity, time of onset, and duration of biological effects of chemicals are functions of dose remains central to toxicological assessments. Thus, the likelihood of detecting an injurious effect in test animals is enhanced by increasing the dose.

Three new issues are surfacing in relation to chronic toxicity testing. The first is the concern that the use of high doses may introduce adverse effects that will be observed only at high doses. These adverse effects may be due to physiological disturbances related to metabolic overload, rather than to toxicity directly attributable to the chemical. When the capacity of normal primary pathways for detoxification and excretion of a chemical is exceeded and secondary pathways come into play, the quality and quantity of metabolites may differ from those produced through the primary pathway. This may result in very different responses by the test animal, which may not represent those characteristic of lower dose exposures that are more typical of human use levels. Thus, test results at extreme levels may well be misleading. The recognition of this situation is one concern associated with use of

the maximum tolerated dose (MTD) in carcinogenesis bioassays. Refinements of this approach, to include studies of the metabolism of the food chemical over a range of dosages from use levels to toxic, are particularly important.

A second issue has to do with increased recognition that additional knowledge of embryonal damage from chemicals and more frequent inclusion of *in utero* exposure are needed in chronic toxicity protocols. Although the appropriateness of using rodents for predicting *in utero* effects in man is controversial, the need for doing so is likely to assume greater importance. As noted earlier, additional research is needed concerning the role of such factors as placental transfer and fetal accumulation of chemicals in producing embryonal tissue damage. Current protocols for evaluating teratogenesis and reproductive damage provide information only on end-stage effects.

The third new issue likely to assume increasing importance arises from attention to the contribution of nutrition to the results of chronic toxicity tests. Deficiencies or excesses of nutrients, or varying ratios of macronutrients (fat, carbohydrates, and protein) and/or micronutrients (vitamins and minerals), may either enhance or inhibit development of a particular toxic effect of a chemical in the diet. Whereas many interactions between dietary constituents and nonnutritive chemicals are now well known, the complexity of the issue requires thoughtful planning and increased appreciation of the significant issues.

Recommendations for animal tests (see page 7) include two relatively recent additions to toxicology testing: genetic toxicology and toxicokinetics. Information obtained from tests in these areas can provide guidance for the conduct and interpretation of subsequent prolonged exposure tests; such tests may substantially improve the predictive value of the animal test results. Positive findings in short-term tests (mutagenesis or cell transformation assays) provide a clear indication of the need for extended testing in animals; demonstration that metabolism of a food chemical in the target species (usually man) is similar to that in the test species supports the predictive value of the test species results.

The Committee believes, however, that neither genetic toxicology, toxicokinetics, nor any single test should be used as a primary basis for regulatory action. This caveat applies to other tests that have not been adequately validated and that have not been shown to have high predictive value in establishing human health risk from food chemicals. This includes most currently available short-term

mutagenesis and carcinogenesis tests, most of the proposed behavioral tests in animals, and many other short-term tests based on pharmacological procedures. Those tests are more properly designated research procedures, not required protocols, unless or until their predictive value has been validated. The distinction between information that may be useful in the evaluation of a specific agent, as opposed to information that would be required for all agents, should be clearly identified in the formulation of new animal testing protocols and in the modification of current test guidelines.

One method for providing judgment or decision points within a risk assessment protocol is to use a "decision tree" or tier approach to evaluate the results of toxicity studies in animals. The recent "Proposed System for Food Safety Assessment" developed by the Scientific Committee of the Food Safety Council (1978) uses such an approach to provide decision criteria for decision points in the proposed scheme. The Food Safety Council's proposal of a priority strategy in selecting compounds to be placed on test and in planning and conducting such studies is likely to be especially useful to toxicologists in the food industry and to the agencies responsible for the regulation of food chemicals. Another approach has been developed by Cramer *et al.* (1978) for use in correlating chemical structure with toxic hazard of food chemicals. It appears that this system, as well as the one discussed above, will be a valuable tool for assessing food chemicals.

Flexibility is a key element in the ultimate usefulness of any series of toxicity tests in animals. Both the regulator and the investigator must be encouraged to use judgment in selecting those tests most likely to characterize fully the toxicity of a specific agent and in modifying the individual tests in a manner that will improve their predictive value. As has been noted previously (ESB/COT, 1975), "There is no substitute for the vigilance of an inquiring and skeptical mind which has assumed the full responsibility for planning, conducting, and evaluating the results of toxicity tests in making safety assessments." If that responsibility is lessened by exclusive dependence on a "check list" approach, the major assurance has been lost that a responsible, perceptive, and efficient investigation will be conducted.

