2. CANCER TESTING TECHNOLOGY

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TESTING METHODS AND GUIDELINES

Present methods are able to identify which substances in the environment are potential carcinogens with increasing frequency and accuracy. Current methods include:

1. Analysis of a substance's molecular structure,
2. Animal tests,
3. Short-term tests, and
4. Epidemiological studies.

To date, knowledge of a substance's molecular structure has not been of general use in predicting carcinogenicity. There have been promising developments in short-term tests, and they already provide economical and quick screening methods. At present, animal tests are the most definitive laboratory evidence for the potential of a substance to produce cancer in humans. Positive results from epidemiological studies are the most convincing evidence for a substance's carcinogenicity in humans.

Analysis of molecular structure provides some information concerning the likelihood that a substance will cause cancer. In most instances, however, present knowledge does not permit useful prediction on the basis of structure alone. An important consideration limiting the usefulness of the approach is that an ingested substance may be metabolized to a different form, and the metabolize may be a carcinogen.

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 best theoretical model must be distinguished from the best practical one. Apart from testing directly in humans, primates would be the best theoretical model. But in order to detect carcinogens of low incidence rates and long incubation periods, the best experiments would involve hundreds of thousands of primates exposed to substances at the same level and by the same route of administration as encountered by humans. Some guidelines would also require that they be followed for at least two generations in order to detect a carcinogenic effect. The best practical model is to use small animals, which have lifetimes of 2 to 3 years.

Standard procedure in animal cancer tests is to feed substances at the "maximum tolerated dose." In the case of saccharin, the "maximum tolerated dose" is 5 percent

of the diet, even though humans are exposed to much lower doses. Contrary to popular opinion, all chemicals do not cause cancer at high dose levels. Many food additives and other chemicals have been tested in animals at this level without causing cancer.

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 one animal. To detect a 1-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. Thus, there are three important variables to be considered in any experiment: strength of the carcinogen, exposure level or dose, and number of animals (or humans) exposed.

These experiments involve a complex target organism, mammals, and there are many other uncertainties about the adequacy of the test protocols and interpretation of the results. Ideally, the only major variable between control and experimental animals should be the absence or presence of the substance being tested, but this state is difficult to attain.

The considerable genetic variation among people and their exposure to other carcinogenic substances affect human susceptibility to carcinogenic agents. This situation causes difficulties in making quantitative estimates of human risk based on data from genetically similar animals in controlled environments.

Examining the relationship of carcinogenicity in test animals to human risk can be divided into three steps:

1. Does the substance produce cancer in the specific experimental situation?
2. What is the significance of this observed effect for the carcinogenic potential in humans?
3. If cancers in humans are likely, what is the expected frequency and location?

Current methodology can answer the first two questions with a reasonable degree of confidence. If the substance produces cancer in test animals and the route of administration is equivalent to that of human exposure, a carcinogenic effect is likely in humans. In only a few cases has it been possible to test whether exposure to a carcinogen produced the predicted frequency of tumors in humans. In those few cases the actual experience was roughly predicted by extrapolation from animal studies. Until more such estimates have been checked, however, caution must be exercised in attaching value to extrapolation estimates.

Short-term tests aid in evaluating the potential of substances to cause cancer. A number of short-term tests are available in varying stages of validation, and some have been used more extensively than others. Because they can be conducted quickly (often requiring only a few weeks) and inexpensively, these tests are useful for screening substances for potential carcinogenicity.

Short-term tests are based on the presumption that cancer is related to cellular

DNA changes and that detection of such changes is predictive for a substance's being potentially carcinogenic. Short-term tests examine the capacity of a substance to cause mutations or other genetic alterations. * A variety of biological systems are used, including bacteria, yeast, mammalian cells in culture, insects, and intact animals. To date, the most widely used method is the Salmonella/Ames test. This method uses several specially constructed strains of *Salmonella* bacteria to detect mutagenic changes resulting from exposure to some chemicals. Rat (or human) liver extracts are included in the test to produce metabolizes from the test chemicals. As mentioned earlier, some chemicals may be carcinogenic only in a metabolized form.

