Chapter V

Methods for Assessing Health Risks

Methods for Assessing Health Risks

The availability and quality of data on the toxicity of contaminants in large part determine the Food and Drug Administration's (FDA) ability to protect public health. Chapter III reviewed the strengths and weaknesses of FDA's procedures for setting action levels and tolerances. This chapter addresses four issues related to the methods used for assessing health risks:

- What is the role of human epidemiology in the setting of action levels and tolerances?
- What are the roles of animal and other toxicological tests?
- Are current testing procedures adequate for the assessment of interactions of toxic substances?
- Are methods for quantitative risk assessment sufficiently developed for application to environmental contaminants in food?

POSSIBLE TOXIC EFFECTS

The possible toxic effects of an environmental contaminant depend on its chemical nature, its concentration in food, and the type of toxic action involved. If the substance is highly toxic and/or is consumed in large quantity, acute toxic effects may occur and the onset of the symptoms would be rapid and severe. If a small amount of a highly toxic substance is consumed, or if the substance is of low toxicity, the health effects may not be seen for many months or years (or may not be observed at all). Potential effects of toxic environmental contaminants in food include systemic toxicity, mutations, cancer, birth defects, and reproductive disorders.

Systemic toxicity involves changes in the structure and function of organs and organ systems: weight change, structural alterations, and changes in organ system or whole animal function. Functional effects may include changes in the lungs, liver, and kidneys, in cardiovascular function, in brain and nervous system activity and behavior, and in resistance to disease (1). Systemic toxicity is studied in whole animal tests.

Some environmental contaminants have been shown in experiments to cause point mutations (which generally affect a single gene) or more extensive effects such as gross changes in chromosome structure or changes in chromosome number. Such genetic effects in humans often cannot be immediately detected. Indeed, damage to the human gene pool may not become apparent for many generations if the deleterious effect is due to a recessive gene (2). However, a chemical's mutagenic potential can be evaluated indirectly from various biological tests involving microorganisms, mammalian cell cultures, insects, and whole mammals (l).

Some environmental contaminants in food may cause cancer. Direct cause-and-effect associations between exposure to a specific chemical and human cancer are difficult to establish because of the complex nature of cancer and the vast number of potential carcinogens to which humans are exposed. In some cases, exposure to one toxic agent may trigger a sequence of events leading to cancer. In others, carcinogenesis may depend on interactions of several factors, combinations of noxious agents, co-factors, and natural or acquired metabolic peculiarities (2). The cancer-causing potential of a sub-

stance is evaluated through animal tests and a variety of short-term tests employing bacteria, insects, or cell cultures (1).

Environmental contaminants may produce birth defects other than inherited mutations. These are called "teratogenic effects. " Malformations may include gross, histological, molecular, and sometimes behavioral anomalies. Human sensitivity to a teratogenic agent during pregnancy is determined by: 1) the time at which the insult is received during gestation, 2) the dose of the compound, 3) transfer of the teratogen to the developing fetus, 4) uterine factors, and 5) dietary factors. The teratogen may work its effect by producing mutations or chromosomal aberrations, interfering with cell division, altering nucleic acid synthesis, inhibiting specific enzymes, or altering membrane characteristics. A seemingly small change may have far--reaching effects, since the fetus is undergoing rapid biochemical, structural, and physiological growth and change (1).

Environmental contaminants can have other effects on the reproductive process in both males and females. Toxic effects may include reduced or altered sperm formation, inhibition of ovulation, increased spontaneous abortion, fetal resorption, and increased numbers of stillborn infants, Teratogenic and reproductive effects are evaluated through animal tests (l).

Given the range of possible adverse health impacts, it is clear that newly discovered environmental contaminants must be subjected to the best available toxicological testing techniques so that any harmful effects can be uncovered. Furthermore, regulators must have information on the possible toxic effects of ingesting small amounts of a substance in food over an extended period of time, perhaps over a lifetime. It would also be desirable to know what effects other toxic substances already present in our air, water, and food may have on the metabolism and toxicity of a new contaminant,

HUMAN EPIDEMIOLOGY

The science of epidemiology seeks to determine the distribution and causes of diseases and injury in humans, It focuses on groups rather than individuals (3).

There are several types of epidemiological studies. Each provides different levels of information on environmentally related disease or injury. The cohort-study method is the best way to develop information on potential toxic effects in a population exposed to an environmental contaminant in food. In a cohort study, individuals are classified into groups according to the levels of exposure, including a control group with no previous exposure to the suspect substance. The groups are then followed over a period of time and studied for differences in disease incidence (3). Such epidemiological studies have an advantage over animal tests. There are some agents known to cause disease in humans that do not produce similar adverse effects in animals (benzene, for example) (4). Human epidemiological data can also be used to directly estimate human risk, thus eliminating the need for extrapolating from animal experiments.

Epidemiological evidence has sometimes weighed heavily in the setting of tolerances or action levels. This was the case in FDA's decisions on mercury and polychlorinated biphenyls (PCBs). However, if epidemiological data are lacking when an environmental contaminant is discovered in food, the usefulness of further epidemiological studies is restricted by inherent limitations of the science.