PREDICTION OF RISK

SELECTION OF TEST PROCEDURES

Historically, the toxicity tests discussed earlier sought to provide information on the risk to humans of exposure to various substances. It is often not possible to make quantitative evaluations of this risk on the basis of animal data. The converse, however, is recognized--chemicals known to be toxic to humans generally produce comparable effects in many animal species. The ability to extrapolate scientific results from animals to man is a fundamental principle without which prediction of risk would have to be derived from data obtained on man alone.

Considerable information can be obtained from the animal testing protocols described in the previous section. Four general categories of tests are proposed: acute, subchronic, chronic, and special. The value of data in predicting risk differs with the type of test. Acute toxicity tests are useful primarily in guiding the selection of test methods and dose levels for subsequent studies. Subchronic tests, on the other hand, are of great value in predicting toxicity and risk for man. Aside from neoplasia, most responses associated with repeated administration of chemicals will develop within the 3-month span proposed for subchronic tests, particularly if studies of reproduction and teratogenesis are carried out in conjunction with the 3-month exposure. Chronic tests that extend over most of an animal's life span are also necessary because prolonged, low-level exposure may be required to detect certain chronic effects, notably carcinogenesis. The fourth category comprises tests designed to obtain information on some special facet of the anticipated action of the chemical.

Any test used to provide data for estimating risk to humans should be carried out in ways that generate valid

data relating dose and response. Test animals must be healthy and of known origin and breeding stock. They must be adequately housed, nourished, and cared for prior to and during the experiment. The number and range of doses, the vehicle, route of administration, and number of test and control animals must be appropriate.

New testing procedures for genetic toxicology, teratology, immunotoxicology, and neurotoxicology are being developed. The potential value of *in vitro* systems is great, provided rapid, reproducible tests with a known predictive validity can be developed. There is great need for developing these new tests in a framework specifically related to the more conventional methodology.

SELECTION OF SPECIES

Selection of the animal species on which the tests are to be performed is fundamental to success. For classical acute toxicity tests, line-bred strains of rodents are customarily used and testing is generally restricted to young adult males. Short-term subchronic toxicity tests generally use young adult male rats of line-bred albino strains. Chronic toxicity tests classically utilize rats, both male and female, and a second species, often line-bred beagle dogs. The species used clearly affects the test results. Currently, selection of species is based on four considerations: the most sensitive species, the species that most resembles man metabolically, the species that most resembles man in function, and the best-characterized species. Each attribute is further discussed below.

Sensitivity to Toxic Effects

Often it is not known which species is most sensitive to a particular toxic effect, but information on analogous compounds may be helpful in making the selection. Moreover, tests in the most sensitive species are no more valuable in predicting effects in man than are tests in other species that respond in a similar manner, but require larger doses. The relative sensitivity of man cannot be predicted without information such as comparative data on metabolism. In the absence of such information for setting tolerances, the no-observed-adverse-effect level (NOAEL) in the most sensitive species provides a conservative tolerance level, because it is based on the assumption that man may be at least as sensitive as the most sensitive experimental animal.

Metabolism

The choice of an animal species that metabolizes the test chemical in a pattern similar to man has substantial scientific merit. This choice presupposes sufficient available information about the metabolism of the chemical (or similar chemicals) in man and other animals. If the species thus identified is an infrequently used animal, particularly a large animal, problems of procurement and housing may outweigh the scientific advantages. The advantage of selecting an animal species on the basis of metabolic similarities with man must be tempered by the realization that either may exhibit marked variability in the metabolism of chemicals, depending on age, sex, genetic factors, and physiologic state.

Function

Even when the metabolism of a compound in an animal resembles that of man, the effects of the active compound or metabolite in that species may not resemble the effects in humans. System and organ functions are necessarily monitored by tests designed to accommodate the species under study as well as to provide relevant information for man. The choice of mammalian species for comparison with man is usually based on similarities of structure and function of such organs as the endocrine glands, blood-forming organs, liver, kidney, brain, and placenta. Non-mammalian species, particularly invertebrates, are divergent in many of these aspects. However, the common view that higher primates or even monkeys are more like man in function and metabolism than are other species is not uniformly true, in spite of the many similarities that may exist.

