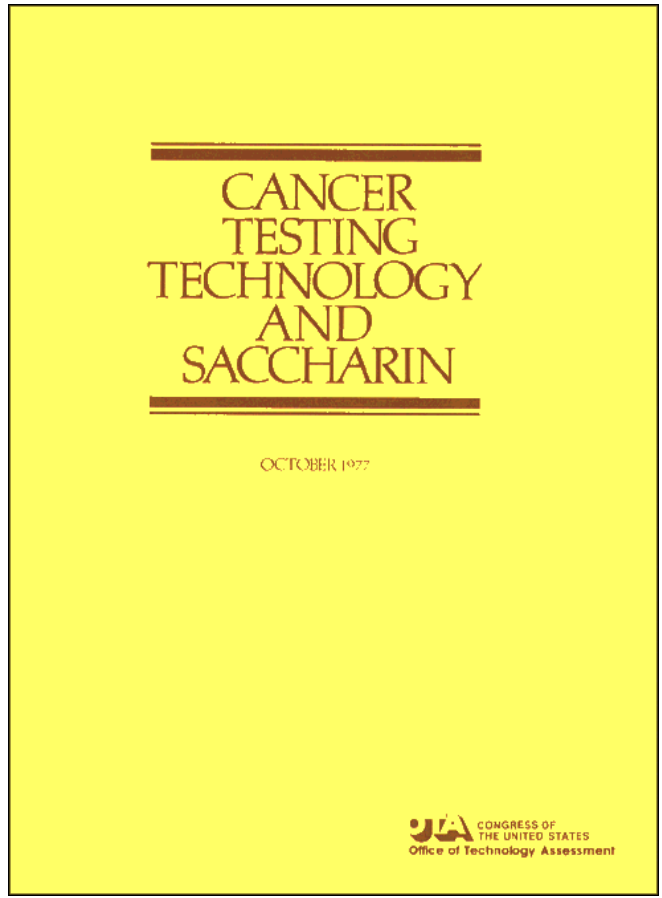
Cancer Testing Technology and Saccharin

October 1977

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Congress of the United States
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WASHINGTON, D.C. 20510

DANIEL DE SIMONE
ACTING DIRECTOR

OCT 17 1977

Committee on Human Resources
U. S. Senate
Washington, D. C. 20510

Gentlemen:

On behalf of the Board of the Office of Technology Assessment, we are pleased to forward the results of the assessment requested by your Committee.

This report provides a balanced and impartial analysis of issues related to the proposal by the Food and Drug Administration to prohibit the use of saccharin in foods. We hope that this analysis will serve as a useful resource not only for the current debate, but also for the continuing evaluation of the broader issues that it discusses.

Sincerely,

Edward M. Kennedy
Chairman

Sincerely,

Larry Winn, Jr.
Vice Chairman

Enclosure

FOREWORD

This assessment of cancer-testing technology and saccharin was requested by the Subcommittee on Health and Scientific Research of the Senate Committee on Human Resources. The study was performed as part of an evaluation by the Congress of the proposed ban on saccharin by the Food and Drug Administration.

The "Delaney Clause" of the Food, Drug, and Cosmetic Act prohibits the use of any food additive that has been shown to cause cancer when ingested by humans or animals. Since saccharin has been shown to cause cancer in laboratory animals, the FDA must ban its use.

Because saccharin is the only non-nutritive sweetener currently available to the American public, its ban has been widely criticized. The debate has prompted questions about the validity of the technology for testing whether a substance causes cancer, as well as the failure to consider the benefits as well as risks of a substance in determining whether it should be prohibited.

The Office of Technology Assessment was requested specifically:

(1) To assess the capacity of current testing methodology to predict the carcinogenic potential of chemicals consumed by humans, with special reference to the validity of extrapolating from results of animal tests to possible human effects.

(2) With respect to that assessment, to evaluate and quantify insofar as possible the potential risks that saccharin consumption might cause cancer in humans.

(3) In view of current methods for measuring health benefits of dietary behavior, to evaluate the potential health benefits, including any psychological benefits, of saccharin available to the general public and to diabetics and other groups with special medical problems.

(4) To assess the potential availability of alternative artificial sweeteners.

In the report, chapter 1 introduces the current debate over cancer-testing technology, saccharin use, and Government regulation. It also explains the scope of the assessment and presents a summary of the findings and conclusions. Chapter 2 examines testing methods and guidelines. Chapter 3 summarizes the results of animal, short-term, and human studies of the carcinogenicity of saccharin. Chapter 4 discusses possible benefits of saccharin, and Chapter 5 evaluates the potential availability of alternative sweeteners. The appendixes contain detailed data on the findings of animal and short-term tests, including the results of short-term tests commissioned by OTA for this assessment.

As an addendum to this report, the results of two epidemiological studies on the relationship between artificial sweetener consumption and human bladder tumors are summarized. Complete studies have not been made available to OTA, and these results were not available when a draft of this report was forwarded to the requesting subcommittee for hearings on June 7, 1977.

This assessment was conducted by staff of the OTA Health Program, with assistance from an advisory panel and consultants. Joyce C. McCann, a member of the advisory panel, coordinated the short-term tests for the study and was the Principal author of appendix II. The report was also reviewed *by the* OTA Health Advisory Committee, chaired by Frederick C. Robbins. This report is a synthesis and does not necessarily represent the views of any of the individuals involved in its preparation.



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1.

SUMMARY

1.

SUMMARY

INTRODUCTION

The Food, Drug, and Cosmetic Act requires that safety of food additives be established "provided that no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animals."

This statement of special treatment for food additives, emphasizing the risk of cancer, is commonly known as the "Delaney clause," named for the legislator who introduced it into what became the Food Additives Amendments of 1958. The clause prohibits the marketing of a food additive that has been shown to be carcinogenic, that is, capable of inducing cancer. It allows no balancing of the risks and benefits of the food additive. In contrast, governmental regulation of other substances has tended to require assessment of both risks and benefits.

Saccharin, a non-nutritive sweetener, has recently been identified by the Food and Drug Administration as a carcinogen. Under the "Delaney clause," its use in foods must therefore be prohibited. Saccharin is the only non-nutritive sweetener currently available to the American public, and it is widely used. The proposed ban of saccharin has prompted debate about the appropriateness of the "Delaney clause." Many people are asking whether a demonstration of carcinogenicity in animals is sufficient reason to keep a substance off the market, regardless of its benefits.

Regulatory decisions concerning substances that have been available to the public for some time (like saccharin) are especially difficult to make. Once a substance is in use, two phenomena commonly occur: (1) additional groups of people are exposed, intentionally or unintentionally, and (2) the use itself becomes perceived by some people as a benefit. Thus, once a substance is introduced into the market, additional information on risks and benefits accrues. In some instances, the information refines the evidence for or against appropriate use. In other instances, risks and benefits become known that are different from those originally examined.

The current debate about saccharin and the "Delaney clause" has also raised questions about the validity of cancer testing technology. The carcinogenicity of a substance is tested by laboratory experiments and epidemiological studies, methods susceptible to scientific protocols and statistical verification. Controlled animal experiments, which test the ability of a substance to cause cancer in animals, provide the most reliable laboratory evidence of carcinogenicity. Animal experiments are expensive and require several years to conduct. Short-term laboratory tests, which are inexpensive and usually require only a few weeks to conduct, have been developed to aid

in evaluating the potential of substances to cause cancer. These short-term tests examine the capacity of a substance to cause mutations or other genetic alterations. Epidemiological studies examine whether exposure to a particular substance causes cancer in humans. Positive epidemiological results are the most convincing of all evidence, but negative results are less certain. Epidemiological studies are very difficult to conduct because they require data from a large number of people, sometimes over a long period of time.

Complicating an evaluation of the risks of cancer is the fact that testing technology is rapidly changing, and standards are constantly being revised. Advances are being made in the amount and quality of data available and in methodologies used to gather that information. In many cases, alterations, in standards occur between the time a test is begun and completed. For example, guidelines for animal studies of carcinogenicity presently call for experiments that require 2 to 3 years to conduct, and changes in the guidelines are being considered. Thus, data from recently initiated and ongoing experiments may not meet testing standards when the experiments are completed. Similarly, short-term tests are in varying stages of development, and they have not been fully validated.

Testing saccharin for carcinogenicity reflects the advancing nature of cancer testing technology. Data about saccharin are available from a number of laboratory experiments and epidemiological studies. However, only the most recently completed studies approach current standards for testing. Data about the carcinogenicity of saccharin from short-term tests are still limited.

Although the “Delaney clause” does not allow the weighing of risks and benefits of a food additive such as saccharin, the current debate has raised the question of benefits nevertheless. Possible benefits of saccharin involve cultural and psychological considerations. Various hypotheses have been advanced about the effect of saccharin’s sweet taste, Some of these hypotheses predict beneficial effects; others predict detrimental effects.

Except when the chemical properties of a specific non-nutritive sweetener are at issue, the potential benefits of these sweeteners lie in their possible contribution to the reduced consumption of sugar. On the other hand, enumerating hypothetical benefits of saccharin does not eliminate the possibility that its use promotes practices that constitute health risks. Conceivably, continuing to provide the sweet taste may lead to greater, not lower, consumption of sugar.

The benefits of saccharin are more difficult to test than the risks. The kinds of questions asked about risks have never arisen for benefits. Specific benefits of saccharin have neither been studied in isolation from other sweeteners nor examined as carefully as the risks from carcinogenic substances. Because of the general lack of relevant literature, the kind of detailed analysis applied to the assessment of risks is not possible for the assessment of benefits. Thus, the analysis of risks is narrower but more thorough than the analysis of benefits,

Because the possible benefits of saccharin are primarily related to its use as a sweetener, the analysis of its benefits also applies to other non-nutritive sweeteners. Therefore, the availability of other artificial sweeteners does not affect the analysis of the benefits of saccharin.

FINDINGS AND CONCLUSIONS

1. Because carcinogenicity cannot be tested directly in humans, indirect methods are necessary. Current methods can predict that a particular substance is likely to cause cancer in humans. The technology for making quantitative extrapolations from animal experiments to human risk is progressing and has been verified in the few cases for which data are available. But this technology does not currently permit reliable estimates of the numbers or locations of cancers that might occur in humans.
2. Three methods are employed:
   A. Animal tests are accepted as valid, reliable predictors that a substance will produce cancer in humans.
   B. Short-term tests provide presumptive evidence of a substance's risk to humans. A positive result in any of the short-term tests warrants suspicion and calls for tests in animals. A negative test indicates that carcinogenicity is less likely, but does not rule it out.
   C. Human epidemiological studies attempt to answer two questions: (1) Is there a positive association between a particular exposure and the occurrence of cancer in humans, and (2) If there is, is it causal? Positive results can clearly show that human populations are at risk. Negative results are more difficult to interpret, but they do not eliminate the possibility of risk.
3. Statutory authorities for regulating carcinogenic substances to which humans may be exposed are not consistent. Unlike the Food and Drug Administration's (FDA) authority under the "Delaney clause, " other agencies regulate carcinogenic substances under general authorities relating to toxic substances. Although attempts are made through implementing regulations to apply consistent standards, such efforts are voluntary, often discretionary, and the legislation sometimes precludes consistency:
   A. The "Delaney clause" reflects the present state of technology in which laboratory methods can predict that a specific substance is likely to cause cancer in humans, but cannot reliably quantify this potential effect.
   B. Other legislative authorities that allow risks to be balanced against other factors in decisions to regulate carcinogenic substances implicitly permit quantitative extrapolations to be made from animal testing to humans.
4. The National Cancer Institute (NCI) guidelines do not provide criteria for classifying an agent as a potential risk to humans. Although they provide criteria for judging whether specific experiments have been properly conducted, they are not mandatory for all Federal agencies.
5. Laboratory evidence demonstrates that saccharin is a carcinogen.
   A. Prolonged ingestion of saccharin at high levels caused a significant increase in the incidence of bladder cancer in rats in three independent experiments. Earlier experiments were not sensitive enough to detect this carcinogenic effect.

B. This evidence leads to the conclusion that saccharin is a potential cause of cancer in humans.

C. There are no reliable quantitative estimates of the risk of saccharin to humans.

6. Epidemiological studies of human experience have not been sensitive enough to determine whether or not saccharin is a carcinogen when ingested.

7. As part of this study, a battery of 12 short-term tests was conducted on pure saccharin, impure saccharin, and impurities in commercial saccharin.

A. Pure saccharin was mutagenic in 3 of 10 completed tests.

B. Impurities were mutagenic in the one system in which they have been tested. These impurities could possibly account for the observed carcinogenicity of saccharin in animals, but they are present in commercial saccharin.

8. Because of its widespread use, the availability of a non-nutritive sweetener is of perceived psychological benefit to many people.

9. Claimed benefits of non-nutritive sweeteners were identified for five groups of users:

A. Diabetics. A non-nutritive sweetener may help in avoiding consumption of sugar and in complying with prescribed dietary therapy.

B. Persons with long-term, low calorie requirements. Substitution of a non-nutritive sweetener for sugar by people on restricted diets could permit them to consume greater amounts of foods containing vitamins and minerals without reducing the consumption of sweets and without increasing total calories.