Beyond the obvious ethical considerations, such studies are of little use when a rapid regulatory decision must be made. At the low levels that environmental contaminants usually occur in food, pathological effects may not occur for many years, Most cancers, for example, have a latency period of at least 5 to 10 years, As much as 40 years may elapse between the onset of exposure and the development of the disease, Thus epidemiological

methods can only be used retrospectively to evaluate the health status of individuals who have been through this 5- to 40-year period. The findings are compared to the health histories of "control" individuals who presumably have not experienced comparable exposures.

Such retrospective studies are vulnerable to scientific criticism: a) it is very hard to find an adequate control group (unlike animals whose entire lives can be in a "controlled" environment) because of the variability in human susceptibility and personal behavior patterns, b) it is impossible to define potential synergisms and/or inhibitions by other substances to which the target group may have been exposed, and c) it is difficult to quantify previous levels of exposure (5).

Retrospective studies suffer other handicaps, They are extremely expensive, difficult, and time-consuming to carry out, A given type of cancer, for example, may occur in the "normal" population at a rate of 1 case for each 100,000 people. An exposure to a toxic substance that increases by a factor of 100 the likelihood of that cancer occurring would raise the incidence to 1 case for each 1,000 people. To ascertain in a statistically satisfactory and compelling way that such an increase had occurred, one would have to do comprehensive medical studies on at least 3,000 people. Assuming an average cost of $200 for each person studied (which is a low estimate considering the costs of physicians" time, laboratory analysis, and the technological assessments necessary to show a person free of a given tumor or disease), the expense for such a study would be $600,000. It would be necessary under most circumstances to find more than one new case of a disease to convince a nonstatistician and even most statisticians. This would double or triple the costs (5). Furthermore, demonstrating that the incidence had increased would still leave the vexing problem of correlating the increase with the exposure to the specific suspect substance.

Such limitations make cohort epidemiology studies inappropriate for determining the toxic potential of a given contaminant. Moreover, in a regulatory scheme designed to prevent serious illness from developing in humans, the time required to generate epidemiological data would preclude its use in initial regulatory decisions. The prospects for developing a human epidemiological method that would meet such regulatory demands are presently hard to imagine.

One area, however, where human epidemiological studies have and will continue to be useful is in the confirmation of suspected chronic-effect data when a population that has inadvertently been exposed to a substance or a hazard (such as radiation) over a long period of time can be identified. In this instance, carefully designed studies can be the "clincher'" which finally provides thorough documentation of the suspected toxicity. Such studies can confirm or refute the adequacy of regulatory actions (in retrospect) (5), Again, however, these kinds of studies are extremely expensive and consume limited resources which might be better spent supporting other types of evaluations using other techniques.

People who fail to understand these limitations criticize reliance on animal tests when epidemiological information is negative or lacking. This fundamental misunderstanding may delay health-related regulatory action and dilute its effectiveness in preventing illness.

ANIMAL TESTS AND OTHER METHODS FOR TOXICOLOGICAL TESTING

Toxicologists have developed a number of techniques to assess the toxicity of a compound. Some tests can be conducted in 90 days or less. These include simple tests such as 2-hour "range finding" to determine the dose of a substance that is lethal to 50 percent of the animals (LD50). More complex tests include 90-day continuous exposure or paired feeding studies, and short-term tests for mutagenesis and potential carcinogenesis. Tests requiring more than 90 days, such as lifetime exposure studies, are generally considered long-term. In addition to the time necessary for exposure and data-gathering, analysis of the results may take up to an additional year, depending on the complexity of the experiment, the number of animals used, and the amount of data collected (l).

Testing methods can be categorized by ''endpoint" as well as duration. Some experiments are based on expected results such as functional changes, birth defects, or cancer, By the use of an appropriate experimental design, several such endpoints can be assessed in the same experimental period (l). Appendix C describes the range of toxicological tests available for assessing the health risks associated with consumption of contaminated food. Each section describes the tests for assessing a given endpoint, and includes a discussion of available long- and short-term tests,

Animal tests provide valuable information for the setting of action levels and tolerances. The animals serve as proxies for humans in cases where data are lacking on the human health effects of a contaminant and experiments involving humans would be unethical, Because of shorter animal lifespans, the effects on several generations can be studied. Furthermore, animals can be raised in controlled environments, thus eliminating many of the factors that complicate human epidemiological studies, In many cases, animal experiments are the only means by which needed information can be obtained. Using a carefully selected battery of 90-day tests, considerable data can be generated on a contaminant's potential toxic effects including its potential for causing mutations and cancer.

Despite widespread scientific agreement on the usefulness of animal tests, several controversies exist. These include the appropriateness of the carcinogen bioassay techniques, the potential of short-term tests for evaluating mutagenesis and carcinogenesis, the lack of emphasis on testing for potential toxic interactions, and the usefulness of methods for extrapolating from high-dose animal test results to low doses in humans.