Several expert committees are evaluating the relative usefulness of short-term tests in detecting the potential mutagenic and carcinogenic hazards of chemicals (39, 41, 34, 47). A retrospective validation procedure has been used to determine the ability of short-term tests to detect chemical carcinogens. For example, several hundred known animal carcinogens and noncarcinogens have been tested (97, 139, 155, 163) in the Salmonella/Ames test, which at this time is the most extensively validated short-term test. About 90 percent of the known carcinogens were positive in the Salmonella/Ames test, and about 90 percent of the known noncarcinogens were negative. The growing list of chemicals for which this concordance is found strengthens the argument that mutagenic agents in short-term tests are likely to be carcinogens. This retrospective validation procedure helps to determine if a specific short-term test accurately detects carcinogens and noncarcinogens. In other words, it helps to determine the validity of the short-term test itself.

After a test has been well validated, it can be reasonably assumed that if a previously untested substance is clearly positive in that test, it will probably be a carcinogen in animals. However, a negative result in a short-term test is more difficult to evaluate: such a result only suggests that the chemical is noncarcinogenic. Negative results are not necessarily definite because short-term tests do not detect promoting agents or cofactors in the carcinogenesis process, and such substances may be important in causing cancer. Also, even though a high percentage of known carcinogens may be positive in a short-term test, no test is perfect. One cannot be sure whether a negative result is simply a "false negative. "

In assessing the potential carcinogenic hazard of a substance to humans, short-term test results must be evaluated in conjunction with other available information from human epidemiological studies and animal carcinogenicity experiments. Data from these three sources are weighed very differently in such an evaluation. For example, a positive result in a human epidemiological study would override a negative result in either of the other two areas, and a positive result in an animal carcinogenicity test would override a negative short-term test.

The ultimate usefulness of short-term tests depends on their accuracy in predicting the carcinogenicity of substances. Increasing numbers of carcinogenic substances are first being identified by short-term tests, for example, nitroquinoline-N-oxide, the fumigant ethylene dibromide, the Japanese food additive AF-2, and the flame retardant Tris. The number of substances is still small, but other chemicals identified as potential carcinogens in short-term tests are now being tested in animals. During the

*Chemical mutagens are substances that can interact with chromosomes to change their molecular structure. Since the chromosomes contain the genetic information in the cell, these interactions can lead to mutations (genetic changes) that will permanently alter one or more of the characteristics of the cell. Mutations can lead to heritable changes if they occur in the germ (sperm or egg) cells, and such changes that occur in other cells (somatic cells) are believed to be important in causing cancer.

next few years, knowledge of the predictive value of short-term tests should be greatly expanded.

It is much more difficult to determine the role that short-term tests should play in regulatory decisions when animal or human carcinogenicity data either are not available or are negative. When information from any nonhuman test is incorporated into decisions about the potential health hazard of a chemical to humans, an element of uncertainty is injected into the decision. The degree of uncertainty depends on how much is known about the ability of the particular nonhuman test to predict accurately the potential of chemicals to cause human cancer. Although some of the short-term tests have been validated quite extensively, they are clearly less certain than animal carcinogenicity tests. Nevertheless, the degree of uncertainty acceptable in each regulatory decision is likely to vary enormously depending upon the extent of human exposure and on the benefits associated with the particular chemical. Some cases may arise in which a regulatory decision may be justifiably based on short-term test data.

Epidemiological studies attempt to answer two questions:

(1) Is there a positive association between a particular exposure and the occurrence of disease in humans?
(2) If there is, is it causal?

Epidemiological studies can provide strong evidence of the causal relationship between exposure and disease, particularly when the findings are positive. Negative findings are more difficult to interpret. Humans are usually exposed to carcinogens in far smaller doses than those used in animals. The effects in humans are consequently less frequent, and it is necessary to examine large numbers of people to detect them. A further reason for caution in interpreting negative findings is that the data on exposure almost always contain elements of uncertainty.