C. The obese and those concerned with avoiding obesity. A non-nutritive sweetener may help in avoiding excessive consumption of sugar.

D. Persons particularly susceptible to dental caries. Non-nutritive sweeteners may aid in reducing exposure to sugared foods, which are highly cariogenic.

E. Persons who must take certain drugs. A non-nutritive sweetener may have benefit in improving the palatability of certain essential drugs, including fluoridated dentifrices and other fluoridated oral health preparations.

10. Whether or not using a non-nutritive sweetener leads to measurable health benefits has never been tested. The Food and Drug Administration has proposed limited use of saccharin as a single-ingredient, over-the-counter drug and as a component of certain drug products, but these uses will be allowed only if such health benefits are proven.

11. The availability of alternative non-nutritive sweeteners is uncertain at this time. The Food and Drug Administration began new hearings on cyclamate

on July 13, 1977. Petitions for four other non-nutritive sweeteners have been filed with the FDA. No predictions on availability can be made on the basis of these petitions.

SCOPE OF THE STUDY

Questions of benefits and risks were the major issues behind the congressional request for this study. The Office of Technology Assessment was asked to undertake four specific tasks:

(1) To assess the capacity of current testing methodology to predict the carcinogenic potential of chemicals consumed by humans, with special reference to the validity of extrapolating from results of animal tests to possible human effects.

(2) With respect to that assessment, to evaluate and quantify insofar as possible the potential risks that saccharin consumption might cause cancer in humans.

(3) In view of current methods for measuring health benefits of dietary behavior, to evaluate the potential health benefits, including any psychological benefits, of saccharin availability to the general public and to diabetics and other groups with special medical problems.

(4) To assess the potential availability of alternative artificial sweeteners.

The request asked the OTA to evaluate saccharin for only one risk, carcinogenicity '(which is the only one known or suspected), but to identify all potential health benefits of its availability. This study is, therefore, not a comprehensive risk/benefit analysis of saccharin. Such an analysis would attempt to weigh all relevant risks against all relevant benefits. The Office of Technology Assessment was asked not only to examine the evidence for the one specific risk and to quantify it, but also to examine critically the testing methods used to generate that evidence.

In addition to the four tasks listed in the request, the OTA commissioned a battery of 12 short-term tests to be conducted on saccharin as part of this study. This study marked the first time that saccharin had been tested by most of these methods. The purpose of conducting these tests was to demonstrate to the Congress the nature of the tests, the speed with which they can be conducted, and their usefulness in regulatory decisions. It also seemed possible that conducting a full battery of short-term tests might help to clarify some of the uncertainties regarding the carcinogenicity of saccharin.

2. CANCER TESTING TECHNOLOGY

2.

CANCER TESTING TECHNOLOGY

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.

3.

SACCHARIN RISKS

The experiment that determined cancer incidence in both generations found more tumors in the second. The numbers of tumors between first and second generations were different, but the probability that the difference would occur by chance alone was about 50 percent. This probability gives little evidence against equality. However, the apparent difference in incidence between the two generations raises a suspicion that *in utero* and breast-feeding exposure may be important factors in saccharin's causing bladder cancer. Although the direction of the difference is consistent with the hypothesis of greater risk to the second generation, the differences are not statistically significant at the 5-percent level.

In one of the two-generation experiments (165,177), an increased number of uterine cancers was associated with ingestion of 5-percent saccharin. This correlation has not been found in the other experiments, and the increase may have been a random event.

To conclude, the two-generation experiments showed that saccharin caused an increase in bladder cancer in second generation animals, especially among males. In the one experiment in which the first generation was also examined, the increase fell just short of the standard test of significance. No cancer of any other site has been convincingly associated with saccharin.

Other Animal Experiments. Publications from one laboratory report that saccharin *promotes* the growth of bladder tumors that were *initiated* by previous exposure to another chemical (69,70). This "cocarcinogenic" activity of saccharin is of potential importance because humans are exposed to many chemicals in addition to saccharin.

Implantation experiments showed that saccharin also causes cancer in mice (5,25). In those experiments, pellets of cholesterol containing saccharin were implanted in the bladders of mice. About 50 percent of animals exposed to saccharin in this way developed bladder cancers, compared to 13 percent in animals exposed to cholesterol only.

The particular combination of chemicals in the cocarcinogenesis experiment and the route of administration in the implantation experiments do not mimic human exposure to saccharin. Taken by themselves, results from these experiments would be considered warnings that saccharin may be a carcinogen. Taken in conjunction with the two-generation experiments, they support the conclusion that saccharin is a carcinogen in rats, and they show that it causes cancer in mice.

No association between saccharin ingestion and cancer was found in experiments using hamsters and monkeys. The hamster experiments were one-generation tests, and just as in the one-generation rat experiments, no association was found (4). Experiments showed that ingestion of saccharin at up to 500 mg/kg body weight for 7 years did not cause illness in monkeys, did not alter their metabolism, and did not cause cancer in the few animals that have died and been necropsied (35). But the monkey experiments, unlike the positive rat experiments, did not involve lifetime exposure to saccharin, used forced feeding procedures, and examined a very small number of animals.

A general problem occurs when discussing experiments on dangerous substances, What conclusions are to be drawn when some experiments show the substance caused cancer in animals and other experiments do not? In the particular case of saccharin, all two-generation experiments have been positive. A number of other

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experiments have led some to conclude that saccharin is not a carcinogen. The Office of Technology Assessment reviewed those experiments and found none comparable in design to the three positive experiments. Furthermore, some others were too insensitive to have detected the carcinogenic effect of saccharin. This statement is no indictment of those experiments; cancer testing is rapidly evolving, and many older experiments are not now considered to be satisfactory. The positive two-generation studies come the closest of all that have been conducted to meeting the current testing standards.

The information gathered in this review of animal studies, especially the uniformly positive two-generation experiments, leads to the conclusion that saccharin should be considered a carcinogen for animals.

EXTRAPOLATION TO HUMANS

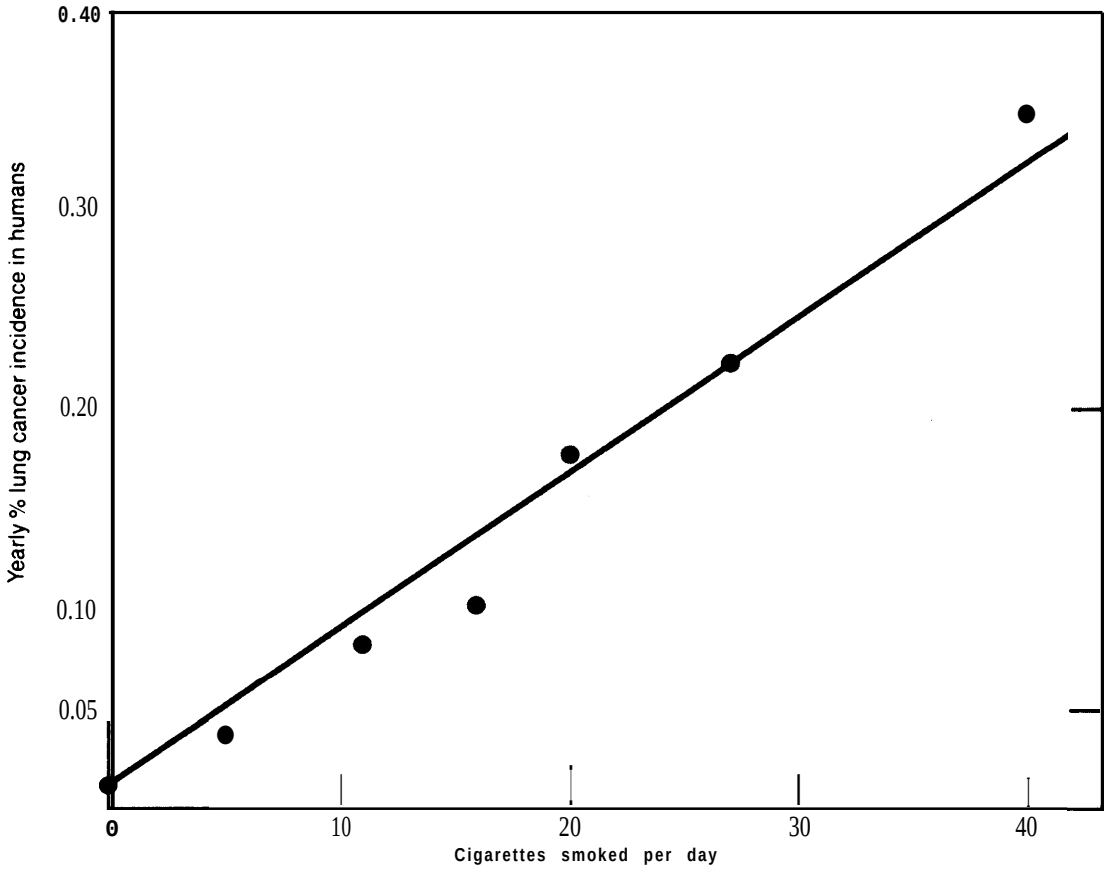
As explained above, standard procedure in animal experiments is to feed substances at the "maximum tolerated dose," which for saccharin is 5 percent of the diet. According to normal dose-response relationships, if cancer is produced in animals at high dose levels, it will also be produced at low dose levels, but in fewer animals.

Substantial evidence for this dose-response relationship exists for animals, but some of the most convincing evidence is derived from human experience. An example of such a dose-response in humans is incidence of cancer resulting from cigarette smoking. As shown in figure 1, the incidence of lung cancer in humans is greater for people who smoke a lot than for those who smoke only a little. At the lowest exposure levels for which there are data, about five cigarettes a day, only a very small fraction of people get lung cancer. But because very large groups of people were examined, these few cases could be detected.

To test these low doses of cigarettes in rats, one would have to design an experiment with thousands of rats in order to be able to detect the same incidence of cancer that occurred in people, a study that would be neither economically nor experimentally feasible. So that a smaller number of animals can be used, only higher doses are tested, a procedure resulting in a higher percentage of animals that develop cancer. In fact, because it is practical to use only about 100 or so animals in a cancer test, very high doses are chosen in an effort to cause cancer in at least 10 percent of the animals. (Cancer in 10 percent of people would clearly be a disaster.) All the evidence that has been accumulated so far suggests that this procedure is reasonable. Therefore, an amount of saccharin equivalent to 800 diet drinks a day was not an unreasonable dose to give to rats; if saccharin causes cancer in rats at such high doses, it is also very likely to cause it at lower doses.

Saccharin was found to be among the weakest carcinogens ever detected in rats, as illustrated in figure 2. The doses of a number of different carcinogens which cause cancer in half of the animals (rats or mice) treated are compared in this figure. There is over a million fold range of doses. In other words, chemical carcinogens are very different in their carcinogenic potencies. For example, aflatoxin (AF-B1), a substance produced by certain fungi and found in moldy peanuts and certain grains, causes cancer in 50 percent of rats at a dose of more than one million times less than the dose of another carcinogen, trichloroethylene (TCE), a chemical that, until recently, was used to extract caffeine in the manufacture of instant coffee. It has been classified as a