The Carcinogen Bioassay

The carcinogen bioassay (6) is a chronic toxicity study requiring 2 years' exposure to the test substance and usually a third year for evaluation of the results. The carcinogen bioassay is used to determine the cancer-causing potential of a compound in males and females of two mammalian species, usually the rat and mouse. The test animals are exposed to the test substance throughout their entire lifespan.

Different groups are exposed to different levels of the substance up to the maximum tolerated dose (MTD). The MTD is the highest dose given during a chronic study that predictively will not alter the animals' normal longevity from effects other than cancer. In practice, the MTD is considered to be the highest dose that causes no more than a 10-percent loss in weight compared to control animals. Throughout the study, animals are examined weekly for signs of toxicity. Animals may be killed at prearranged times and their tissues examined for signs of cancer. At the completion of the study, all remaining animals are killed and detailed examinations are made of their tissues.

Animal tests for carcinogenicity have been questioned because the results are generalized to humans while the animals are fed

much larger doses of the suspected substance than would be consumed by people. However, an earlier OTA report entitled *Cancer Test*ing Technology and Saccharin (7) found that "animal tests are the best current methods for predicting the carcinogenic effect of substances in humans. All substances demonstrated to be carcinogenic in animals are regarded as potential human carcinogens; no clear distinctions exist between those that cause cancer in laboratory animals and those that cause it in humans. The empirical evidence overwhelmingly supports this hypothesis. "

The report also found that the rationale for feeding high doses was sound.

> The rationale for feeding large doses of a substance in animal tests is as follows. As the dose of a substance that causes cancer is increased, the number of exposed animals that develop cancer also increases. To conduct a valid experiment at high dose levels, only a small number of animals (perhaps several hundred) is required. However, to conduct a valid experiment at low dose levels, a very large number of animals is required. (The smallest incidence rate detectable with 10 animals is 10 percent or 1 animal. To detect a l-percent incidence rate, several hundred animals would be required.) Another important variable is the strength of the carcinogen. The stronger the carcinogen, the greater will be the number of animals getting cancer at a particular dose (7).

If data from a carcinogen bioassay are available at the time an environmental contaminant is discovered in food, the results provide crucial information to guide the regulatory decision. However, if no data or inadequate data exist, a newly commissioned carcinogen bioassay would require 2 to 3 years for completion. In this case, the carcinogen bioassay could be used to revise an initial action level.

Short-Term Tests for Mutagenesis and potential Carcinogenesis

Chemicals are evaluated for mutagenicity not only to detect potential carcinogens but also to detect the serious hazard posed by mutagens. In the long run, chemical compounds causing mutations and teratogenic effects may create a greater burden on society by increasing the incidence of genetic disease and birth defects than by causing cancer (2,11).

A number of short-term tests and batteries of tests have been developed to assay chemicals for mutagenicity (1,2). Many of the same tests are also viewed as screens for carcinogens on the assumption that cancer arises following damage to the genetic material of cells (2). The ability of these tests to detect carcinogens is currently undergoing validation (8). The Environmental Protection Agency (EPA) currently uses some of these tests to identify mutagens (9, 10). Presently there is no universal agreement on a "best" set of tests.

Environmental contaminants in food are screened to identify those capable of causing genetic damage, to determine the types of damage they cause, and to evaluate the risks they pose to the general population and certain subpopulations.

The major difficulty in designing tests to detect mutagens and potential carcinogens is the need to reflect the metabolic capabilities of man as nearly as possible while remaining economically realistic. Whole animal tests more nearly reflect responses of man and are therefore useful in estimating risk to humans. Tests using lower organisms are less directly applicable to humans, but they are simpler and more economical to perform. As a consequence they are more popular for use in mutagen screening than animal tests. Risk estimates based on tests in lower organisms have been proposed (12) but are not accepted as sufficient evidence for setting action levels and tolerances.

Many chemicals are not directly mutagenic, but once ingested or absorbed are converted into mutagenically active derivatives. Activation therefore poses important practical and conceptual problems that must be dealt with in evaluating the mutagenic potential of environmental agents. The practical problem is one of designing tests to identify

mutagens that adequately mimic the metabolic capabilities of an intact animal. The conceptual problems focus on the fact that evidence of an agent's mutagenicity is not sufficient to evaluate the actual risk posed to an individual or a population by exposure to the agent (2).

The ability of an agent to cause genetic damage in an intact animal depends not only on the mutagenic potential of the agent or its metabolizes but on a number of other factors as well. The fate of any chemical substance entering the body is determined by a dynamic process involving its absorption, distribution, biochemical alteration, and excretion. Many mutagenic agents are formed by the action of intestinal bacteria. Consequently, the route of exposure to an agent can play a significant role in determining its activity. Enzymatic processes in the body inactivate as well as create mutagens. Many active mutagens may exist in the body as intermediates in the metabolic pathways that process the parent compounds. As a result, the active mutagen may have a short lifespan and be distributed only in those tissues that possess high levels of activating enzymes (2).