Degree of Characterization

Selection of species on the basis of characterization, i.e., availability, uniformity of strain, length of life span, background information, size, cost, and so forth, is undeniably important. As a practical matter, therefore, only a comparatively small number of species are used. The type of test and the selection of species are based on conventional protocols that have demonstrated predictive value in the past. Although the development of new tests, incorporating new species of animals, is to be encouraged, it should be recognized that the use of conventional protocols is often to be preferred because of the large data base that already exists.

METHODS OF PREDICTING RISK

The ideal test for predicting risk would be carried out in a species in which physiological or biochemical functions and metabolism of the compound closely resemble those in man. A complete range of doses and times of exposure would then be evaluated, such that acceptable doses for man could be directly estimated from the data. However, in practice, adequate information for direct extrapolation is seldom attained, because it would necessitate very large numbers of animals and prolonged periods of time.

In the absence of the ideal test, and in an effort to achieve reliability in estimating risk, the following principles are used in selecting protocols: (1) A range of doses is chosen that starts below the dose at which effects are observed in animals and extends to the level at which fatalities occur. (2) Both acute and chronic exposure are studied, thereby adding duration of exposure to the range of doses. In chronic studies, tests of organ function and morphological examination for structural alterations are performed in various organs. (3) More than one species of animal is used in critical tests because the predictability of data from animals to man is thus greatly increased. If one species exhibits a toxic response to a substance, there is a potential risk to man; if the same risk is demonstrated in a second species, the probability that the toxic response will occur in man is increased. (4) Data relating dose and response are used to estimate risk. The toxicity of noncarcinogenic chemicals is perceived to be dose related. That is, occurrence of a toxic phenomenon is predicted to be more likely at a higher dose and less likely at a lower dose.

As a generalization, the dose-response principle is incontrovertible; however, under certain limited circumstances, such as carcinogenesis, it may not hold. The concept of a "threshold dose," below which a toxic effect does not occur, is an exception to the principle because it projects no dose-response relationship in the range of doses from zero to threshold. This concept embodies the principle that below the threshold dose the rate of metabolism and excretion of the compound exceeds the rate of development of the toxic phenomenon. In the case of carcinogens, there is disagreement as to whether there is a threshold dose for the production of cancer by certain chemicals. Whereas consideration of the presence or absence of threshold is of theoretical interest, there is currently no clear means of predicting risk on the

basis of the true shape of the dose-response curve in the range between the lowest observed effect and zero dose.

Two methods are of importance in analysis of the problems of predicting incidence and severity and of extrapolating data both from high dose to low dose and from the test species to humans. One method is based on establishing a safety or uncertainty factor from data obtained in the tests; the other is based on extrapolation below the no-observed-adverse-effect level, using a defined line or point. These methods are discussed below.

Uncertainty Factor

This factor (Safe Drinking Water Committee, 1977), previously termed a safety factor, is used to set the accepted maximum dietary level of a food chemical. This level may vary from 1/10 to 1/1,000 of the highest level at which no effect in animals was observed. The purpose of use of the factor is to identify a level of intake that is below that which would produce an adverse health effect in man. The uncertainty factor varies with the kind and amount of data available. One component depends on the animal species in which the toxicity data were obtained. For many purposes, an arbitrary factor of 100 below the no-observed-adverse-effect level has been used (WHO, 1967). One factor of 10 allows for differences within species; the second factor of 10 accounts for between species differences (e.g., from rat to man). Clearly, the type of effect appearing in animal tests also influences the selection of the uncertainty factor. The nature of the deleterious effect in a subchronic or chronic toxicity test influences the weight ascribed to the toxic effect, and the uncertainty factor is adjusted in accordance with a judgment as to the seriousness of the effect. Whenever there is some question as to the importance of a toxic finding, a larger uncertainty factor might be used initially and altered as more data become available. As uncertainty factors are influenced by the quality and quantity of the available data, it is inappropriate to propose applying a rigid factor.