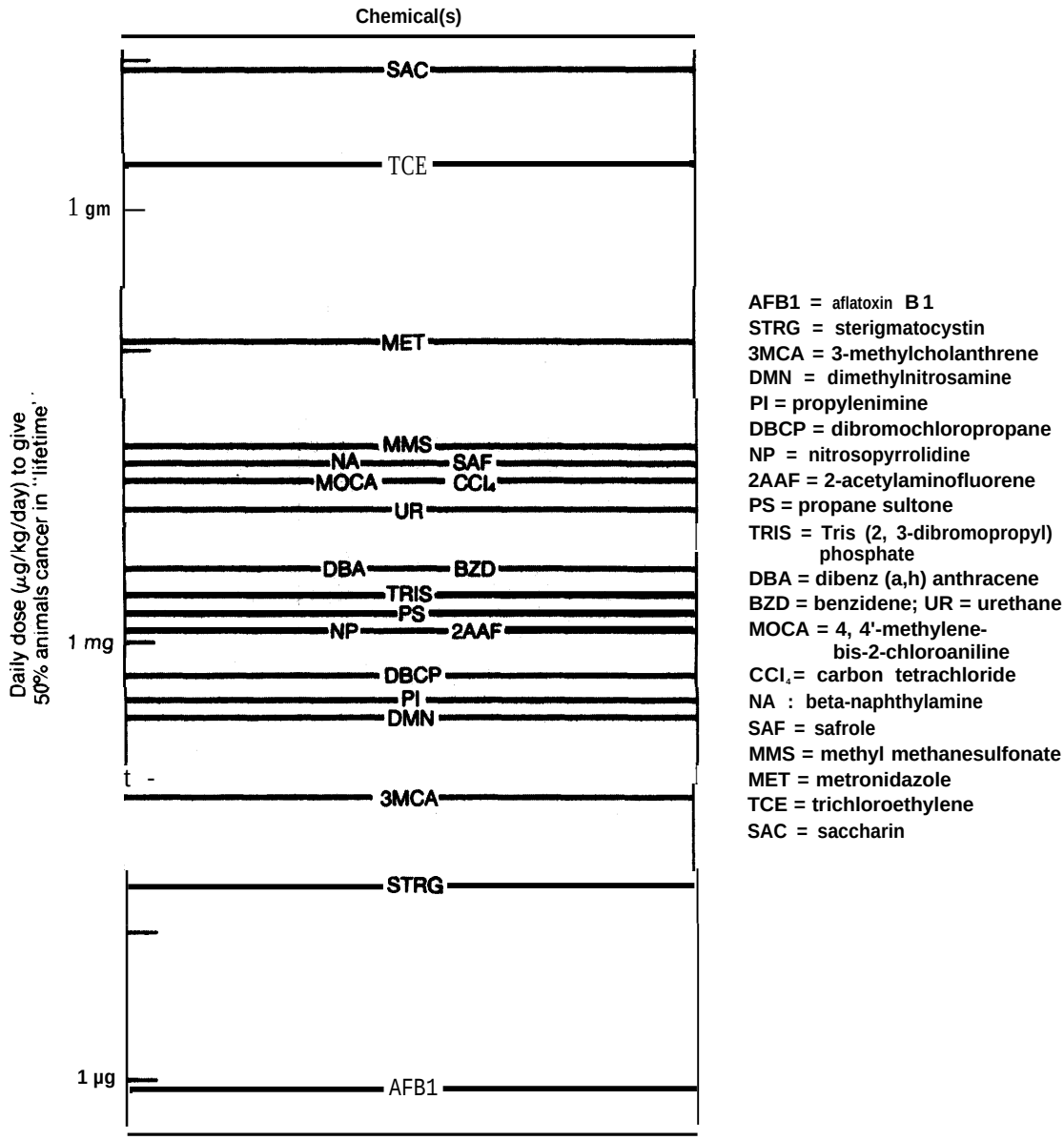
Figure 1-Incidence of Lung Cancer in Humans



food additive because small amounts are left as residue in the coffee, and it was recently banned by the FDA under the "Delaney clause." If this millionfold range of carcinogenic potency in rats has any correspondence in people, it is clear that a tremendously different degree of human risk results from eating a peanut butter sandwich with a trace of aflatoxin in it as compared to drinking a cup of decaffeinated coffee containing the same amount of TCE. Where does saccharin fall on this millionfold scale? It actually extends the scale in the weak direction—it is slightly weaker than TCE.

Some evidence suggests that the potency of carcinogens in rodents may be a rough indicator of their potency in people. The evidence is admittedly fragmentary and subject to considerable uncertainty. However, to compare the strength of carcinogens in animals and people requires data on people. Because controlled experiments cannot be conducted on people, available information is limited to the few studies in which epidemiologists have been able to determine that a substance caused human cancer. This information is very difficult to obtain; estimates of dose levels of substances that have caused human cancer have been possible for only six substances. In most of these cases, a rough correlation exists between potency in rodents and in people (107). Given the enormous range of biological potencies of carcinogens possible, this rough correlation is quite important. If this same correlation holds for saccharin, then it seems reasonable to predict from the rat studies not just that saccharin

Figure 2-Carcinogenic Potency of Chemicals in Rats and Mice



Data calculated from experiments in the literature by Sawyer, Hooper, Friedman and Ames (unpublished). (Though it is clear that there is a millionfold range in carcinogenic potency, the exact location of individual points may change slightly as the calculations are refined.)

is likely to cause cancer in people, but also that it is likely to be a relatively weak carcinogen in people. It must be kept in mind that while some extrapolations have been validated, others are more complex. In the case of diethylstilbestrol (DES), for example, the chemical caused cancer in the liver in animals, but in the female reproductive organs in humans. Nevertheless, although the animal experiments did not predict the organ site, they did show that the chemical was a risk.

Is saccharin likely to be so weak a carcinogen in people that it should cause no concern? Probably not, for several reasons. *First,* in assessing potential human risk, one must take into account not only the potency of the carcinogen, but also the number of people who are likely to be exposed to it and the amount of the carcinogen to which they are likely to be exposed. For example, if one group of people is exposed to a weak carcinogen and another group to a more potent carcinogen, the actual number of cases of cancer can be larger in the group exposed to the weak carcinogen if either (a) the number of people in the group exposed to the weak carcinogen is much greater than in the group exposed to the more potent carcinogen; or (b) the number of people exposed in the two groups is the same, but the group exposed to the weak carcinogen is exposed to much higher doses. In the case of saccharin, because so many people are consuming saccharin in substantial amounts, risk estimates range up to several thousand expected new cases of cancer each year. This number of cases is substantial and, in fact, constitutes a large fraction of the current total incidence of bladder cancer, *Second,* the degree of uncertainty in these extrapolations is sizable and maybe wrong by a factor of 10 or possibly even 100, a factor that would raise the potential risk to clearly unacceptable levels. *Third,* saccharin is not the only carcinogen to which people are exposed, and the total body burden of carcinogens is of greatest concern. Any increment, even a relatively small one, to an already substantial burden of carcinogens must be taken very seriously.

SHORT-TERM TESTS

A number of sensitive short-term tests have been developed for use in predicting whether substances are likely to cause cancer. It seemed possible that conducting a rapid and coordinated battery of short-term tests on saccharin might clarify some of the uncertainties of the animal cancer tests, Government and industry officials are discussing how short-term test results should affect regulatory decisions concerning substances to which humans are exposed. The saccharin test battery, which took about 3 months to complete, illustrates one way short-term tests can be applied to a particular regulatory problem.

Twelve short-term tests on saccharin were commissioned by the OTA; 10 have been completed. The battery of tests was designed to determine, as definitively as possible within the time limits of this study, whether highly purified saccharin* is mutagenic or interacts with DNA. The test battery included many of the most sensitive short-term tests available. Criteria for including a test in the battery were: (1) sensitivity and validity for detecting carcinogens; (2) complementarily with the other tests and with test literature on saccharin; and (3) ability to be completed within the time constraints. Saccharin had been tested previously in only two** of these twelve short-term tests. The experiments reported here were conducted by the developers of the tests or recognized experts, who generously donated their time to this study. The

*All tests were conducted using the same sample of saccharin that was used in the most recent Canadian carcinogencity tests in rats, This material, even though highly purified, still contains very small amounts (about 20 ppm) of impurities and is referred to as “impure saccharin. ” For this reason, all participating laboratories also received a sample of saccharin that had been specially purified to remove essentially all traces of impurities and is referred to as “pure saccharin. ”

**Several sex-linked recessive lethal tests in *Drosophila* have been published, with somewhat conflicting and uncertain results. Results obtained by Stolz, et al. (162) using the *Salmonella/Ames* test were independently confirmed for the OTA test battery.

results of these tests are discussed in appendix II, and a list of principal investigators is presented in table 38 of appendix II.

Results from three tests—sister chromatid exchange, mouse lymphoma, and" chromosome aberration—were positive. Highly purified samples of saccharin were weakly active in these tests, and the results are clearly suggestive that saccharin itself has mutagenic properties. The results should be regarded with some caution, however. The responses were very weak in the three tests, even at the high dose levels tested. And the value of the sister chromatid exchange, mouse lymphoma, and chromosome aberration tests in predicting carcinogenicity has not yet been firmly established. However, validation of the tests has begun by testing a number of carcinogens and mutagens and a few noncarcinogens, with promising results.

The seven other completed tests represent the gamut of short-term tests. Their results were negative, a fact that complements the generally negative results already reported in the literature (85). However, their negative results neither invalidate nor cast suspicion on the positive results. Short-term tests differ in their ability to detect dangerous substances, and it is conceivable that a substance would be positive in only some of the tests.

The ten experiments described above tested highly purified saccharin, but all the saccharin used in animal cancer testing contained some levels of impurities. Some reviewers of the saccharin literature have suggested that impurities might be carcinogens. Therefore, two laboratories have used the *Salmonzella/Ames* test to test impurities in the saccharin used in the 1977 Canadian Study of rats (67).

A sample of the saccharin used in the 1977 Canadian Study was chemically fractionated and found to contain about 20 parts per million (ppm) of impurities. Approximately 12 different impurities were present, but they have not been specifically identified or separated. Mutagenic tests on the residue containing the 12 impurities have yielded positive results in two separate laboratories (162,171). Whether the mutagenic impurities account for the carcinogenic activity of the saccharin in animals is unclear. However, results of the positive short-term tests on highly purified saccharin are consistent with the conclusion that saccharin itself is a carcinogen.

EPIDEMIOLOGICAL STUDIES

Three kinds of epidemiological evidence have been examined.

1. In Great Britain, where a large increase in saccharin consumption occurred during World War II, time trends in per capita consumption of saccharin and of cigarettes have been compared with trends in death rates from cancer of the bladder (12). Increased cigarette smoking can account for the steady increase in bladder cancer mortality among males born after 1870. However, no inflection of the curve of mortality occurred following the sharp increase in saccharin consumption during World War II. These data, while revealing no association between saccharin and cancer, cover only two or three decades of increased saccharin use. Furthermore, it is not possible in such general statistical reviews to sort out specific effects of a chemical that only an unspecified proportion of the general public might use.

2. In series of patients with cancer of the bladder (so-called "cases") and unaffected persons (so-called "controls"), some information has been obtained on their use of saccharin or beverages containing saccharin and their medical histories with respect to diabetes. In none of several studies of this type—in the United States, Canada, and Britain (11,80, 109)—were statistically significant differences found between cases and controls. Data on saccharin use were in many instances incomplete and were not available for those patients who had died of bladder cancer, making comparisons difficult.
3. Series of patients with diabetes-a group that was shown to have heavier saccharin use than the general population (1 1)—have been followed over many years to determine the cause of their deaths. The observed numbers of deaths from cancer have been compared with the numbers expected at the same time on the basis of cancer mortality rates in the general population of the same age and sex. This methodology permits assessment not only of bladder cancer risk, but of risk of all cancers that are likely to cause death. In the two studies conducted to date—one in the United States (79) and one in Britain (13)—no significant excess of bladder cancer mortality was observed. In both studies a small excess of cancer of the pancreas was seen. Significant deficits of certain cancers (mouth, lung and esophagus) were observed, probably related to low rates of use of tobacco and/or alcoholic beverages by the diabetics. Much of the experience of these diabetics was of too short a duration to allow full evaluation of the cancer rates. In most cases, which individuals took saccharin and which did not was not actually known.

Although the epidemiological evidence fails to document an association between saccharin use and bladder cancer in humans, this finding must be interpreted with caution. Adequate evidence is simply not available. Epidemiological studies can provide very strong evidence of the causal relationship between environment and disease—particularly when they are positive. Most of the major known carcinogens for man-cigarette smoke, ionizing radiation, asbestos, sunlight, beta-naphthalamine, and nickel—were identified by epidemiological studies in man before they were identified as carcinogens in experimental animals. However, negative epidemiological studies are more difficult to interpret. Humans are usually exposed to carcinogens in doses far smaller than are used in animal experiments. The effects are consequently less frequent, and the number of people whose experience needs to be judged to detect the cancer effect is much greater.

Lack of certainty as to the validity of the data on exposure in case-control studies is a further reason for reservations in interpreting negative findings. Only a portion of the population uses saccharin, and the use of statistics from the total population may dilute any association that exists among the users—perhaps to the point of making it unobservable. Such a dilution may be responsible for lack of correlation between bladder cancer and the sharp increase in British consumption of saccharin.

In the followup studies of diabetics, the same problems exist. The problem of small numbers is particularly evident in the British study (13), where there were only four deaths from bladder cancer (5.8 expected). Furthermore, in the British cohort of diabetics, information from a different sample indicated that by the end of the study period, only 23 percent of the survivors would have taken saccharin daily for 10 years, and only 10 percent for 25 years or more (11). Numbers are larger in the

American cohort, but no information exists on the use of saccharin by the American patients (80).

The period between exposure and appearance of cancer is a problem common to all these types of studies. This latent period introduces two kinds of difficulties—it again "dilutes" study groups so that they contain fewer individuals who are truly at risk than appears to be the case, and allowance cannot easily be made for the latent period because its duration is unknown. The latent period is almost certainly a matter of years, rather than months, but experience with known carcinogens in humans ranges from 2 to 40 or more years. In the context of saccharin, the latent period may be a particular problem in the studies of diabetes. A large proportion of diabetics have onset of the disease late in life and may not survive the latent period, dying of other causes before developing bladder cancer.

In all three types of epidemiological studies, making the association of primary interest is complicated by extraneous but important variables such as occupation and cigarette smoking. Such variables may not only introduce a false association, but may also hide an association that does exist.

The conditions of the two-generation animal tests are not likely to have been frequently duplicated in the epidemiological studies. The implications for humans of the apparent sex difference in susceptibility of rats are also unclear. Epidemiological data on human bladder cancer indicate that the sex difference (an excess in males compared to females) is explained by differences in exposure to known occupational carcinogens and cigarette smoke, variables that could hardly have been relevant in the animal test.