There are four major factors determining the ability of an environmental agent to produce a genetically adverse effect in an organism, The first is the metabolic response of the organism to the agent. This response will determine the distribution, lifespan, and fate of the mutagen in the body tissue. The second is whether the mutagen damages the genetic material in cells. The third is the response to the genetic damage by the DNA repair machinery in the cell. Fourth is the type of lesion the agent is capable of producing in the genetic material.

Short-term tests for detection of mutagenesis and potential carcinogenesis fall into four categories:

- procedures that can be carried out directly in human populations,
- intact animal tests,
- tests employing cultured mammalian cells, and
- tests employing micro-organisms.

Several procedures that can be carried out directly in exposed human populations have been found to be indicators of the presence of mutagenic agents. These procedures include analysis of white blood cells for chromosome aberrations (cytogenetic analysis) (13), analysis of blood and/or urine of the presence of mutagenic agents (14), analysis of semen for abnormal sperm (15), and the detection of extra chromosomes in sperm (16).

Intact animal tests usually use as test animals either mice or fruit flies [Drosophila melanogaster). The dominant lethal test detects lethal damage resulting primarily from chromosomal aberrations by treating male mice with test agents and mating them. The uteri of the pregnant females are examined for fetal death and resorption. Because the endpoint is detected in the offspring of the treated animal, mutation in the germinal cells (the sperm) is detected by this method. The specific locus test involves the crossing of two genetically distinct strains of mice with visible markers, such as spotted coats. Mutations are detected by the appearance of recessive traits in the offspring. The utility of this testis restricted because of the number of animals needed. Cytogenetic screening of white blood cells or bone marrow cells from treated animals detects damage to chromosomes. The micronucleus test detects chromosome breaks in bone marrow cells of treated animals (17).

The use of cultured mammalian cells for mutagenesis testing is a great simplification over the use of intact animals. At the same time, the cells retain much of the mammalian metabolism and genetic characteristics. Moreover, human cell cultures can be used where exposure of people to toxic agents could not be allowed. The major weakness of cultured mammalian cells as test systems is that they are isolated from the metabolic activity of the intact animal (2).

Several strategies have been developed to try to approximate the metabolic capabilities of the intact animal in mammalian cells, The simplest and most widely used activation system employs homogenates of mammalian

liver. Either the liver homogenate is incubated with the test substance before it is applied to the cell culture, or the test substance and the homogenate are included in the test plates. A major difficulty with the liver microsome activation system is that it cannot be prepared with reproducible enzyme levels from one batch to the next. Storage conditions also alter the activities of the preparations, Furthermore, not all the activation mechanisms present in the intact animal are represented in the liver homogenate. The greatest advantage of the liver homogenate method is that it preserves the simplicity of in vitro testing (18).

Methods for mutagen screening have been developed using many micro-organisms, including fungi, bacteria, and viruses present in bacteria. The most widely known system is the "Ames test" which employs specially bred strains of Salmonella bacteria. Figure 5 illustrates the steps in the Ames spot test. The Ames plate test allows quantitative comparisons to be made of mutagenic potential (19). Numerous other tests have been devised which are in varying stages of validation (20). Some of these are described in appendix C. The great appeal of these techniques is that they are rapid, simple, and inexpensive. No animal colony is required, and the necessary technical skills are those required for conventional bacteriology.

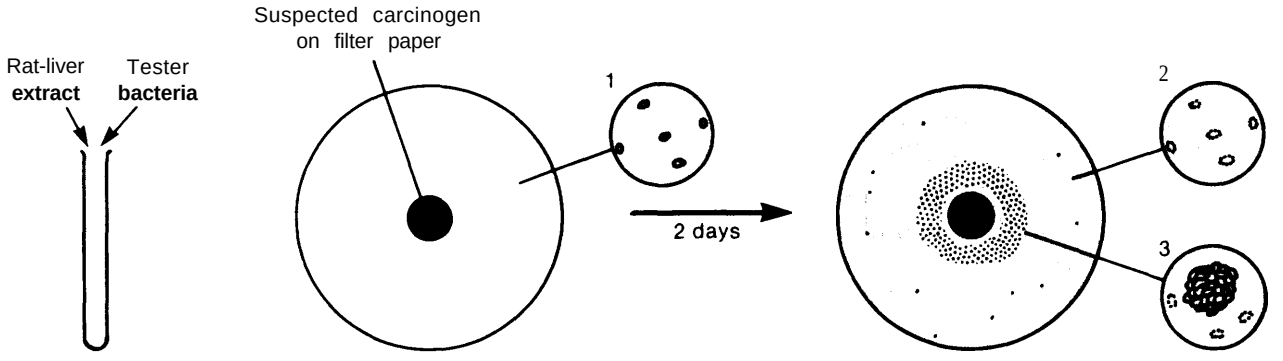
These tests have been criticized because the cell structure of bacteria is very different from the cell structure of mammals. Validation studies now underway are attempting to evaluate 30 mutagenicity assay systems for their ability to predict chemical carcinogenicity (8).