Extrapolation from Dose-Response Data

Mantel and Bryan (1961) proposed that extrapolation from an existing dose-response curve for carcinogenesis could be used to establish "virtual safety." They further suggested that their proposed procedure was not limited to carcinogens and could be used for other compounds. The Mantel-Bryan procedure starts with establishing acceptable

risk. In the case of carcinogenesis, "virtual safety" has been defined as occurrence of the toxic effect in 1:100,000,000, with a statistical confidence limit of 99 percent for a specified dose.

The slope of the dose-response curve below the lowest observed effect, or some other selected data point, must be estimated. Although direct extrapolation from existing dose-response curves could be used instead of a selected slope, it should be recognized that different results will be obtained by direct extrapolation when different methods of curve fitting are employed (ESB/COT, 1975). A conservative slope (such as a slope of 1) is often selected. This selection is practical because there is no assurance that direct extrapolation of dose-response curves below the level of response will result in more accurate prediction than will use of an arbitrary slope. Mantel and Bryan suggested that a slope of 1 probit for each 10-fold change in dose be used as a conservative slope. The more gradual the slope, the more conservative will be the estimate of "virtual safety." Modification of the dose-response extrapolation methods to include an evaluation of the significance of the age at which a toxic effect might occur has been proposed. Friedman (1974) has noted the need for more data to improve the accuracy of the arbitrary choices in the Mantel-Bryan approach: namely the probability estimate (how valuable is a 1:100,000,000 risk estimate?), the statistical confidence interval (is 99 percent too high?), and the slope (would a steeper slope give a closer fit to the acceptable present day utilization of known food additives?).

In addition to the Mantel-Bryan procedure, other statistical models have been proposed for extrapolating from data in estimating risk. Several reports expand on the probit model suggested by Mantel and Bryan or discuss newer models including one-hit, multi-hit, and multi-stage models (Hoel *et al.*, 1975; Mantel *et al.*, 1975; Cornfield, 1977; Van Ryzin and Rai, 1980). Some examples of these models can be found in the reports of the Safe Drinking Water Committee (1977, 1980) and the Scientific Committee of the Food Safety Council (1978).

Selection of Method

Both the uncertainty factor and the risk estimation methods just described have been used for predicting risk, and both require experienced judgment in selecting appropriate parameters. As Mantel and Bryan (1961) have pointed out, uncertainty factors for estimating acceptable dose levels of

drugs have been used routinely in extrapolating pharmacologic data from animals to man. In cases where the toxic or carcinogenic effect appears only after repeated administration of the chemical, risk estimation of the Mantel-Bryan type may be appropriate, although in this case a factor for species differences is not included. In cases where the variability of the toxic response between individual animals is known, the uncertainty factor may be appropriate. At present, a combination of methods offers maximum information for the final judgmental process used in assessing the data and deriving conclusions from mathematical procedures.

It is not possible to identify precisely those tests that will be most appropriate for estimating toxic effects in each individual case. On the other hand, to establish batteries of tests that must be routinely applied to all substances will result in unnecessary expenditures of time and effort. In addition, there is cause for concern that use of animal tests that have not been validated as to applicability to man will unduly emphasize toxic effects, despite the lack of validation. Tests that have not been validated as applicable to man and that generate weak evidence of toxicity are of little help in making risk estimations or regulatory decisions.

In the tests used to assess effects of food chemicals, data on various physical and biochemical parameters will be obtained in the subchronic and chronic studies. From these observations it will be necessary to decide which effects are deleterious and to decide how the various dose-response curves might be used to evaluate risk. Generally, irreversible or life-threatening effects will be assigned greatest importance. The lowest dose at which any deleterious effect is found is also particularly significant. However, it should be recognized that the lowest effective dose is related directly to the sensitivity of the test employed, and the proper objective is not to identify the most sensitive test, but to identify the one that most accurately predicts effects in man.

Observations on Animals

Because of physiological, biochemical, morphological, and functional similarities among mammals, the results of many indices of chemical effects measured in laboratory species are assumed to be predictively transposable to man. In animal studies, both the dose and the duration of exposure can be exaggerated in relation to the exposure of man, and in many, perhaps all, situations the probability of

detecting a toxic effect is increased with increasing dose and duration.

Despite interspecies similarities, however, there are many metabolic, functional, and other differences that inevitably result in uncertainty in species-to-species extrapolations. Although the uncertainty inherent in such extrapolation tends to diminish as the number of species showing similar responses increases, it never vanishes completely. There always remains uncertainty in extrapolating from one species to another, because no two species are identical.