UNRESOLVED QUESTIONS

Several questions concerning the carcinogenic effect of saccharin remain unanswered:

1. What is (are) the carcinogenic agent(s) in commercial saccharin?
   No conclusions can be drawn as to whether it is the chemical saccharin itself, one or more of the impurities, or combinations of both.
2. What is the significance of the increased sensitivity of the male rat bladder as compared to that of the female?
   Differences in bladder cancer incidence between human males and females can be explained by exposure factors. The factors contributing to the difference in the test rats are not known.
3. Is the carcinogenic effect limited to the bladder?
   The induction of tumors in a specific location in test animals does not necessarily predict that the carcinogenic effect in humans will occur in the same site. But it has been shown that a substance that causes cancer in test animals is likely to cause cancer in humans.
4. Does in utero and breast-feeding exposure lead to greater risks of cancer from saccharin?
   In the one experiment which examined both first-and second-generation rats, the cancer incidence of the first (F_o) generation was just short of being statistically significant ($p = 0.075$), while the cancer incidence of the second

(F_1) generation was significant (p = 0.003). The probability of the difference between F_0 and F_1 cancer incidence being significant, however, is only about 50 percent. It is therefore not clear whether additional testing would show that saccharin causes cancer only in the second generation, or whether the first generation is also susceptible. If the second generation is more sensitive, two possibilities explain the differences between F_0 and F_1: 1) the additional length of exposure for the second generation in the gestational and suckling periods; or 2) the F_1 generation may be susceptible due to the special circumstances of in *utero* and/or breast-feeding exposure.

5. What are the mechanisms by which saccharin causes cancer?
Although there have been great advances in understanding the mechanisms whereby some chemicals induce cancer, nothing is known of the mechanisms by which saccharin may cause cancer. Research elucidating the mechanism could enhance future assessments of the human risk of saccharin and could conceivably shed light on the problem of bladder cancer in general. Many chemical carcinogens are converted in the body to highly reactive metabolizes that bind to key components of the cell to initiate cancer. Humans may form such reactive metabolizes to a greater or lesser degree than test animals, thereby influencing the relative susceptibility to the carcinogen. Although saccharin is excreted largely unchanged, a minor metabolize could become important at high doses.

4.

SACCHARIN BENEFITS

4.

SACCHARIN BENEFITS

POTENTIAL BENEFITS

As part of this study, the OTA was asked "to evaluate the potential health benefits, including psychological benefits, of saccharin availability to the general public and to diabetics and other groups with special medical problems."

Many Americans use saccharin, like to use it, and want to continue using it. Its use is widely perceived to result in health benefits, but the claim that saccharin use is essential for the continued health of any segment of the population has not been tested,

Except for the formulation of some drug products, the uses of saccharin are identical to those of the entire class of non-nutritive sweeteners. In contrast to the assessment of the specific risk of cancer from saccharin ingestion, this inquiry stops at the point of identifying *potential* benefits. Although conclusions can be reached on the existence of the risk of cancer, if not on its magnitude, no conclusions can be reached on either the existence or magnitude of benefits.

No scientific data were found to prove or to disprove that use of a non-nutritive sweetener leads to any health benefits. As it is noncaloric and nonfermentable, an artificial sweetener would not promote the health problems associated with excessive calories and fermentable acid formation characteristic of sugar. But the benefits of using non-nutritive sweeteners rest primarily with the avoidance of sugar and not in the use of the sweetener itself.

The proclivity to ingest sweet substances is an innate biological characteristic of newborn animals and humans. It appears to be alterable, and such alterations induced by cultural practices may increase or decrease this proclivity. The preference for a particular level of sweetness is an individual matter, but beyond a certain level this preference becomes an aversion. Too much sweetness is avoided as much as lower levels of sweetness are desired. At an appropriate concentration in foods, sweetness might be used to increase adherence to certain dietary practices that could result in improvement or maintenance of health.

The benefits of a non-nutritive sweetener such as saccharin are unusually difficult to assess, in large part because it has become integral to the diet of many Americans and, in part, because of the absence of controlled studies on its effectiveness in any particular situation. Indeed, benefits may be identified only through the response to the removal of non-nutritive sweeteners from the food supply.

Potentially profound social consequences and the possibility of emotional, unpredictable, and even irrational responses may follow any attempt to change well-established dietary practices. Three contemporary factors may increase the emotional

responses to the removal of non-nutritive sweeteners: (1) its relationship to weight control in a society preoccupied with body weight; (2) the innate biological desire for sweet foods, supported by a culture accustomed to sweets; and (3) the current heightened concern with civil liberties and personal freedom that has produced strong pressures for permitting people to choose even potentially harmful personal practices, such as smoking.

Potential benefits were identified by answering the question: Who would be at risk if non-nutritive sweeteners were not available? Potential benefits derive from the use of saccharin in diets designed to avoid sugar and maintain sweetness for the following groups of people:

1. diabetics,
2. those requiring long-term, low-calorie diets,
3. the obese and those concerned with avoiding obesity, and
4. those particularly susceptible to dental caries.

Additionally, because of its sweetness, lack of bulk, and chemical inertness, saccharin has been included in some drugs to disguise their unpalatable taste. Thus, saccharin is also of potential benefit to:

5. those who must take certain drugs.

No scientific evidence is available to show that saccharin is indispensable for the diets of any of the first four groups of users; For those people, sugar is a problem, and saccharin provides sweetness. The use of sugar can be reduced without any substitute for its sweetness. However, because sweetness is a desired quantity in our society, retention of a sweetening agent may be a psychological benefit.

Another view suggests that the *removal* of saccharin is a potential benefit. According to this view, the ready availability of saccharin has fostered a greater craving for sweets than would have occurred in its absence, and its removal might reduce this craving and decrease the likelihood of other persons developing it. If so, long-term benefits in decreased consumption of nutritive and non-nutritive sweeteners would outweigh short-term risks and inconveniences.

Diabetics. Weight control and avoidance of sugar are important components in the treatment of adult-onset diabetes. No evidence, other than anecdotal testimonials, is available to support the contention that use of non-nutritive sweeteners leads to these desired consequences.

Nonetheless, access to artificial sweetness might be considered an important factor in complying with prescribed diet therapy. In fact, according to the Interim Regulations on Saccharin (21 CFR 121.4001), saccharin at present is supposedly used only for “a valid special dietary purpose . . . in accord with current special dietary food regulations and policies or if the use or intended use is for an authorized technological purpose other than calorie reduction. ”

Juvenile-onset diabetes presents many of the same dietary problems, which are, if anything, complicated by peer pressure. Many of the social activities of a young diabetic’s peer group center around the consumption of sweet snacks and beverages. The availability of foods sweetened with saccharin enables diabetics to participate in these activities as equals. If such products became unavailable, young diabetics would be able to participate only at some risk to their health, or elect not to participate and perhaps suffer from a feeling of being excluded or different. Saccharin-sweetened

snacks may be an important psychological benefit for young diabetics. The criteria for determining if saccharin is useful for weight control and for diabetes management would be difficult to define in areas in which cultural pressures are important, such as in the management of the juvenile-onset diabetic.

Persons with low-calorie requirements. Persons on long-term, low-calorie diets must exercise careful management. If a food, such as sugar, lacking in vitamins and minerals (micronutrients) constitutes a sizable percentage of total calorie intake, the remaining food may not contain adequate amounts of essential nutrients. Substitution of a non-nutritive sweetener for sugar could permit the consumption of greater amounts of foods containing micronutrients.

Saccharin is not essential for management of such dietary problems. Other alternatives are available, such as taking vitamin and mineral supplements or eating foods other than sugar.

The obese and those concerned with avoiding obesity. A strong case has not been made for the effectiveness of any single aid in the treatment or prevention of obesity. One can argue either that the availability of non-nutritive sweeteners has not prevented the occurrence of obesity or that the prevalence and severity of obesity would have been greater in their absence. The impact on human behavior of removing non-nutritive sweeteners from the food supply is not known. If persons now using them shift to sugar, their calorie intake may increase with predictable consequences; if they do not shift to sugar, these consequences will not ensue. Neither animal nor human data permit conclusions as to which of these consequences is most likely to occur, An increase in weight, however, might have more than simple cosmetic consequences. If large, it would lead’ to increased risks of hypertension, diabetes, hyperlipidemia, and associated cardiovascular diseases.

Persons might shift from a non-nutritive sweetener to sugar in order to satisfy their desire for sweet food and drink, a satisfaction to which they have become accustomed. Also, adolescents might consume soft drinks containing sugar because of peer pressure.

Persons particularly susceptible to dental caries. The essential elements in dental caries are the presence of acid-producing bacteria, fermentable sugars, and susceptible teeth. Controlling or preferably eliminating one or more of these factors would minimize the incidence of caries. Simple saccharides, especially sucrose, are particularly cariogenic (cause caries) when added as a sweetener in snacks and beverages that come in frequent contact with the tooth surface.

Almost everyone is susceptible to a moderate amount of dental caries. However, some people with dry mouth conditions and some adolescents succumb to a rampant type of dental decay when they use sugar. This dental condition is defined as “a suddenly appearing, widespread, rapidly burrowing type of caries resulting in early involvement of the pulp and affecting those teeth or dental surfaces usually regarded as immune to ordinary decay” (19). Rampant dental caries are found in about 10 percent of the population, particularly in the New England and Northwest sections of the country.

Non-nutritive sweeteners, if used to substitute for all the sugar in the diet, probably would help patients control rampant caries. But with the exception of this one group of people, total substitution of all sugar in all foods and beverages would not be feasible.

Partial substitution of sugar by non-nutritive sweeteners may have some effect on dental caries, but the magnitude of such an effect is difficult to predict. As long as some sugar remains in the diet and if foods containing sugar are eaten frequently during the day, the potential for cariogenic, acidogenic bacterial plaques to form on the tooth surface is present and the effect possible.

Because non-nutritive sweeteners cannot be fermented by the oral flora, they are not cariogenic. However, adequate data on the correlation between use of non-nutritive sweeteners and dental caries do not exist. Neither positive nor negative results have been reported with respect to the role of non-nutritive sweeteners in preventing caries.

Persons who must take certain drugs. Many drug products are sweetened artificially to increase palatability without increasing bulk. Palatability may be relevant in patient compliance with prescribed drug therapy. Because of its pleasing taste, a sweet additive can make medicinal more acceptable. In addition, saccharin is the best sweetener known in terms of chemical inertness, which is important for maintaining the stability of a drug product.

If non-nutritive sweeteners were unavailable, which drugs would be affected? If some drugs cannot be formulated without them, which of these drugs are medically necessary? If drugs can be formulated only with considerably altered taste, how would this taste affect patient compliance in following prescription regimens? Assessing the benefits of non-nutritive sweeteners in drugs requires a review of drug safety and efficacy.

REGULATING SACCHARIN AS A DRUG

The FDA proposal to ban saccharin as a food additive also includes proposals to allow it as a single-ingredient, over-the-counter (OTC) drug and to ban it as a nonmedical ingredient in drugs. The latter proposal would remove saccharin as an inactive ingredient in drugs unless it affords an overriding benefit: “If saccharin is included as a pharmaceutical aid, an adequate showing that there are not technically feasible alternatives to saccharin, or an adequate showing that the drug product containing saccharin provides a substantial health benefit that would not be available without the use of saccharin” must be made.

Because there is no “Delaney clause” for determining the safety of drug products, the conditions under which they are used would be relevant. Safety and efficacy are separate criteria, and the Food and Drug Administration balances the benefits of a drug product against its risks.

A general test for whether saccharin benefits users of certain drugs would be based on 1) whether the therapeutic component of the drug product is efficacious, and 2) whether no technological alternatives are available for saccharin as a component of such drug products. Because certain fluoridated dentifrices and other fluoridated oral health preparations are considered drug products, these products would also be included in such determinations.

Efficacy of drug products must be shown through “substantial evidence,” which is defined as “evidence consisting of adequate and well-controlled investigations, including clinical investigations, by experts qualified to evaluate the effectiveness of the

drug involved, on the basis of which it could fairly and responsibly be concluded by such experts that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling or proposed labeling thereof" (21 U.S.C. Section 505(d)). These criteria address the question of the benefits of saccharin or any other non-nutritive sweetener for weight reduction and management of diabetes.

Because present use of saccharin falls under food additives and not drug regulations, the supposed restriction of use for "a valid special dietary purpose" is not enforced, and saccharin is widely available. If saccharin were removed as a food additive and classified as an over-the-counter drug, this restriction not only would be more strictly enforced, but its validity would first have to be proven, Though difficult, tests to meet the criterion of "substantial evidence" could be developed to see whether saccharin does lead to measurable benefits in weight reduction and weight maintenance.

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5.

ALTERNATIVE SWEETENERS

5.

ALTERNATIVE SWEETENERS

Saccharin is the only non-nutritive sweetener currently available to the American people. Other artificial sweeteners, however, have been used at other times and in other countries. Still other alternatives are currently under investigation. Sorbitol, xylitol, and mannitol are nutritive sweeteners. Non-nutritive sweeteners whose names have been occasionally mentioned in the literature include stevioside, osladin, d-6 chlorotryptophan, and SRI-oxime V. Six other non-nutritive sweeteners-cyclamate, aspartame, neohesperidan dihydrochalcone, miraculin, monellin, and thaumatin I and II—have been discussed in the literature more extensively. This review is limited to these six.