Bacteria also lack the enzyme systems that are the principal mammalian mutagen-activating enzymes. This criticism is partially overcome by the use of liver homogenates described above. Finally, each test is sensitive to one particular type of genetic damage. Therefore, the best approach is to use a battery of short-term tests designed to test for the different types of genetic damage.

Interactions of Two or More Substances

Tests designed to detect effects from interactions of two or more substances are of lim-

Figure 5.—Steps in the Ames Spot Test



MUTAGENESIS is detected in the Ames test by mixing an extract of rat liver (which supplies mammalian metabolic functions) with tester bacteria (which cannot grow because a mutation makes them unable to manufacture histidine, a necessary nutrient) and plating the mixture on an agar **medium so** that a thin layer of bacteria covers the medium evenly, as is shown on a microscopic scale (1). In this "spot assay" a dose of the chemical to be tested is placed on a disk of filter paper on the tester bacteria. After 2 days most of the *his* bacteria have died for lack of histidine (2), but DNA damage caused by the chemical diffusing out from the **disk has given rise to mutations, some of which result** in reversion of the *his*$^-$ mutation. The histidine-making revertant bacteria proliferate, forming visible colonies (3).

SOURCE Redrawn with permission Devoret R Bacterial Tests for Potential Carcinogens *Scientific American* 241 (2) 40 August 1979

ited use for regulatory actions. This does not mean that such information is not important. It rather reflects the rudimentary nature of our present understanding of interactions, the complexities of the tests, and the difficulties in interpreting the results in a fashion meaningful to regulators.

Six different types of interactions may occur when two or more chemicals interact in a biological system. The effects produced may differ in magnitude from those caused by any one of the chemicals alone.

- If the interaction of two substances produces an effect equal to the sum of the individual effects, the response is called summation or addition.
- If the interaction of two substances produces an effect greater than would be anticipated from the sum of the individual effects, the response is known as potentiation or synergism.
- If the effect is less than the sum of the two would predict, the response is antagonism.
- When an inert substance, having no observed effect at a given dose, enhances the effect of another simultaneously administered chemically, the effect is usually referred to as activation.
- If an inert substance decreases the effect of another chemical administered simultaneously, the effect is called inhibition.
- Finally, there may be no interaction and each chemical would exert its own effect independently of the other (2 1).

Once ingested, a chemical may exert an action locally in or on the stomach or intestines. While such effects possibly could be produced by environmental contaminants, they are not likely to be observed at the low levels normally encountered in food. The more serious concern is the systemic effects that may occur after absorption from the gastrointestinal tract.

Following absorption from the gastrointestinal tract, additive chemicals may produce their effect by acting on a target organ, by acting on different target organs or systems, or by acting differently on different organs to produce the same effect. Most of the interactions are at the biochemical level and the mechanisms are still being studied.

The key consideration is whether present testing technologies are adequate to provide data that are useful in making regulatory decisions. Techniques have been developed to study the interactions of drugs, other chemicals, and physical agents such as radiation. Such techniques should be applicable to the study of the effects of environmental contaminants. However, their scope is usually limited to the study of two component interactions because of the large number of test animals required and the difficulties in interpreting the results. For example, a relatively simple test involving two agents in a factorial design would require about 100 animals. A more recent study (22) of the combined effects of cadmium, mercury, and lead used 50 to 60 animals in each of 15 different treatment groups. The results indicated that a particular combination of the three metals could be synergistic, antagonistic, or additive, depending on the relative doses employed (table 13).

Table 13.—Combined Effects of Cadmium (Cd), Mercury (Hg), and Lead (Pb)

Metal combinations[a]	Interaction[b]		
	Antagonism	Addition	Synergism
Pb/Hg	0.64		
Hg/Pb			14.7
Pb/Cd	0.73		
Cd/Pb			2.3
Hg/Cd		1.0	
Cd/Hg	0.54		
(Pb + Hg) / Cd			1.2
(Hg + Pb) / Cd			1.9
(Pb + Cd) / Hg	0.61		
(Cd + Pb) / Hg			1.4
(Hg + Cd) / Pb			18.4
(Cd + Hg) / Pb			1.7

[a]For each combination, the metal or metals in the numerator were held constant at or near the essentially no-effect level (LD 1). The dose of the metal in the denominator was increased to obtain a dose-response curve. When a pair of metals appear in the numerator, the first was fixed at its LD 1 and the second was increased to obtain an LD 1 for the pair.

[b]Interactions are expressed as the ratio of the dose of the increased metal alone needed to attain the LD 50 to the corresponding value for that agent in the presence of a single metal or a metal pair. If the ratio is greater than 1, synergism has occurred. If the ratio is equal to 1, the interaction is additive. If the ratio is less than 1, the interaction is antagonistic.

SOURCE: Adapted from J. Schubert, E. J. Riley, and S. A. Tyler, "Combined Effects in Toxicology—A Rapid Systematic Testing Procedure: Cadmium, Mercury, and Lead." *Journal of Toxicology and Environmental Health* 4:763, 1978.