Knowledge of toxic effects in laboratory animals cannot ensure absolute protection against injury for every individual in the population, because the population includes persons of different ages and physiologic and disease states as well as hypersensitive or allergic individuals and persons with extreme food habits. Because most risk studies are conducted on healthy adult animals receiving standardized laboratory diets, the results would be expected to be more applicable to healthy adult humans on good diets than to those on nutritionally inadequate or imbalanced diets. There is much need for research to extend the validity of conclusions to the more extreme or abnormal human situations.

The aim of the series of tests is to determine the maximum dose level that is without discernible adverse effect. Clearly, duration of experimental exposure as well as dosage must be finite, and scientific judgment must be applied in the selection of test dosages and duration of exposure. The maximum dose level that has no observed adverse effect is of decisive importance in risk evaluations. It is this dose that is translated, after applying a suitable uncertainty factor or dose-response extrapolation, into a maximum acceptable level of intake for man. The value of arriving at an estimated no-observed-adverse-effect dose for humans is to provide a basis for achieving minimum risk, though that risk can never be established as zero. The reasons for the residual uncertainty are numerous and include the following:

- The size of the test groups is small relative to the population to which the data are to be applied, so that there is only a probability estimate of the variation to be expected in a large population.
- No-observed-adverse-effect dose levels vary with the species, strain, sex, age, physiological state and other factors in the test animals. Hence, the failure to

observe an adverse effect under one set of defined experimental conditions does not preclude the possibility of an effect under other conditions. No matter how many types of response may be observed in toxicological studies, it is conceivable that one or more tests not employed in a study might be more appropriate for man and reveal an effect not previously observed.

- Although it is desirable to identify the dose at which no adverse effects are observed, it is sometimes difficult to determine if a deviation from a "normal" or control response is indeed an adverse effect. For example, reversible changes in the level of a normal constituent of blood or tissue, in organ weight, in morphology, or in function may be indicative of a compensatory state that may or may not be indicative of injury.

Observations on Man

The ideal species in which to study risk of food chemicals for man is man himself. The nature, usefulness, and limitations of this approach have been succinctly discussed (WHO, 1961, 1967, 1970).

The ultimate assessment of the risk from ingesting a food chemical derives from years of widespread consumption by man under varying conditions of use. Even here, the absence of recognized adverse effects does not, of itself, constitute adequate assurance of absence of risk. The possibility always exists that adverse effects, because of their subtle, rare, or slow development, may not be recognized as due to the chemical in question.

Epidemiological studies in which the subjects consume food and food chemicals under normal conditions of use are difficult to carry out. Even with the most careful attention to control, the data reflect a complex interplay of such factors as intake, age, sex, race, dietary habits, and variable physiological, social, and environmental circumstances. To conduct a meaningful survey requires rigid control, careful selection of the sample population, and large numbers of subjects and measurements.

Controlled experimental studies in man, though desirable for prediction of effects of general distribution of a chemical in food, have limited predictive value for a number of reasons:

- The number of subjects is usually not adequate to disclose reactions that occur infrequently.
- The feasible duration of an experiment is but a relatively short segment of man's total life span. Thus,

observations relating to low-level, long-term effects, such as carcinogenesis, are impossible with present knowledge and methodology.

- Tests are usually carried out on a restricted category of subjects, such as healthy adult males, and do not yield information on variation in susceptibility due to sex, pregnancy (including susceptibility of the embryo), age, race, and environmental factors.
- The variety of experimental tests, histological observations, and range of doses used legitimately in man is severely limited in comparison to those employed in experimental animals. In extended studies, the low levels of consumption of a food chemical that are dictated by ethical considerations do not provide adequate data from which risk can be computed. No degree of risk can be measured if no toxicity develops. Neither the toxicity nor the risk associated with food chemicals can be measured quantitatively in man in a controlled experiment that meets accepted ethical standards.
- In order to control the intake of a chemical accurately, it is frequently given by capsule or in some other manner not precisely that characteristic of use in foods.
- Because the subjects must by law be informed of the experiment and of any potential risk, psychological reactions may distort the results unless the experiment is very carefully designed.
- Lapses of cooperation by the subjects reduce the dependability of the data.