Positive epidemiological evidence can confirm the effect in humans predicted by animal tests. Sometimes an epidemiological study provides the first evidence that a substance is carcinogenic in humans. A carcinogenic substance is most easily detected if the cancer has a short induction time and a high incidence, or if the cancer is a rare one. The usual sensitivity limits of even a properly conducted study make detecting a carcinogen with a low incidence unlikely. A long induction time also makes detection difficult.

Thus, positive or negative epidemiological evidence could make a strong case for or against the existence of a carcinogen with a high incidence and short induction time. Negative evidence alone would not provide the basis for a case against a carcinogen with an expected low incidence and/or long induction time. In such cases, the negative epidemiological evidence might, however, indicate the upper limits of the incidence of cancer from that substance.

Guidelines for carcinogenicity testing have been established, and they have general, not specific, applications. They apply to (1) animal tests, (2) short-term tests, (3) epidemiological studies, and (4) extrapolation from experimental data to the evaluation of human risks. None of the criteria expressly states the necessary conditions leading to conclusive evidence that a chemical is carcinogenic in humans. Guidelines discuss the kinds of evidence to be considered, but the conclusion is dependent on the circumstances of the individual cases. The most commonly used guidelines are those issued by the National Cancer Institute (NCI), sometimes altered by suggestions from the National Academy of Sciences (NAS). These guidelines have considerable influence on the Federal agencies that regulate carcinogens. An example

(described below) is the draft proposal prepared by the Occupational Safety and Health Administration (OSHA).

FEDERAL AUTHORITY OVER CARCINOGENIC SUBSTANCES

With two exceptions, Federal laws do not directly address the issue of carcinogenicity. Instead, they specify regulatory authorities for particular classes of substances. Usually, regulation applies to the toxicity or general dangers to health posed by the substances. Substances can be divided into those occurring in the general environment; present in the workplace; ingested or contacted as foods, drugs, or cosmetics; or products that may be used by consumers in the home, in recreation, etc.

Only the Food, Drug, and Cosmetic Act and the Toxic Substances Control Act contain provisions that relate directly to carcinogens. Both distinguish the procedures to be followed in regulating carcinogenicity from those for general toxicity. Seven other statutes are related to carcinogenicity, but they make no distinctions between carcinogenicity and general toxicity (see table 1).

In the past, Federal regulations* have set standards for exposure to carcinogenic chemicals on a case-by-case basis. Efforts have been made to regulate carcinogenic substances more uniformly. For example, a current draft document from the Department of Labor proposes to set standards for worker exposure to cancer-causing chemicals under the Occupational Safety and Health Act (P.L. 91-956) through the use of three uniform job-health standards. Each carcinogen or suspected carcinogen would be placed into one of three categories. Each category would have its corresponding uniform standard. Allowable exposure levels may vary depending on the substances, even within the same category.

A substance will be classified as a Category I Toxic Material ("confirmed" carcinogen) based on positive evidence found in any of the following:

- Humans.
- Two mammalian test species.
- One mammalian species, if the results are replicated in the same species in a separate study.
- A single mammalian species, if the results are supported by multitest evidence of mutagenicity.

In developing this proposal, the Occupational Safety and Health Administration has attempted to incorporate the guidelines for testing (referred to earlier). If adopted, this proposal would establish general criteria for determining when a substance should be considered carcinogenic in humans. It sets no general criteria for quantifying human risk, but permits such estimates to be made for individual substances as part of the risk/benefit determination.

In conclusion, the present state of carcinogenesis testing technology is best reflected by the "Delaney clause." Demonstration of a substance's carcinogenicity in humans or animals is sufficient for banning it from the food supply. The "Delaney clause" does not require quantification of risk and does not allow risk/benefit balancing. It differs therefore from those authorities that include carcinogenicity under general toxic effects. Those authorities implicitly allow quantitative estimates to be made for the purpose of balancing risks against other factors, such as economic impact or health benefits.

*Except for those regulations issued by the FDA pertaining to the "Delaney clause."