Various problems make it unlikely that any of these substances, with the possible exception of cyclamate, will be approved for marketing in the immediate future.

- Cyclamate, aspartame, and neohesperidan will not be considered for approval until the necessary toxicity data are submitted and reviewed.
- Aspartame, while stable in dry form, is unstable in alkaline solutions, and the activity of the sweetener declines with storage. Primary use of aspartame would be in dry products.
- Miraculin, monellin, and thaumatin I and II were all isolated from fruits native to tropical West Africa. It would be necessary to produce these fruits in the United States in order for mass marketing of the sweetener to be economically feasible.
- Neohesperidan dihydrochalcone is characterized by a sweet sensation that is slow in onset, long in duration, and accompanied by an aftertaste similar to licorice or menthol.
- Monellin is characterized by a lingering sweetness.
- Thaumatin I and II are unstable at high temperatures as well as having a licorice aftertaste.

Other salient characteristics of these non-nutritive sweeteners and saccharin are summarized in table 3.

CYCLAMATE

Cyclamate was introduced into the market in 1950 and placed on the generally recognized as safe (GRAS) list in 1959. In 1970, however, FDA banned the use of cyclamates in all foods and drugs. This action was taken because experiments on the chronic toxicity and metabolism of a combination of cyclamate and saccharin resulted in bladder tumors in the rats tested (76,1 18). In addition, cyclohexylamine, a

Table 2—Salient Characteristics of Non-Nutritive Sweeteners

Sweetener	Origin	Relative Sweetness to Sucrose	Taste Characteristics	Stability	Use
Cyclamates	cyclohexane sulfanic acid	30-180	no aftertaste	stable	dry products beverages foods drugs
Aspartame	methyl ester of dipeptide, L-aspartyl-L-phenyl-alanine	160-220	no aftertaste	stable in dry form unstable in alkaline solutions and at prolonged cooking temperatures decline of activity with storage	table use cold breakfast cereals chewing gum dry bases for: beverages instant coffee, tea gelatins puddings fillings dairy product (analog) topping
Neohesperidan dihydrachalcone	conversion of: —neohesperidan in Seville orange *C. aurantium* —naringin in grapefruit *C. paradisi* —hesperidin in *C. sinensis*	1,000-2,000	slow in onset long in duration aftertaste similar to menthol or licorice		
Miraculin	miracle berry, *Synsepalum dulcificum*		sour foods taste sweet long in duration (1 to 2 hrs) quality of sweetness—"good"		chewing gum candies dessert puddings fruit-flavored drink mixes
Monellin	serendipity berry, *Dioscoreophyllum cummissii* Diels	800-1,500	long in duration (1 or more hrs)	stable	
Thaumatin I, II	katemfe fruit, *Thaumatococcus daniellii* Benth	2,600	licorice aftertaste	unstable at high temperature	previous use: bread fruits wine tea
Sodium Saccharin	$C_6H_4CONNaSO_2$	200-700	bitter aftertaste	stable at room temperature heat labile	dry products beverages foods drugs

metabolize of cyclamate, reportedly has caused not only chromosomal abnormalities and testicular atrophy in test animals, but also dermatitis and convulsions in humans, when inhaled or applied to the skin (76,136).

Many long-term studies of cyclamate's carcinogenicity and cocarcinogenicity have been conducted in laboratory animals since the removal of cyclamates from the market. All have been negative. Studies of cyclohexylamine, however, have not been conclusive.

In light of these additional tests, Abbott, one of the original manufacturers of cyclamate, petitioned FDA in November 1973, to permit the use of the sweetener in specific dietary foods. In September 1974, FDA asked Abbott to withdraw its petition until data could be provided on the safety of the product. On April 20, 1977, a preheating conference was held to establish a schedule for disclosure and submission of documentary materials. A hearing on cyclamate began on July 13, 1977.

ASPARTAME

In March 1973, G.D. Searle and Company filed a petition for aspartame, a white crystalline powder intended for use as a tabletop sweetener. FDA approved aspartame as a food additive for a number of foods in July 1974, Objections to the regulations were filed, and FDA announced its intention to convene a Public Board of Inquiry. Prior to the establishment of the Board, however, an investigation of the records from animal studies indicated the need for a comprehensive review of some of the data. The Public Board of Inquiry was postponed, and in December 1975, the regulation to permit the use of aspartame was stayed (16).

Objections to aspartame centered on the potential risk of brain damage, primarily in infants and children (57). It has been suggested that large doses of aspartame or combinations of aspartame and monosodium glutamate could cause brain damage in young children. Young mice in one study have developed brain damage similar to that caused by glutamate and aspartame when administered aspartame by feeding tube (118). It has also been reported that lesions were produced in the hypothalamus after aspartic acid and glutamic acid were administered in very large single doses to newborn rodents (118).

Elevated levels of phenylalanine, an amino acid present in aspartame, are associated with the development of mental retardation. Ingestion of aspartame may be harmful to those individuals with phenylketonuria (PKU), a disease characterized by the inability to degrade phenylalanine. The relationship between ingestion of aspartame and metabolism of phenylalanine has been the subject of several studies. A study of 45 adults, all parents of known PKU patients, found acceptable levels of serum phenylalanine among subjects who used aspartame over a 28-week period (83). A study of 126 children and adolescents found that aspartame, when used over a 13-week period, had no significant effect on plasma levels of phenylalanine (52).

Aspartame may not be marketed until the review is completed and all questions raised about its safety resolved.

NEOHESPERIDAN DIHYDROCHALCONE

FDA has recently received petitions from two manufacturers asking for approval of neohesperidan dihydrochalcone, a product derived from bitter citrus flavanones (substances present in the rind of grapefruits and sweet oranges). In August 1975, Neutrality Products, Inc., submitted a second petition—the first having been withdrawn when necessary toxicology tests were requested—for the use of the sweetener in mouthwash, toothpaste, and chewing gum. That petition is still incomplete, pending receipt of the toxicology data.

In March 1977, California Aromatics and Flavors, Inc., a Division of Research Organic/Inorganic Chemical Company, asked for approval of the use of neohesperidan dihydrochalcone as a sweetener. Until the use for the sweetener is specified by the manufacturer and the necessary toxicology tests are identified by FDA, no action may be taken toward the approval of its petition.

Although FDA might approve these petitions, the taste characteristics of neohesperidan dihydrochalcone could discourage widespread use. Its sweet sensation is slow in onset, usually long in duration, and accompanied by a slight licorice aftertaste.

MIRACULIN

Miraculin is a glycoprotein found in the berries of the Nigerian fruit, *Synsepalum dulcificum.* These berries are commonly eaten by children in West Africa. “Miracle fruit” was first described in the literature ‘in 1852. It is a taste modifier that causes sour foods to taste sweet. The sweet sensation is long lasting-often up to 2 hours.

The Miralin Corporation began test marketing miracle fruit as a GRAS food item in 1973, In September 1974, a petition was filed to affirm GRAS status of the fruit for use in foods as a sweetening agent or flavor enhancer. After a preliminary evaluation, however, the petition was denied because the information on consumption in the United States and in existing scientific studies to support the GRAS determination was found to be inadequate. The data were also considered insufficient for the issuance of a food additive regulation at that time, The product was immediately removed from the market.

In May 1977, the FDA announced that it will not permit the *marketing* of “miracle fruit” because its safety for long-term use has not been demonstrated.

MONELLIN

Monellin is a sweetener isolated from the fruit of the West African plant, Dioscoreophyllum *cumminii* Diels. The fruit is often referred to as the “serendipity berry.” Research is being conducted on the reasons for monellin’s sweet taste. The commercial possibilities for marketing the sweetener are not being investigated at this time.

No petitions for affirmation of GRAS status or food additive classification have been filed with the FDA.

THAUMATIN I, II

In 1972, two proteins, Thaumatin I and II, were extracted from the Nigerian fruit, *Thaumatococcus danielli* Benth. The seeds from the fruit have been used in West Africa since 1839 to sweeten bread, fruits and wine. The interest in thaumatin, like that in monellin, centers on the reasons for its sweet taste.

No petitions for affirmation of GRAS status or food additive classification have been filed with the FDA.

APPENDIXES

Appendix I

SACCHARIN ANIMAL TEST DATA

INTRODUCTION

Data presented here show that consumption of high levels of saccharin is associated with increased incidence of bladder cancer in rats. The analysis is presented in two parts. First, the evidence for the carcinogenicity of saccharin in laboratory animals is reviewed. Second, methods of extrapolation from animal data to human exposure are explained, and some models are applied to the data from the two-generation rat studies to arrive at some estimates of the potential effect in humans.

Current Guidelines for Animal Testing. The National Cancer Institute (NCI) has published guidelines for testing suspected carcinogens in laboratory animals (121). Salient points include:

1. Groups of 50 animals of one sex and one strain should be started on the experiment at 6 weeks after birth or at weaning. Control groups should also contain 50 animals. (In practice 100 animals [50 M, 50 F] should be used at each dose).
2. The chemical should be administered by a route that mimics human exposure.
3. At least two doses, MTD (maximum tolerated dose) and MTD/2 or MTD/4 should be administered.
4. Treatment should be continued long enough (in practice generally 24 months) to produce a maximum response.
5. Animals should be sacrificed (usually at 24 months) and necropsied according to detailed pathology procedures.
6. Tests should be conducted in two species, and the results of the more sensitive one given greater consideration.

Additionally, a subcommittee of the National Academy of Sciences (115) has recommended that:

7. Exposure to the chemical for two generations should be considered. This procedure exposes the animals of the second generation to the chemical in *utero,* which may represent the most sensitive stage of the animal's life.

While none of the carcinogenicity tests of saccharin meets all of these criteria, the experiments considered positive most closely approach the current standards. Because of the test conditions or the small number of animals tested, some other experiments would not have detected the carcinogenic effects of saccharin.

TESTING OF SACCHARIN IN RATS

Since 1949, at least 10 feeding experiments have been carried out in rats to test potential carcinogenic effects of saccharin. Only four of these experiments have been published; three in refereed journals. The others have remained in the files of the sponsoring institutions.

Rats have been used in three types of feeding experiments. First, in most experiments weanling animals were started on diets containing saccharin and fed such diets for 2 years or until death. Second, the most convincing experiments involved feeding of saccharin over two generations. This design exposed second generation animals to saccharin from the moment of conception until termination of the experiment. Third, in cocarcinogenesis experiments, saccharin was fed to rats that were also exposed to a single low dose of a known carcinogen.

Many of these studies have been reviewed by subcommittees of the Committee on Food Protection, National Academy of Sciences (NAS) (114, 116, 117), and by Reuber (141). In general, the NAS committees found the evidence for the carcinogenicity of saccharin unconvincing, but no NAS committee has reviewed the 1977 Canadian Study (67). Reuber's analysis of the data from the same experiments led him to conclude that a number of experiments have shown saccharin to be a carcinogen. '

The Office of Technology Assessment found Reuber's review (141) invaluable as a guide to literature that was hard to locate. However, the OTA analysis disagrees in detail with many of Reuber's conclusions. Wolfe and Johnson (189) cited Reuber's analysis in their testimony before the House Committee on Interstate and Foreign Commerce. At that hearing, they pointed out that high doses of a potential carcinogen are required to produce a detectable number of cancers in small numbers of experimental animals. This argument is generally accepted. Reuber, however, drew attention to a few cancers that occurred at a dose of 1/100 or 1/10 of the maximum dose administered but did not occur at any higher dose. Such results are inconsistent with the accepted argument. If the cancers were induced by saccharin ingestion, more cancers should have occurred at all higher doses. In many examples cited by Reuber, no increase in cancers occurred with increasing doses. It appears likely that the few cancers mentioned were spontaneous ones that occurred by chance in saccharin-fed animals. All experiments that Reuber cited as positive are mentioned below, and the OTA analysis is compared to his.

A. Two-Generation Rat Feeding Experiments

1. 1977 Canadian Study (67)

(a) Experimental Design

Two groups of 100 (50 M, 50 F) Charles River (COBS) rats were used. The control group was fed a standard laboratory ration. The experimental group received the same ration but with saccharin, purified of ortho-toluenesulfonamide (OTS), added to comprise 5.0 percent of the diet. (The shorthand designation "5-percent rats" will be used for such experimental animals.) The diet was adjusted weekly to maintain a constant saccharin dose. The experiment began when the rats were 30 days old. At

age 100 days, members of the F_0 (parental) generation were mated. The F_0 generation was continued on the saccharin diet, and the F_1 (offspring, progeny, or second) generation animals were fed the same diet received by their parents, At death, each animal was subjected to "gross and microscopic examination. "

(b) Results

Weight: F_0 and F_1 animals weighed less than controls throughout the experiment (table 4).