It is clear that there is no substitute for appropriate and well-executed studies with animals. Such studies are more likely to provide a substantial background of biological data from which to judge risk than are studies in man alone. The greatest advantage of animal experiments is that the dose can be raised, even to excessive levels, above expected intakes. It is a principle in toxicology that the toxic effect of a chemical is related to both dose level and duration of exposure. Novel toxic effects on an organ may arise as the dosage increases. However, many toxic effects can be seen after a short period of exposure to high dosage of the substance of interest, whereas, the same effects may occur only after prolonged exposure to lower dosages. Thus, well-designed lifetime toxicity tests in animal species with relatively short life spans can provide results that have greater value for predicting safety in man than can the results of tests of similar time duration in man.

Optimum Conditions

The ideal situation for estimating risk would be obtained by a combination of the following: (1) extensive toxicologic tests in appropriately selected animal species, with emphasis on effects on target organs or tissues, mechanisms of action, and metabolic rate; (2) suitably controlled tests in man; and (3) epidemiological studies in man. Even this conscientious program of study cannot completely dispel uncertainty regarding those harmful effects that are difficult to attribute to specific causes because they develop slowly, are of subtle nature, or occur infrequently. In practice, however, due to ethical standards and considerations of time, effort, and cost, estimations of risk must usually be made on the basis of a less than ideal spectrum of data.

FEDERAL REGULATIONS

Federal statutory authority to regulate the safety of the U.S. food supply was first enacted as part of the Federal Food and Drugs Act of 1906 (U.S. Congress, 1906). Food was declared to be adulterated, and thus illegal, if it contained any added poisonous or other added deleterious ingredient which may render such article injurious to health. Two early Supreme Court cases broadly interpreted the word "added" to exclude only food constituents placed in the food by nature itself, and construed the provision to prohibit only those added ingredients that might be injurious under their actual conditions of use (U.S. Code, 1914, 1916).

When the 1906 Act was replaced by the Federal Food, Drug, and Cosmetic Act in 1938 (U.S. Congress, 1938), a distinction was made between natural and added food constituents. The general food safety provisions in Section 402(a) of the 1938 Act declare an "added" food constituent to be adulterated if it may render the food injurious to health, and a natural food constituent to be adulterated if the quantity of the substance in the food "ordinarily render[s] it injurious to health."

The Food Additives Amendment of 1958 (U.S. Congress, 1958) was passed by Congress to require premarketing approval of some, but not all, food ingredients. The 1958 Amendment defines the term "food additive" to include those food ingredients (whether of natural or synthetic origin) that are not (1) generally recognized as safe (GRAS) by qualified experts, (2) approved by FDA or USDA for use in food prior to 1958 (a "prior sanction"), or (3) within other specified exemptions. Only "food additives" as so defined are required to be approved for safety by FDA prior to marketing. All other food ingredients may be used without such premarketing approval. On the other

hand, FDA retains regulatory authority over GRAS and prior-sanctioned food ingredients under the general safety provisions in Section 402(a) of the 1938 Act.

Shortly after enactment of the 1958 Amendment, FDA issued a list of food ingredients it regarded as GRAS, and thus not food additives. As a result of the cyclamate ban in 1969, FDA began a reevaluation of this so-called GRAS list. Committees of the Food and Nutrition Board have undertaken surveys of the manner and level of use of GRAS food ingredients (FNB, 1972b, 1979). The Select Committee on GRAS Substances (SCOGS) of the Federation of American Societies for Experimental Biology (FASEB), under contract with FDA, agreed to evaluate the risk involved in use of these GRAS substances. SCOGS submits a report to FDA on each substance reviewed, expressing the judgments of the experts who serve on SCOGS on the degree of risk presented by the substance, stated in the form of one of the following four conclusions:

- There is no evidence in the available information on _____ that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future.
- There is no evidence in the available information on _____ that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.
- While no evidence in the available information on _____ demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.
- The evidence on _____ is insufficient to determine that the adverse effects reported are not deleterious to the public health when it is used at levels that are now current and in the manner now practiced.

For those few substances for which a scientific evaluation of potential hazard cannot be made because of a serious deficiency in relevant data, a fifth category has been established:

- In view of the almost complete lack of biological

studies, the Select Committee has insufficient data upon which to evaluate the safety of _____ as a food ingredient.