The testing for interactions among toxic substances is further complicated by the necessity to limit the number of substances studied, Because of the large number of animals required, and the difficulties in interpreting results, the number of substances tested are confined to those that may be most important, Deciding what is ‘‘important” to study out of the vast number of toxic substances to which we are exposed is a difficult problem, Perhaps a reasonable approach would be to focus on those to which people are known to be exposed (see chapter VII). The choice could also be based on structure-activity relationship or known mechanism of action, Chemical selection for testing is being reviewed and evaluated in an ongoing OTA study entitled “Assessment of Technologies for Determining the Carcinogenic Risk from the Environ merit.”

The study of the interactions of two or more substances is an area in which far more basic research is required before meaningful information can be generated for regulatory decisions. At the present time, no satisfactory methods exist for testing the interactions of more than two chemicals.

Extrapolating From High Doses to Low Doses

Quantitative risk assessment. based on mathematical models, is often proposed as an alternative to the current use of safety factors (chapter 111) (24,25). This approach is now most extensively employed in assessing carcinogenic risks (26). The technique is also being investigated as a means to evaluate other types of irreversible toxic effects (23). The following discussion of mathematical extrapolation of risk involves only its uses in determining risks from chronic low-level carcinogenic insults.

Mathematical models can be used to estimate the number of extra cancers that are likely to be caused by the presence of a carcinogen in the food supply. Models may be used to estimate a tolerance based on calculations of a “safe dose’” for human consumption. They can also forecast the likely change in the number of extra cancers that would accompany some projected increase or decrease in the level of human exposure occurring either as a result of regulatory action or inaction, The technique is therefore used to calculate benefits to be derived from reducing human exposure to a substance.

The typical carcinogen bioassay uses around 100 animals at each experimental dose. If a particular experimental dose produces evidence of a lifetime increase in cancer of 1 in 10, this increase can be measured using 100 animals. But if the increased cancer risk is less than 1 in 100, this increase will often not be detected even with a 100-animal feeding experiment. The extra human risk resulting from environmental exposure is usually much smaller than 1 in 100 (perhaps on the order of 1 in 100,000) for any given chemical over a lifetime exposure. It would not be practical to conduct an experiment with enough animals to directly measure this small an increase in risk (30).

For these reasons, carcinogen bioassays use (in addition to a control dose of zero) several doses at which the projected extra cancer incidence may be 1 in 10 or larger. These high-dose data are then used to estimate the extra risk at a very low dose where the extra risk may be no larger than 1 in 1,000,000. These problems are often referred to collectively as the “low close extrapolation problem” (30).

A low-dose extrapolation involves the choice of a mathematical function to model the dose-response relationship and the choice of statistical procedures to apply to the mathematical function (24-29). The choice of mathematical function is crucial to the outcome of a low-dose risk estimate. If the assumed relationship between tumor occurrence and dose does not apply in the low-dose regions to which the extrapolation is being made, a serious overestimate or underestimate of the “safe dose’” may result. Even though different dose-response models may agree in the observable response range, they could differ by many orders of magnitude at low-dose levels. Furthermore, there is no experimental way to

predict the shape of the dose-response curve at very low doses,

Because of the possible disparity of dose-response functions when extrapolated to low doses, the dose-response function must be selected not only on the basis of how well it can be made to fit experimental data but also on the basis of known (or at least plausible) information on the biological mechanisms through which a chemical induces or promotes cancer. This is a major source of uncertainty in extrapolation procedures, since the exact biological mechanisms through which carcinogenesis may occur are unknown (30).

Several theories on the nature of the process by which cancer develops serve as the bases for different low-dose extrapolation models. Some "linear" models project that the relationship between the dose received and the cancer risk is a straight line at low doses and that there is no threshold. Other models level off at low doses and therefore predict that a threshold dose exists below which the agent has no carcinogenic effect (24-30).

Newer models take into account the effects of metabolic activation and detoxification upon carcinogenic dose response (26), These "kinetic" models encompass free toxic substances, metabolizes, deactivators, and the possible interactions of these substances, Only a "steady-state" situation is studied in that the variation over time of the concentrations of these agents is not considered. If deactivation of the carcinogen is 100-percent efficient, the model predicts a threshold dose below which there is no carcinogenic risk. However, in a naturally occurring process, it is likely that deactivation would be less than 100-percent effective. A number of modifications to the model allow for imperfect deactivation. These situations rule out a threshold and would lead directly to a model for which carcinogenic response varies linearly with dose at low dose,

The mechanisms through which most carcinogens produce cancer are not sufficiently understood so that the shape of the dose-response curve can be predicted with certainty, As pointed out earlier, experiments of sufficient size to permit direct experimental investigations of the dose-response curve at low dose cannot be conducted. There are plausible arguments that the dose-response curve is linear at low dose for many carcinogens, On the other hand, no mechanism that would lead to a more cautious dose-response relationship has been seriously proposed except for some dose-response relations in radiation-induced carcinogenesis.