Table 4.—Animal Weights in 1977 Canadian Study
(grams)

	Weeks on test						
	0	5	15	48	80	110	124
F_0 Male controls	111	320	518	701	791	745	633
5-percent rats	112	298	462	614	682	658	614
Percent difference*	0	7	11	12	14	12	03
Female controls	**98**	206	282	364	448	488	467
5-percent rats	**99**	190	262	323	379	409	375
Percent difference	0	8	7	11	15	16	20
F_1 Male controls	90	315	501	720	756	736	673
5-percent rats	**72**	**272**	452	630	677	686	632
Percent difference	20	**14**	10	12	10	7	6
Female controls	81	**192**	272	372	447	504	499
5-percent rats	67	**180**	261	331	379	396	394
Percent difference	17	**6**	4	11	15	21	21

'Calculated as: $\frac{\text{weight of controls-weight of 5-percent rats}}{\text{weight of controls}} \times 100$

Life Span: No significant differences between controls and experimental (table **5).**

Table 5.—Mean Time to Death in 1977 Canadian Study
(days)

Diet	F_0		F_1	
	Males	Females	Males	Females
Controls	686±22	695±25	665±22	699±19
5 percent	679±22	731±25	623±22	706±19

Bladder Cancer Incidence: Increases in males of both generations and in F_1 females (table 6).

Other Cancer Incidence: The pathologist for the experiment is still examining other organs, but so far, cancer of no other organ has been associated with saccharin ingestion. The pathology had not been completed by October 1977.

Fertility, gestation, live delivery, and lactation index: No significant differences between controls and experimental.

Table 6.—incidence of Bladder Tumors in 1977 Canadian Study

(Rats with tumors/Rats examined (Percent))

	F_0			F_1		
	Benign	Malignant	Total	Benign	Malignant	Total
Males						
Controls	1/36(3%)	0/36(0%)	1/36(3%)	0/42(0%)	0/42(0%)	0/42 (0%)
5-percent rats	4/38(11%)	3/38(8%)	7/38(19%)	4/45(9%)	8/45(18%)	2/45 (27%)
Females						
Controls	0/38(0%)	0/38(0%)	**0/38(0%)**	0/47(0%)	0/47(0%)	0/47 (0%)
5-percent rats	0/49(%)	0/40(0%)	0/40(0%)	0/49(0%)	2/49(4%)	2/49 (4%)

(c) Discussion

The author's discussion of this experiment was not available in October 1977,

(d) Comments by Others

These data led to the Canadian Government's decision to ban saccharin (66). The FDA's proposed ban is based on this study (50). Reuber accepts the data and conclusions as evidence for the carcinogenicity of saccharin (141).

(e) OTA Comments

OTA has not been able to evaluate this entire study because it is not yet completed. In particular no information is available about bladder stones, urothelial changes, or tumors at other sites. Only one dose level of saccharin was tested. This experiment was primarily directed toward assessing the carcinogenicity of orthotoluenesulfonamide (OTS), a contaminant previously found in commercial saccharin. A committee of the National Academy of Sciences (NAS) had suggested that OTS might be responsible for the carcinogenicity associated with saccharin in earlier experiments (116). The 1977 Canadian experiment clearly showed that OTS is not a carcinogen (data not presented in this report).

Unlike some carcinogenicity experiments, no animals were sacrificed for necropsy at scheduled intervals during this experiment. Animals were examined daily for clinical signs of tumors, and those diagnosed as probably having tumors were isolated and examined twice daily. Moribund animals were sacrificed for necropsy. Less than 1 percent of animals died unobserved and were lost to the experiment because of autolysis.

The mean time to death for animals in the experiment is shown in table 5. It ranged from about 21 to 25 months. In this experiment, large numbers of animals survived long enough to develop tumors.

Throughout the experiment, animals on the 5-percent saccharin diet had lower weights than controls. The results from the experiment show that male rats were more sensitive to saccharin than females and that saccharin caused bladder tumors.

These tumors showed only low invasiveness and no metastasis (62). Both F_0 and F_1 were examined. Tumors were found in both generations but only in males in F_0. The higher frequency in F_1 may be related to that generation's in utero exposure, but the difference in cancer incidence between F_1 and F_0 is not statistically significant.

2. 1973 FDA Study (49)

(a) Experimental Design

The design was similar to the 1977 Canadian Study. Six groups of 96 (48 M, 48 F) Charles River rats (Sprague-Dawley) were fed diets supplemented with different amounts of sodium saccharin: 0, 0.01, 0.1, 1.0, 5.0, or 7.5 percent of diet. Histological examinations were conducted on only the F_1 (second) generation rats.

(b) Results

Weight: Rats fed 5- and 7.5-percent saccharin were about 15-percent lighter than controls and those eating lower levels of saccharin.

Life Span: No significant differences between the controls and experimental rats.

Stones and Parasites: Analysis of data presented in this paper showed that there was no association between bladder stones and bladder cancer. Neither was there any correlation between parasitic worms, or their eggs, and bladder cancer.

High Doses of Sodium: One group of animals was given a diet containing a sodium salt at the same level as that ingested by the 7.5-percent sodium saccharin-fed rats. There was no increase in tumors.

Tumor Incidence: At death or sacrifice each animal was examined for macroscopic tumors (table 7), and organs were excised, fixed, and stained for subsequent microscopic pathology. Some results from the microscopic examinations are shown (tables 8-1 1).

Table 7.—incidence of Macroscopic Tumors in Rats Surviving 18 Months or More in 1973 FDA Study

Dose (Percent)	Rats with tumors/Rats examined (Percent)	
	Male	Female
0	2/29 (7%)	1 /27(4°/0)
0.01	2/28 (7%)	3/30(1 0°/0)
0.1	5/29 (14%)	3/32(9°/0)
1	3/28 (11%)	5/32(1 6°/0)
5	4/24 (17%)	7/29(240/o)
7.5	8/26 (36%)	9/32(280/o)

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Other Pathologies: A particular type of kidney hyperplasia "colyceal polyposis" occurred more frequently in the 7.5-percent rats than controls ($p < 0.05$). This hyperplasia is not considered to be precancerous,

Table 8.—incidence of Neoplasms in 1973 FDA Study

Dose (Percent)	Rats with neoplasms/Rats examined (Percent)			
	18 Months		24 Months	
	Males	Females	Males	Females
0	**4/7(57%)**	**5/6(83 %)**	19/29(65%)	21/27 (78%)
0.01	—	—	10/28(36%)	32/30 (107%)
0.1	—	—	14/29(48%)	31 /32 (97%)
1.0	—	—	**12/28(43%)**	20/32 (62%)
5.0	—	—	**9/24(38%)**	34/29 (117%)
7.5	0/7(0%)	3/5(60%)	**20/26(77%)**	38/32 (119%)

Table 9.—incidence of Bladder Tumors in Rats Surviving 18 Months or More in 1973 FDA Study

Dose (Percent)	Rats with tumors/Rats examined (Percent)		
	Papillomas of the Urinary Bladder	Carcinomas of the Urinary Bladder	Total Tumors of the Urinary Bladder
Male Rats			
0	**0/25(0%)**	1/25(*4%)*	1/25(*4%)*
0.01	**0/16(0%)**	*0/16(O%)*	*0/16(O%)*
0.1	**0/27(0%)**	*0/27(O%)*	*0/27(O%)*
1.0	0/22(0%)	*0/22(o%)*	*0/22(o%)*
5	0/21(0%)	*1/21(5%)*	*1/21(5%)*
7.5	1/23(4%)	*6/23(23%)*	*7/23(30%)*
Female Rats			
0	**0/24(0%)**	**0/24(0%)**	**0/24(0%)**
0.01	**0/23(0%)**	**0/23(0%)**	**0/23(0%)**
0.1	**0/24(0%)**	**0/24(0%)**	**0/24(0%)**
1.0	0/30(0%)	0/30(0%)	0/30(0%)
5	0/28(0%)	0/28(0%)	0/28(0%)
7.5	0/31(0%)	2/31(6%)	2/31(6%)

Table 10.—Incidence of Mammary Gland Tumors in Rats Surviving More Than 18 Months in 1973 FDA Study

Dose (Percent)	Rats with mammary gland tumors/Rats examined (Percent)		
	One/Rat	Two or More/Rat	Total
Male Rats			
0	5/29(17%)	1/29(3%)	**6/29(20%)**
0.01	8/25(32%)	6/25(24%)	**14/25(56%)**
0.1	9/27(33%)	0/27(O%)	**9/27(33%)**
1.0	8/27(30%)	0/27(O%)	**8/27(30%)**
5	7/25(28%)	0/25(O%)	**7/25(28%)**
7.5	7/24(29%)	0/24(O%)	**7/24(29%)**
Female Rats			
0	5/26(19%)	1/26(4%)	**6/26(23%)**
0.01	8/30(27%)	6/30(20%)	**14/30(47%)**
0.1	9/34(26%)	4/34(12%)	**13/34(38%)**
1.0	8/30(27%)	4/30(13%)	**12/30(40%)**
5	7/27(26%)	5/27(18%)	**12/27(44%)**
7.5	7/32(22%)	2/32(6%)	**9/32(28%)**

Table11 .—Incidence of Urinary Bladder Hyperplasia in 1973 FDA Study

Dose (Percent)	Rats with hyperplasia/Rats examined									
	Months on Saccharin								Total	
	6		12		18		24			
	M	F	M	F	M	F	M	F	M	F
0	1/29	1/48	1/12	0/7	0/7	0/6	8/25	2/24	10/73	3/85
0.01	1/31	0/49	1/18	0/5	1/6	0/4	3/16	0/23	6/71	0/81
0.1	0/35	0/48	0/15	0/3	0/4	0/5	4/27	0/24	4/81	0/81
1.0	0/32	0/50	1/15	0/4	0/7	0/6	3/22	3/30	4/76	3/90
5	3/20	1/37	0/16	1/16	0/7	0/7	3/21	3/28	6/64	5/88
7.5	4/18	3/35	4/15	0/5	4/6	0/5	7/23	7/31	19/62	10/76

(c) Discussion

The authors of this study concluded that bladder tumors were associated with ingestion of the maximum amount of saccharin. Other experts agreed with the classification of the urinary bladder tumors reported by the authors of the study (116,181).

(d) Comments by Others

The NAS committee (1 16) agreed with the conclusions of the authors, but considered it unfortunate that histological examinations were not carried out on the F_0 generation. It also suggested that the OTS impurity in the saccharin might be the active carcinogen. The 1977 Canadian Study (55) eliminated the basis for that objection because saccharin free of OTS was associated with a higher incidence of bladder cancer than controls, and OTS was not,

The committee speculated that ingestion of large amounts of sodium saccharin might lead to bladder stones and that the stones might be the causative agent for bladder cancer. Reuber (141) analyzed the evidence from this experiment and concluded that although 67 percent of the treated male rats at 12 months had stones, no rat with stones developed either hyperplasia or tumors.

The NAS committee also suggested that another agent, parasites, might have been the causative agent, but there is little evidence to support the suggestion. No bladder parasites were observed, and indeed, there appears to be little reason for assigning a carcinogenic role to the parasites (147).

Reuber agreed that the high levels of dietary saccharin are correlated with bladder cancer. He further argued that urinary bladder hyperplasia is a precursor of cancer and that those data, as well as the total number of tumors and number of mammary tumors, further strengthen the causal relationship between saccharin and cancer.

Dr. M.A. Weinberger, Director, Division of Pathology, Food and Drug Administration (FDA), wrote a memo in 1974 (181) agreeing with the pathology of the FDA Study. He stated that: (1) the incidence of tumors in the 7.5-percent rats was significantly different from that in the controls, (2) there was no evidence for stones or parasites playing a role in the genesis of the tumors, (3) tumors of no organ other than the urinary bladder were associated with saccharin, and (4) the bladder tumors were of low invasiveness and resembled those found in the WARF Study (165, 177).

(e) OTA *Comments*

Table 7 is derived from data presented in a preliminary report of this experiment (51), and from data that were cited by Reuber (141). Table 8 is based on the final report (49) and includes data from microscopic pathological examinations. Reuber concluded that total tumor incidence increased in parallel with increasing dose, but the more complete data do not support that conclusion, In males the incidence in controls was higher than at all doses except 7.5 percent. In females there were two peaks, one at 0.1 percent, the other at 5 and 7.5 percent.

Only the 7.5-percent dose in F_1 males and females was associated with an incidence of bladder cancer greater than controls. Similarly, the incidence of bladder hyperplasia increased only at the highest dose. In fact, low doses of saccharin were associated with a lower incidence of hyperplasia than in controls.

A large increase in number of mammary tumors was noted at the lowest dose (0.01) percent), but the incidence did not increase with higher doses. By this measure, there is again no clear-cut relationship between dosage and effect. These data are examples of cases that Reuber considers positive, and OTA does not.

None of the other experiments described here reported high rates of bladder hyperplasia or mammary gland tumors. The data in the WARF study are complete enough to suggest that such pathologies would have been noticed and reported if they had occurred. Furthermore, no such pathologies have been associated with saccharin ingestion in the 1977 Canadian Study (67).