The work of SCOGS is still in progress. By early 1980, approximately 400 substances, involving approximately 120 chemicals and their analogues, had been reviewed by SCOGS and reports submitted to FDA. FDA publishes these reports in the *Federal Register* with proposed regulations determining whether the substance should retain its GRAS status, be regulated as a food additive, be subject to appropriate restrictions, or be banned entirely from use.

In addition to the GRAS review, FDA regulates food additives (i.e., those food ingredients that are not GRAS or prior sanctioned) by evaluating their safety prior to actual use in food. The manufacturer of a new food additive is required to submit to FDA a food additive petition containing all relevant safety data. FDA may then approve its use without restriction, approve it with restriction, or disapprove it. A large number of food additives have been approved by FDA for both direct and indirect uses since the enactment of the 1958 Amendment. FDA has considered, but not yet undertaken, a program for a cyclic review of all food additive regulations to make certain that the status of previously approved food additives remains justified.

Because the validity of the scientific testing conducted on GRAS ingredients and food additives is critical to any scientific review of these substances, FDA has issued standards governing the practices under which laboratories conduct such tests. FDA's good laboratory practices (GLP) regulations were published in final form in December 1978 and became effective in June 1979 (DHEW, 1978). The stated objective of these regulations is to assure "the high quality of non-clinical laboratory testing required to evaluate the safety of regulated products."

SUMMARY

Experimental studies in animals, supplemented when possible by data from humans, are used to predict the risk to man resulting from the use of food chemicals. The use of animals in toxicity testing is predicated on the principles that effects in animals are applicable to man and that the likelihood of detecting an injurious effect in animals is enhanced by increasing the dose. The 2-year chronic test remains the most reliable predictor of potential carcinogenicity of food chemicals in man. Ideally, tests in animal species are combined with clinical tests and epidemiological studies in man.

A number of new procedures in chronic toxicity testing are being developed. Studies of the metabolism of food chemicals are increasingly required or recommended as part of safety guidelines or industry protocols. They should provide data for more reliable predictions of human response to food chemicals. Genetic toxicology and toxicological pharmacokinetics are recent additions to the battery of toxicology tests. These and other short-term tests are important tools for predicting risk in man and are often used effectively in conjunction with the chronic 2-year tests. Most of the *in vitro* short-term tests for carcinogenesis should not be used as the primary basis for establishing or quantifying risk, as none have been shown to have high predictive value in establishing human health risk. Furthermore, to establish fixed lists of tests and apply them routinely in risk assessment is unjustified--this approach can lead to unnecessary expenditures of time and effort, to failure to detect certain toxic effects, and to unwarranted emphasis on the results even when their applicability to man is unsubstantiated. Toxicity testing sequences must remain flexible and investigators and

regulators must be permitted to exercise scientific judgment in applying them.

Numerous difficulties remain. It is not yet possible to predict effects of multiple chemical agents; tests must still be done with the chemical mixture in question. The scientific basis for predicting validity of testing protocols designed to detect damage to reproductive systems and induction of teratogenic effects requires additional research. These evaluations should be expanded beyond current protocols directed at effects visible at or after birth to include *in utero* studies. Interactions of macro- and micronutrients with nonnutritive constituents in the diet often are not considered in toxicity tests. The use of a high dose (such as a maximum tolerated dose) to shorten response time is now being questioned, because it may introduce effects peculiar to the high dose itself as a consequence of overloading normal metabolic pathways with additional and different sets of metabolites.

A number of principles are followed in the selection of protocols for animal studies used for estimating risk to man. The range of doses selected extends from below the level at which no adverse effects are observed to the level at which fatalities occur. Both acute or single doses and chronic exposure are studied. More than one animal species is used. Dose-response data are used to predict risk, and the intensity of response is assumed to correlate with dose rate. The question of whether or not there is a "threshold dose," below which no response in the development of cancer occurs, remains unresolved.

Conventional protocols have been developed only on a limited number of species with a demonstrated sensitivity, metabolism, and function similar to those in man. Availability, uniformity of strain, relatively short life span, and appropriate size and cost make certain of these species especially suitable. Development of tests incorporating new species is encouraged. However, conventional protocols are often preferred for evaluating risk because a large data base already exists. As improved methods in genetic toxicology, teratology, neurotoxicology, and *in vitro* systems are validated, they should be incorporated into or replace current conventional methodologies.