TABLE 1 -Federal Regulation of Carcinogenic Substances

	(a) Administered By:	(b) Type of Sunstances Regulated	(c) Specific Procedures for Regulating Carcinogens?	(d) If "C" Does Not Apply, How are Carcinogens Regulated	(e) Benefit-Risk Analysis or Consederation of Factors Other Than Safety	Regulating	(g) Relationshli to Other Federal Statutes
I(a) Federal Food, Drug, and Cosmetic Act–food provisions	Food and Drug Administration,	Foods, food additives, other substances or residues m food	Yes, in several sections (food additives, color additives, residues of animal drugs)	For other sections, general safety is the criterion	Risks dominate; no such analysis permitted if color or food additives or residues from animal drugs are carcinogenic; If a naturally-occurring substance in food is carcinogenic, technological feasibility of removing it may be weighed against the health risk.	Carcinogenic food and color additives, and foods with carcinogenic residues of animal drugs,* must be banned; otherwise discretion is not prohibited	The Act takes precedence m areas of foods and related substances; for residues from pesticides there is an interagency memorandum of agreement between FDA and EPA
I(b) Federal Food, Drug, and cosmetic Act–drug provisions	Food and Drug Administration,	Drugs and substances in drugs	No	Carcinogenicity is considered as a risk of the drug; used in weighing safety against usefulness	Explicitly require the benefits and the risks (safety) of a drug must be considered in regulating.	Yes, FDA may permit carcinogenic drugs or substances in drugs to be marketed if the risks outweigh the risks	Takes precedence m the area of foods
I(c) Federal Food, Drug, and Cosmetic Act–cosmetic provisions	Food and Drug Administration,	Cosmetics and substances in cosmetics	No	Action is taken on the basis of adulteration [unsafe or injurious)	No benefits to health are presumed; risks predominate m analysis; those "cosmetics" claiming positive health benefits are treated as drugs.	Banning takes place based on the discretion allowed by the adulteration sections of the Act; public health is only criterion	Takes precedence in the area of cosmetics
2." 'Toxic Substances Control Act	Environmental Protection Agency	Substances such as foods, drugs, cosmetics, tobacco are not covered; all non-excluded substances are covered but if other Acts cover such substances those Acts take precedence	Carcinogenic and certain other substances are to receive priorty attention; a ruling must be made on carcinogens within a specified tune: but regulatory action is based on toxic@	Toxicity; cancer regarded as a priority class of toxicity	Explicitly required by the Act.	All regulatory actions are at the discretion of EPA	See Column "b"
3-6 Clean Air Act; Water Pollution Control Act; Safe Drinking Water Act; Federal Insecticide, Fungicide, and Rodenticide Act	Environmental Protection Agency	Pollutants in the respective areas of the environment	No	As environmental pollutants posing danger to pubic health; toxicity	Permitted	All regulatory actions are at the discretlion of the Commission	At the discretion of the EPA, these Acts take precedence over the Toxic Substances Control Act
7- Consumer Product Safety Act	consumer Product Safety Commission	Substances used by consumers (at home, m recreation, etc.)	No	As hazardous products, or imminent hazards	Explicitly required by the Act	All regulatory actions are at the discretion of the Commission	Not applicable to substances covered by Food and Drug Act; close relationship to Hazardous Substances ACt
8. Federal Hazardous Substances Act	Consumer Product Safety Commission	'Hazardous substances (in effect, it primarily covers household products)	No	As hazardous substances; toxicity is criterion	Not explicitly mentioned; has been interpreted as allowing it, and the Commission uses such analyses	Banning is at the discretion of the Commission: certain labeling requirements are non-discretionary	Not applicable to substances covered by Food and Drug Act
9. Occupational Health Act	Occupational Safe and Health Admm., Dept. of Labor	Hazardous substances in the workplace	No	As toxic substances; there are proposed implementing regulations dealing specifically with carcinogens	Permitted by the Act; required by the implementing regulations	Yes	Takes action when other Federal agencies have not, for workplace hazards

"There is some Judicial opinion that for animal drug residues, if regulated under general safety some risk/benefit analysis must be made, even if carcinogenicity is indicated.