3. Wisconsin Alumni Research Foundation (WARF) Study (165, 177)

(a) Experimental Design

This experiment was similar in design to the 1973 FDA Study, but the number of rats in each group was smaller. Five groups of 40 Sprague-Dawley rats (20 M, 20 F) were used, and sodium saccharin was added to the rations at O-, 0.05-, 0.5- or 5.0-percent levels. F_1 rats born to mothers ingesting saccharin were maintained on the identical diet for 100 weeks. "Most" tissues of F_1 rats (not F_0) were sectioned histologically. No rats were sacrificed at scheduled intervals. Rats that became moribund or died during the test were necropsied, and survivors were necropsied at 100 weeks.

(b) Results

Weight: (F_1 generation): 5-percent rats had reduced weights at weaning and gained weight more slowly, but reached the same levels as controls.

Life Span: No significant differences between controls and experimental.

Hematology: No significant differences between controls and experimental.

Reproduction: No significant differences between controls and experimentals.

Tumor Incidence: Results of histological examination of tissues from the rats are shown in tables 12-14.

Table 12.—Total Number of Tumors in 1974 WARF Study

Dose (Percent)	Rats with tumors/Rats examined	
	Males	Females
o	3/20	1 2/20
0.05 "	2/20	6/20
0.5	2/20	9/20
5.0	14/20	18/20

(c) Discussion

The authors concluded that overall tumor incidence was increased in the 5-percent males compared to controls. Furthermore, they pointed out that five squamous cell carcinomas of the uterus and seven transitional cell carcinomas of the bladder were seen only in saccharin-fed groups.

The authors underline the sporadic appearance of some tumors by mentioning that of the tumors seen in three or more animals, one (subcutaneous adenofibroma) occurred most frequently in the control population.

Table 13.—Incidence of Urinary Bladder Tumors in 1974 WARF Study in Rats Surviving 18 Months or Longer

Dose (Percent)	Rats with tumors/Rats examined			
	Males		Females	
	Benign	Malignant	Benign	Malignant
0	0/12	0/12	0/1 6	0/1 6
0.05 " : : : : : : : : : : :	0/10	0/10	0/1 4	1/1 4
0.5	1/12	0/12	0/1 5	0/1 5
5.0 ....., ...	1/1 5	7/15	0/20	0/20

Table 14.—Incidence of Ovarian and Uterine Tumors in 1974 WARF Study

Dose (Percent)	Rats with tumors/Rats examined	
	Benign	Malignant
o	0/20	1 /20
0.05" :	0/20	1 /20
0.5	1 /20	2/20
5.0	2/20	4/20

(d) Comments by Others

The NAS committee (116) leveled the same criticisms at this experiment that it did at the 1973 FDA Study (49). They suggested that OTS, bladder stones, or parasites might be the active carcinogen.

Reuber (141) accepted the conclusions of the authors and also drew attention to the increased number of female reproductive system tumors at high doses.

(e) *Comments by OTA*

No symptoms of acute toxicity were noted except slower weight gain in the 5-percent rats.

This experiment suffers from the small number of animals, but its results are consistent with the other two-generation experiments. Ingestion of saccharin at the highest dose resulted in an increase in male bladder cancer. The increase in female reproductive cancers was not seen in other two-generation experiments, and it is not considered to be an important finding.

The high number of spontaneous tumors in the female rats contrasts to other experiments in which the spontaneous incidence was nearly equal between the sexes or higher in males. Even so, bladder cancers were associated with the highest dose of saccharin in males,

The following tumors were noted to occur only in control animals in this experiment: adrenal adenoma, islet cell adenocarcinoma, papilloma with squamous metaplasia of the uterus. Such findings underline the difficulty of interpreting small numbers. Furthermore, the following tumors occurred in 1/40 controls and in 1/160 experimental: pituitary carcinoma, thyroid adenocarcinoma, subcutaneous fibroma, subcutaneous fibroadenoma, subcutaneous sarcoma, and subcutaneous adenocarcinema.

4. Summary: OTA Discussion of the Two-Generation Feeding Experiments

There is general agreement among the authors of the experiments, the NAS committees, and Reuber that:

(1) Exposure of rats to 5-or 7.5-percent saccharin from the moment of conception to death was associated with an increased frequency of urinary bladder cancers. These tumors were of low invasiveness and had no reported metastasis.

(2) These same conditions resulted in slower weight gain in all experiments and lower adult weights in two of the three experiments.

Reuber's and OTA's analyses of these data further argue that:

(3) Bladder tumors were not associated with stones or parasites in at least two of these experiments.

(4) Increased frequencies of bladder hyperplasia were associated with 7.5-percent saccharin in the 1973 FDA Study.

Additionally, OTA notes that:

(5) In all experiments more bladder cancers were found in males.

These experiments cannot be interpreted as showing a threshold for saccharin induction of bladder cancers at about 5- or 7.5-percent dietary saccharin. The frequency of tumors at the highest doses is so low that larger numbers of animals would have been needed to detect cancers at lower levels.

If these data fit a no-threshold model, an almost linear relationship between dose and tumor number might have been seen in the 1973 FDA Study in which both 5- and 7.5-percent doses were used. While no such relationship was seen, the data do not eliminate there being one. The design of the other two experiments precludes any conclusions being drawn about dose response.

The NCI Guidelines have suggested that carcinogenicity testing is best conducted at doses that produce no apparent toxicity (121). In all cases in which increased numbers of tumors were detected (5 and 7.5 percent), weight gain was not normal, and in two of the three, final weights were less than those of the controls. Reduced final weights and slower weight gain are symptoms of toxicity. The difference in weights

in the 1977 Canadian experiments exceeds the 10-percent difference acceptable to the NCI, but the weight difference is greater among females, who had fewer tumors than the males.

B. One-Generation Rat Feeding Experiments

1. 1948-49 FDA Study (46)

(a) *Experimental Design*

Groups of 3-week-old Osborne-Mendel Rats, 20 per group (10 F, 10 M) were fed saccharin at 0,01-, 0.1-, 0.5-, 1- or 5-percent levels for 2 years. The control group included 54 rats. Animals were to be carried on the experiment for 2 years, but some died earlier. At death or sacrifice, most rats were examined both grossly and microscopically, but bladders were not included in the list of examined organs.

The microscopic slides as well as some preserved organs from this experiment were held at FDA, and in 1969, Long and Habermann (93) examined those samples. All bladders were described as "grossly normal." The one sectioned for histology was normal. Paraffin blocks of kidneys were sectioned and examined.

(b) Results

Weight: 5-percent rats were somewhat lighter than controls; other saccharin-fed rats did not differ from controls.

Life Span: No difference was observed between saccharin-fed and control animal groups.

Tumors: In the 5-percent rats, seven rats were found to have thoracic lymphosarcomas, which is near the incidence seen in control rats. However, four of those seven rats had abdominal lymphosarcomas, a much higher frequency than the usual 1:15-20 for abdominal: thoracic lymphosarcomas.

Long and Habermann's (93) reexamination of the 1948-49 FDA materials produced a more detailed exposition of the incidence of lymphosarcomas. Table 15 is taken from Long and Habermann's pathology reports.

Table 15.—Incidence of Lymphosarcomas in 1948-49 FDA Study

Dose (Percent)	Rats with tumors/Rats examined (Percent)		
	Lymphosarcomas of the Thorax	Lymphosarcomas of the Abdomen	Total Lymphosarcomas
o	9/54 (17%)	0/54(0%)	9/54 (1 7%)
0.01"	8/14 (57%)	0/1 4(0%)	8/14 (57%)
0.1	5/1 6 (31 %)	0/1 6(0%)	5/16 (31%)
0.5	2/1 5 (1 3%)	0/1 5(0%)	2/15 (13%)
1	1/1 8 (6%)	0/1 8(0%)	1/18 (6%)
5	7/1 7 (41%)	3/1 7(1 8%)	10/17 (58%)

Other Pathologies: Long and Habermann presented the data concerning kidney lesions in table 16.

Table 16.—Incidence of Kidney Lesions in 1948-49 FDA Study

Dose (Percent)	Rats with lesions/Rats examined (Percent)		
	Epithelial Hyperplasia	Calcification	Venous Thrombosis
0	**1 /33 (3%)**	1/53 (2%)	0/53 (0%)
0.01	**0/13 (0%)**	0/13 (0%)	0/3 (0%)
0.1	**0/15 (0%)**	0/15 (0%)	0/15 (0%)
0.5	1/15 (7%)	2/15 (13%)	0/15 (0%)
1	3/18 (17%)	1/18 (6%)	0/18 (0%)
5	13/17 (76%)	13/17 (76%)	4/17 (24%)

(c) Discussion

The authors concluded that saccharin produced no adverse effects at doses less than 5 percent, that the 5-percent dose caused only slight toxic effects, and that only the ratio of abdominal to thoracic lymphosarcomas was remarkable. Long and Habermann (93) drew attention to the increased number of thoracic lymphosarcomas at 0.01 percent and noted that the incidence was significantly greater than in the controls. They tempered that conclusion with comments about the decreasing incidence at all higher doses except 5 percent. They attached less importance to the number of abdominal lymphosarcomas than did the original report of this study (46).

(d) Comments by Others

The NAS committee (116) quoted the original observation that saccharin-fed rats had increased incidence of abdominal lymphosarcomas (46) but made no other comments. Reuber (141) also cited the increased incidence of abdominal lymphosarcomas, and he treated the renal pathologies as evidence for a precancerous condition.

(e) Comments by *OTA*

The lymphosarcoma data present some difficulties in interpretation. Four groups of 20 control animals were used, and the incidence in the controls ranged from 0/20 to 4/20. Fitzhugh et al. (35) did not present these data in tabular form; in fact, they did not mention lymphosarcomas at doses less than 5 percent, but did mention that the ratio of abdominal to thoracic lymphosarcomas was remarkably high. They found the ratio to be 4:7, rather than the 1:15 to 1:20 they expected. Long and Habermann (93) did not consider this difference to be significant and pointed out that the abdominal tumors occurred only in animals with thoracic tumors. The distribution of lymphosarcomas across the dose range has two peaks, one at 0.01 percent, the other at 5.0 percent. This distribution may reflect a difference in frequency of spontaneous tumors among the groups,

The renal pathologies may be considered to be examples of acute toxicity. Indeed, their frequencies might fit a threshold model. If the kidney lesions are precancerous, such cancers must develop quite slowly because no excess of kidney

tumors has been noted in any experiments, An excess of kidney calyceal polyposis was noted in 7.5-percent animals in the 1973 FDA Study, but that condition is not precancerous. Furthermore, a careful examination of 600 saccharin-fed rats did not find an excess of kidney lesions (112).

2. Lessel Study, 1948-49 (91)

(a) Experimental Design

Groups of 40 Boots-Wistar rats (20M, 20F) were fed rations of O-, 0,005-, 0.05-, or 5. O-percent saccharin for 2 years. This experiment was intended to complement the FDA 1948-49 Study.

(b) Results

Body Weight: The 5-percent rats weighed less than other groups.

Life Span: The 5-percent rats had decreased lifespans.

Food Consumption: The 5-percent rats ate more even though they gained less weight and died earlier.

Tumors: Animals were examined for tumors at death or sacrifice (table 17), and the urinary bladder was examined for pathology (table 18).

Table 17.—Number of Survivors and Number of Tumors in 1948-49 Lessel Study

Dose (Percent)	Males			Females		
	Survivors at 2 Years	Tumors		Survivors at 2 Years	Tumors	
		Benign	Malignant		– Benign	Malignant
0	6	1	1	9	5	4
0.005 : : : : : :	12	2	1	13	6	3
0.05	8	4	2	10	4	0
0.5.	8	2	2	9	0	1
5.0.	3	1	1	2	2	0

Table 18.—Male and Female Rats Ingesting Saccharin With "Gross Abnormalities" of the Urinary Bladder[a] in 1948-49 Lessel Study

Dose (Percent)	Rats with abnormalities/Rats examined (Percent)	
	Males	Females
0	2/20 (1 0°/0)	0/20 (0°/0)
0.005	1 /20 (5%)	0/20 (0%)
0.05	4/20 (20%)	0/20 (0%)
0.5.	1 /20 (10%)	0/20 (0%)
5	5/19 (26%)	3/18 (17%)

[a]Rats surviving 6 months or longer.

(c) Discussion

The author concluded that tumor incidence was unaltered at all dose levels.

(d) Comments by Others

The NAS committee (116) quoted from Lessel's reports extensively and summarized by saying that his detailed description of examination of bladders (table 18) ". . . underscore some of the problems encountered in long-term testing. " It then commented that the study was incomplete because not all bladders were examined microscopically.

Reuber (141) drew attention to the observations that one 5-percent rat had bladder mucosa hyperplasia, and two early bladder tumors were seen in 5-percent females. One tumor was associated with a stone; the other was not.

(e) Comments by OTA

All but one of the tumors reported in table 17 were detected at 22 to 24 months. Because of the small number of animals alive at 24 months, it is difficult to assess the significance of the numbers of tumors.

The highest incidence of bladder "gross abnormalities" (table 18) was associated with the highest dose of saccharin. These abnormalities occurred at a higher dose than did the majority of the tumors.