In the event an effect potentially deleterious to man is observed in animals, a maximum acceptable level of intake for man may be estimated. This level is based on the maximum dose for which no adverse effects were observed in animals, to which an uncertainty factor or risk extrapolation method is then applied. The acceptable level for

man ranges from 1/10 to 1/1,000 of the no-effect level observed in animals; it varies with the kind and amount of data available on animals and human exposure to similar or identical materials naturally occurring in foods. There is always some uncertainty in extrapolating data from one species to another. Risk evaluations are usually performed on healthy adult animals on nutritionally adequate diets. Research is needed to extend the data from such protocols to more aberrant situations.

Adverse effects may escape detection when studies or observations are done on man, if these effects are subtle, rare, or slow in developing. Studies on man are limited by ethical considerations and hampered by the fact that the studies last less than life span, are performed on a limited number of subjects and subject categories, and are limited as to number of tests and doses. Therefore, well-designed life-span toxicity tests on relatively short-lived animals can give more information for prediction of risk in man than can tests of similar duration in man. This does not imply, however, that tests in man should not be done whenever feasible.

In conclusion, each protocol or guideline has its own unique set of assets and problems. Tests used in the past have largely been validated, but there are limitations to each. New tests should be introduced into the existing system with care and only after they are validated. Reliable tests must not be replaced by less reliable ones. Value judgment remains of great importance.

Complete absence of risk (absolute safety) for man from use of any chemical substance cannot be assured. What must be accepted is a balance between a low level of risk and the beneficial effects of a food chemical. In assessing risk there must also be a balance between the effort expended in evaluating risk and the consequences of failure to detect an effect.

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APPENDIX: DEFINITION OF TERMS

The following terms are often encountered in discussions and in the literature relative to the evaluation of food chemicals. These terms as defined by the Committee are:

Food Chemical: A substance or a mixture of substances, other than a basic foodstuff, that is intentionally added to a food as a result of any aspect of production, processing, storage, or packaging. The definition excludes chance contaminants.

Toxicity: The capacity to produce adverse effects in a biological system when a substance is injected, inhaled, absorbed, ingested, or produced within the body. Toxicity is quantifiable in terms of the dosage or dosage rates that produce adverse effects in a sample population of test organisms.

LD_{50} (Lethal Dose 50): The dosage calculated from a dose-response curve to kill 50 percent of the exposed population

No-Observed-Adverse-Effect Level (NOAEL): The dose level at which no adverse effects in structure, function, or behavior are observed

Mutagen: An agent which produces expressable and heritable changes in genetic material

Teratogen: An agent that initiates *in utero* a deviation of form, chemical content, or function resulting in abnormality in the fetus or newborn

Carcinogen: An agent that significantly increases the incidence of malignant neoplasms in treated animals, compared with untreated controls

Tumor or Neoplasm: A new growth of cells that may be benign or malignant when evaluated by conventional histological criteria and biological behavior. A tumorigen is a chemical which produces a tumor.

Benign Neoplasm: A population of cells that exhibits a

degree of autonomy, little or no cellular atypism, and does not metastasize or invade normal tissues

Malignant Neoplasm: A population of cells that exhibits variable degrees of cellular atypia and autonomy and that metastasizes or invades and destroys normal tissues

Probability: An expression of the occurrence of an event based on a defined distribution

Significance: The probability of the occurrence of an event that is sufficiently above random to merit consideration

Risk: The probability that an adverse effect will occur under specified conditions

Acceptable Risk: A risk that is judged by society to be outweighed by corresponding benefits or one that is of such degree that it is considered to pose no significant potential for adverse effects

Hazard: The probability that an adverse effect will result from use of a substance in the quantity and manner proposed for its use

Safety: Previously defined as "the practical certainty that injury will not result from the substance when used in the quantity and in the manner proposed for its use" (FNB, 1970). Since it is impossible to prove a negative, safety is an absolute which cannot be demonstrated.

Metabolic Overloading: A dosing schedule that exceeds the capacity of the normal metabolic pathways for a food chemical

Tolerances: Acceptable limits of variability. The limits are arbitrary decisions based on available evidence.

Uncertainty Factor: A term recommended to replace safety factor: A number that reflects the degree or amount of uncertainty that must be considered when experimental data in animals are extrapolated to man (Safe Drinking Water Committee, 1977)