In view of these uncertainties, it would seem reasonable to base estimates of added cancer risk on a mathematical model that encompasses low-dose linearity until the mechanisms through which the carcinogen operates are understood sufficiently to conclusively rule out low-dose linearity,

Caution must be used in interpreting the results of low-dose extrapolations, particularly when they are applied to humans, Table 14 demonstrates that different models applied to the same data sets yield differing estimates of virtually safe doses. Virtually safe doses based on the multistage model are identical with those based on the one-hit model when there is only one experimental dose. This is illustrated with Data Sets I and

Table 14.—Virtually Safe Doses Computed From Three Different Data Sets and Three Different Models

Data set	Dose-response model	Virtually safe doses (lower 95-percent confidence bounds for dose) in ppb corresponding to extra risk of 1/1 ,000,000
I[a]	Probit	14.2
[a]	One-hit and multistage	.511
I[b]	Probit	.180
I[b]	One-hit and multistage	.0551
II[c]	Probit	1.29
II[c]	One-hit	.235
II[c]	Multistage	.205

[a] Kimbrough et al. (1975). rat study with Aroclor 1260. using number of animals with hepatocellular carcinomas.
[b] Kimbrough et al. (1975), rat study with Aroclor 1260. using number of animals with liver neoplastic nodules.
[c] Industrial Bio-Test (1971) rat study with Aroclor 1260. using number of animals with liver neoplastic nodules.

SOURCE Adapted from K S Crump and M D Masterman Assessment of Carcinogenic Risk From PC Bsin Food OTA Working Paper 1979

II. The multistage model also yields virtually the same value as the one-hit model for Data Set III. However, as explained in appendix D, the multistage model can yield higher values for the virtually safe dose than the one-hit model whenever the data exhibit upward curvature and are inconsistent with the one-hit model (30).

Caution also must be exercised when comparing calculations of extra lifetime risks. Table 15 summarizes the results of an FDA risk assessment of PCBs (31) with a similar risk assessment commissioned by OTA (30). Differences can arise in such calculations when different methods are used to extrapolate from animals to man. The extrapolation can be performed on the basis of milligrams per kilogram of body weight per day, parts per million of substance in the diet, milligrams of substance per square centimeter of body surface area per day, or milligrams of substance per kilogram of body weight per lifetime.

Table 15.— Extra Lifetime Risks of Cancer Associated With Consumption of PCBs in Food

Dose (g/day)	Extra lifetime risk/100,000	Upper limit of new cancers/year
FDA'		
9 2'	44	21
14 9'	72	34
201[c]	98	47
OTA[b]		
3 3(!	013	4
3.7[e]	035	11
127f	5	

[a]Based on NC I bioassay and total malignancies for males and females
[b]Based on Kimbrough 1975 study and hepatocellular carcinomas
[c]Based on highest consumers 90th percentile of fish species contaminated with PCBs if tolerances set at 2 5 ppm
[d]Based on FDA Total Diet Study 1976
[e]Based on FDA Total Diet Study 1975
[f]Based on average intake of people consuming more than 24 lbs year Lake Michigan fish

Differences also arise when varying animal data serve as the basis for the risk assessment, And, of course, the choice of the model also affects the final outcome. In this case, both extrapolations were based on milligrams of PCB per kilogram of body weight per day. Both FDA and OTA applied a linear model: however, different assumptions were made on the amount of PCBs that would be ingested and on the size of the exposed population. FDA's risk assessment therefore applies to the 15 percent of the total U.S. population expected to consume the fish species known to be most highly contaminated with PCBs. The OTA calculations are based on the average daily intake of PCBs based on FDA's total diet study (3.3 and 8.7 μg/day) and on estimates of the PCB intake of people who consumed more than 24 lbs of Lake Michigan sport fish per year.

Although many different dose-response models exist and can be used for low-dose risk assessment, it appears prudent at this time to use a model that does not arbitrarily rule out low-dose linearity. Models are still being developed and when" appropriate could be used to guide regulatory decisions. Dose-response models for low-dose risk assessment provide a useful technique for assessing the possible added risk attributable to environmental contaminants. Such models also might well be used in place of safety factors in future procedures adopted for food contaminant regulations. The major impediment to the widespread use of the models in environmental food contamination considerations is the lack of data on which to perform the analysis.

CHAPTER V REFERENCES

1. SRI International. "Assessment of Methods for Regulating 'Unavoidable" Contaminants in the Food Supply, " OTA Working Paper, 1978.
2. Legator, M. S. "Short Term Procedures for Determining Mutagenic/Carcinogenic Activity," OTA Working Paper, 1979.
3. MacMahon, B., and T. F. Pugh. *Epidemiology Principles and Methods*, Little, Brown and Company, Boston, Mass., 1970.
4. Ray, V. "Are Benzene Effects Limited to the Chromosomal Level?, " *Banbury Report 1—Assessing Chemical Mutagens: The Risk to Humans*, Cold Spring Harbor Laboratory, 1979.
5. Jackson, R. S. "The Limitations of Applying Human Epidemiology to the Regulator Pro-

cess for Toxic Substances, Particularly Carcinogens," OTA Working Paper, 1979.
6. Sontag, J. M., N. P. Page, and U. Saffiotti. Guidelines for Carcinogen Bioassay in Small Rodents, DHEW Publication No. (NIH) 76-801, Government Printing Office, Washington, D. C., 1976.
7. Office of Technology Assessment. Cancer Testing Technology and Saccharin, Government Printing Office, stock no. **052-003-00471-2,** Washington, D. C., 1977.
8. U.S. Department of Health, Education, and Welfare. National Toxicology Program Annual Plan Fiscal Year 1979.
9. U.S. Environmental Protection Agency. FIFRA Testing Guidelines, 1978.
10. U.S. Environmental Protection Agency. TSCA Test Standards, 1979.
11. Neel, J. V. "Mutation and Disease in Humans, " Banbury Report l—Assessing Chemical Mutagens: The Risk to Humans, Cold Spring Harbor Laboratory, **1979.**
12. Committee 17, Council of the Environmental Mutagen Society, "Environmental Mutagen Hazards," Science 187:503, 1975.
13. Kilian, D. J., and D. Picciano. "Cytogenetic Surveillance of Industrial Populations, " Chemical Mutagens, Principles and Methods for Their Detection, Vol. IV, A. Hollaender (cd.), Plenum Press, New York, **1976.**
14. Legator, M. S., L. Troung, and T. H. Connor. "Analysis of Body Fluid Including Alyklation of Macromolecules for Detection of Mutagenic Agents, " Chemical Mutagens, Principles and Methods for Their Detection, Vol. V, A. Hollaender and J. DeSerres (eds.), Plenum Press, New York, 1978.
15. Heddle, J. A., and W. R. Bruce. "On the Use of Multiple Assays for Mutagenicity, Especially the Micronucleus, Salmonella, and Sperm Abnormality, Assays in Progress, " Genetic Toxicology, P. Scott, B. A. Bridges, and F. H. Sobels (eds.), 1977.
16. Kapp, R. A. Mutation Research, in press, 1979.
17. Kilbey, B. J., M. Legator, W. Nichols, and C. Ramel. Handbook of Mutagenicity Test Procedures. Elsevier, Amsterdam, 1977.
18. Ames, B. N., W. E. Durston, E. Yamasaki, and F. O. Lee. "Carcinogens and Mutagens: A Simple Test Combining Liver Homogenates for Activation and Bacteria for Detection, " Proceedings of the National Academy of Sciences 70:2281, 1973.
19. Ames, B. N. "A Bacterial System for Detecting Mutagens and Carcinogens, " Mutagenic Effects of Environmental Contaminants, H. E. Sutton and V. I. Harris (eds.), Academic Press, New York, 1972.
20. Devoret, R. "Bacterial Tests for Potential Carcinogens, " Scientific American 241(2):40, 1979.
21. Fingl, E., and D. M. Woodbury. "General Principles, " The Pharmacological Basis of Therapeutics, L. S. Goodman and A. Gilman (eds.), 5th edition, MacMillan Publishing Co., Inc., New York, 1975.
22. Schubert, J., E. J. Riley, and S. A. Tyler. "Combined Effects in Toxicology—A Rapid Systematic Testing Procedure: Cadmium, Mercury, and Lead, " Journal of Toxicology and Environmental Health 4:763, 1978.
23. Van Ryzin, J., personal communication, **1979.**
24. Interagency Regulatory Liaison Group, Work Group on Risk Assessment. "The Scientific Bases for Identification of Potential Carcinogens and Estimation of Risks," Journal National Cancer Institute 63:241, 1979.
25. Scientific Committee of the Food Safety Council. "A Proposed System for Food Safety Assessment ," Food and Cosmet. Toxicol. 16, Supplement 2, 1978.
26. Cornfield, J. "Carcinogenic Risk Assessmerit, " Science 198:693, 1977.
27. Crump, K. S., D. G. Heel, C. H. Langley, and R. Pete. "Fundamental Carcinogenic Processes and Their Implications for Low-Dose Risk Assessment, " Cancer Research 36: 2973, 1976.
28. Guess, H. A., K. S. Crump, and R. Pete. "Uncertainty Estimates for Low-Dose-Rate Extrapolations of Animal Carcinogenicity Data, " Cancer Research 37: 3475, 1977,
29. Rai, K., and J. Van Ryzin. "Risk Assessment of Toxic Environmental Substances Using a Generalized Multi-Hit Dose Response Model, " Energy and Health, N. Breslow and A. Whittemore (eds.) SIAM Press, Philadelphia, Pa., p. 94, 1979.
30. Crump, K. S., and M. D. Masterman. "Assessment of Carcinogenic Risk from PCBS in Food, " OTA Working Paper, 1979.
31. U.S. Food and Drug Administration. An Assessment of Risk Associated with the Human Consumption of Some Species of Fish Contaminated With Polychlorinated Biphenyls (PCB's), **1979.**