The conclusion reached by Reuber (141) that saccharin increased tumors in males has to be qualified because of the low incidence of tumors in the control males. In all studies except this one and WARF, spontaneous tumors occurred more frequently in males or at about the same frequency in both sexes. The incidence seen in males here may be lower than the average incidence if larger numbers of controls had been studied. Alternatively, of course, it might be argued that the incidence in control females was abnormally high, and that the female data might have been positive if a better determination of spontaneous incidence had been made, These opposing arguments again point up the problems of trying to analyze data from too few animals.

3. National Institute of Hygienic Sciences (Tokyo) (123)

(a) Experimental Design

A control group of 54 rats was fed a standard laboratory diet. The experimental group of 54 rats was fed a diet increasingly rich in saccharin according to the schedule given in table 19. Rats were killed and inspected for cancers at various times from 6 to 24 months.

Table 19.—Saccharin Feeding Schedule for Rats in Japanese Study (undated)

Days	Dose (Percent)
0 - 20	2
21 - 60	3
61 -150, ...	4
Greater than 150	5

(b) Results

Life Span: There was no apparent effect of saccharin on life span.

Tumors: The number of tumors found was reported; some of those results are shown in table 20.

Table 20.—Incidence of Tumors in Rats Necropsied at 24 and 28 Months in Japanese Study (undated)

Months	Rats with tumors/Rats examined	
	Control Rats	Saccharin Rats
24 months	5/1 1	5/1 1
28 months	0/3	**5/6**

(c) Discussion

The author's discussion of these experiments is not available.

(d) Comments by Others

Reuber (141), based on data not quoted here, concluded that the saccharin-fed rats had twice as many tumors as controls.

This experiment was not mentioned by any NAS committees (114, 116, 117).

(e) Comments by OTA

Table 20 is an excellent (although extreme) illustration of the difficulties of interpreting some experiments. The numbers are very small and do not lend themselves to interpretation. For example, given *5/11* positive control rats at 24 months and 0/3 positive controls at 28 months, it is difficult to come to any conclusion about the frequency of spontaneous tumors.

4. Litton Bionetics Study (119)

(a) Experimental Design

Two studies (Saccharin I and II) were run in parallel. In each experiment, 52 Charles River Rats (26 M; 26 F) were fed either 1- or 5-percent saccharin for 104 weeks. The control group for each experiment was 40 rats (20 M, 20 F).

Complete necropsies were performed.

(b) Results

Life Span: Feeding of saccharin did not affect longevity (see table 21), but an epidemic of chronic murine pneumonia killed many l-percent male rats in Saccharin II.

Tumors: Animals were necropsied and examined for cancers (table 21).

Other Pathologies: One 5-percent male had bladder hyperplasia in Saccharin I. There were 17 cases of glomerulonephritis among the 26 l-percent males in Saccharin I; none in Saccharin II.

Table 21.—Survivors, Tumors, and Pneumonia in 1973 Litton Bionetics Study

Dose (Percent)	Survivors at 18 Months		Incidence of Pneumonia		Tumors in Males		Tumors in Females	
	M	F	M	F	Benign	Malignant	Benign	Malignant
Saccharin I								
0	20/20	18/20			10	0	27	1
1	24/26	21/26			12	1	27	1
5	21/26	24/26			17	2	26	0
Saccharin II								
0	18/20	19/20			14	3	13	0
1	18/26	26/26	18/26		11	1	23	1
5	23/26	23/26	11/26	4/26	3	1	25	2

One 5-percent female rat had a urinary bladder papilloma (Saccharin II).

(c) Discussion

The authors concluded that saccharin was not associated with carcinogenesis.

(d) Comments by Others

Reuber (141) reports that these data were analyzed by statisticians at the NCI, who concluded that tumor incidence was higher in the female rats in the Saccharin 11 experiment. The males in Saccharin II were stricken with pneumonia, and the number of early deaths may have caused the reduced incidence of tumors seen in that group. It was also concluded (113) that there were no increases in malignant tumors. The NAS committee (116) was apparently interested only in bladder tumors. Its discussion of these experiments noted only that a single bladder papilloma was found in a 5-percent female.

Reuber (141) presented figures somewhat different from those in table 21 which supported the conclusion that the total number of tumors was higher in saccharin-fed female rats than in female rats in Saccharin II.

(e) Comments by OTA

This experiment is unique in that females appeared to be more sensitive than males to saccharin. The males in Saccharin II had a high incidence of pneumonia, and some animals died. While this factor may account for that group's having fewer tumors, it could have had no effect on Saccharin I in which females were also more sensitive than males.

The frequency of spontaneous tumors in female controls between the two experiments is quite different. The significant difference between experimental and control females in Saccharin II depends on the much lower spontaneous rate in that experiment. There was no increase in tumor incidence as the saccharin dose was increased fivefold from 1 to 5 percent.

Toxic effects, bladder hyperplasia and chronic glomerulonephritis were noted in some saccharin-fed rats, but not in both experiments. The incidence of glomerulonephritis was higher in the l-percent than in the 5-percent male rats in Saccharin 1.

5. Bio-Research Consultants (122)

(a) Experimental Design

Twenty-five male Charles River Rats (derived from Sprague-Dawley) were fed O-, 1-, or 5-percent saccharin diets from the age of 8 weeks through 104 weeks or longer. All animals that died after the first 6 months were necropsied except those badly autolyzed. Two experiments were run, using saccharin from different sources.

(b) Results

Body Weight: Saccharin had ". . . no significant effect upon body weight. '

Life Span: The l-percent rats in one experiment ". . . showed increased mortality after the first year of study, " but the difference was not considered significant.

Tumors: Results are presented in table 22.

Table 22.—Number of Tumors in Male Rats in 1973 Bio-Research Consultants Study

	Rats with tumors				
	Study 1		Study 2		Control
Dose (Percent)	1	5	1	5	o
Animals in Group	13	12	15	14	16
Tumors					
Pituitary Adenoma	**9**	**5**	7	6	7
Parathyroid Adenoma	**0**	**0**	0	0	1
S.C. Fibroma or Fibrosarcoma	**2**	**0**	1	1	3
Adrenal—Medullary	1	1	3	1	2
—Lymphangioma	1	**0**	0	0	0
Breast—Adenocarcinoma	1	**0**	0	0	0
Bladder—Noninvasive CA	**0**	1	1	0	1
—Papilloma	1	**0**	0	1	0
Stomach—Epidermoid CA	**0**	**0**	1	0	0
—Papilloma	**0**	1	1	1	0
Kidney—Tubular Adenoma	1	0	0	0	0
Spleen—Hemangioma	0	1	1	0	0
Skin—Epidermoid CA	0	1	0	0	0
Lymphoma	0	0	1	0	0
Lymph Node Hemangioma	0	0	0	1	0

(c) Discussion

The authors concluded that their data did not show a correlation between saccharin and cancer.

(d) Comments by Others

The NAS committee (116) concurred with the authors' conclusion. Reuber (141) eliminated pituitary adenomas from his considerate ion because of its high incidence in all groups, and he concluded that saccharin was associated with higher incidences of total tumors and malignant tumors.

(e) Comments by OTA

Using the data in table 22 and eliminating the pituitary tumors from all groups and the parathyroid adenoma from the controls (because ‘‘neck organs were not included in the tissues to be examined under the contract’ ‘), OTA obtained the numbers shown in table 23. There is little difference in occurrences among the groups and no increase between the l-percent rats and the 5-percent rats.

Table 23.—Tumors Other Than Pituitary in Male Rats in 1973 Bio-Research Consultants Study

Dose (Percent)	Rats with tumors/Rats examined		
	Group 1	Group 2	Control
o			6/1 6
1	7/1 3	9/1 5	
5	5/1 2	5/1 4	

The data in table 22 show that the number of bladder lesions was not increased by saccharin ingestion. Only in the l-percent rats of Group 2 was there an increase in malignant tumors. No such increase appeared in either the 5-percent group or in the other l-percent group. The significance of the l-percent increase is therefore not clear.

6. Schmahl Study (152)

(a) Experimental Design

Groups of 104 Sprague-Dawley Rats (52 M, 52 F) were fed diets containing saccharin beginning at age 70 to 90 days until the animals died. After death, each animal was necropsied, and every bladder was examined histologically. These groups ingested either O-, 0.2-, or O. S-percent saccharin.

(b) Results

Weight Gain: No significant differences between experimentals and controls.

Blood Chemistry and Pressure: No differences between experimental and controls.

Life Span: No significant differences between experimental and controls.

Tumors: No significant differences between experimentals and controls.

(c) Discussion

The author concluded that there was no detectable carcinogenic effect of saccharin.

(d) **Comments** *by Others*

The NAS committee (116) listed this experiment in a table but made no comment about it.

Reuber (141) accepted the conclusion of the authors that no bladder cancers

were caused by saccharin but faulted the study for a number of technical reasons. He concluded, from inspection of the data, that the incidence of lymphomas and leukemias was higher in the 0.5-percent rats.

(e) Comments by OTA

This study has numerous shortcomings, but it is not immediately apparent why Reuber (141) singled this one out for criticism. As in other experiments: (1) this one was not published in a refereed journal (but see next paragraph); (2) histological examinations were carried out only when "during the dissection, conspicuous conditions were found, (but histology was performed on all bladders); and (3) the rats had a high spontaneous frequency of tumors. Additionally, the results were not separated by sex. Results are stated as number of tumors, and the number of animals examined is not given. The higher dose of saccharin was tenfold less than those used in the positive two-generation experiments.

Schmahl has published a paper in German. OTA did not review that paper, and the amount of overlap between data reviewed here and those presented in the German publication (151) is unknown. The translation available to OTA reports one lymphosarcoma in the controls, four in the 0.2-percent group, and two in the 0.5-percent group. The total is higher among the experimentals, but it does not increase with dose. In the case of leukemia, there was one in the controls, zero in the 0.2-percent group, and one in the 0.5-percent group.

7. Munro et al. (112)

(a) Experimental Design

Groups of 120 Charles River CD (COBS) rats (60 M, 60 F) were fed diets containing saccharin from weaning until 120 weeks. Constant dose levels were obtained by adjusting saccharin amounts at weekly intervals. The levels fed were O, 90, 270, 810, or 2430 mgm saccharin/kgm body weight/day. (The maximum level is approximately 5 percent, and these levels have been converted to percent of saccharin in the diet in the data presented here.)

Animals were visually examined each day for clinical signs of toxicity. Blood chemistry, urine chemistry, body weights, and food intake were determined. Animals dead or moribund during the experiment and all survivors at 120 weeks were subjected to detailed gross necropsy.

(b) Results

Body Weight: The 5-percent rats, both M and F, had reduced body weight and slight diarrhea at all times after 10 weeks.

Life Span: "A dose-related increase in mortality was observed in treated male rats. . but not in female rats. . ."

Hematology Examinations: No difference between controls and experimental.

Urine Chemistry Composition: No difference between controls and experimental.

Examination for Bladder Parasites: No ova of T. *crassicauda* were observed in urine, and no parasites were observed in any bladders.

Pathology: Detailed tables of neoplasms and other histological findings are presented, The incidence of lymphomas and leukemias found in this study are given in table 24.

Table 24.—Number of Leukemias and Lymphomas in Examined Animals in 1974 Munro Study

Dose (Percent)	Rats with leukemias and lymphomas/Rats examined	
	Males	Females
o ..	2/57	5/56
0.2	2/51	3/56
0.6	5/54	2/52
2	2/52	1 /56
5	7/54	1 /54

(c) Discussion

Authors state "saccharin administration was not accompanied by an increase in tumor incidence. "

(d) Comments by Others

The NAS committee (11) discussed only the incidence of urinary bladder tumors. They concurred with the authors' conclusion that there was no relationship between dosage and incidence of tumors as shown in table 25.

Table 25.—Number of Urinary Bladder Cancers in 1974 Munro Study

(Rats with tumors)

Tumors	Dose (Percent)									
	o		0.2		0.6		1.7		5	
	M	F	M	F	M	F	M	F	M	F
Angiosarcoma	1	0	0	0	0	0	0	0	0	0
Transitional Papilloma	0	0	1	0	0	1	2	0	0	0

Reuber (141), discussing the same data, cited the NAS report (116), which reported that two of the four bladder papillomas were reclassified as carcinomas. He concluded that the small number of bladder tumors suggest ". . that saccharin may well be carcinogenic for the urinary bladder. " He drew attention to the incidence of leukemias and lymphomas in males, concluding that saccharin-fed males displayed slightly increased incidence of tumors compared to control males.

Reuber (141) also pointed out that certain "unusual or rare tumors" were observed in treated, but not in control rats as shown in table 26.

Table 26.—Tumors in Saccharin-Fed and Not in Control Rats in 1974 Munro Study

	Tumor	Seen in
1.	Hepatocellular Carcinoma (liver)	0.6 percent males
2.	Adenocarcinoma (prostate)	0.6 percent males
3.	Endometrial Adenocarcinoma	1.7 percent females
4.	Endometrial Adenocarcinoma	5.0 percent females
5.	Malignant Mesenchymal Tumor (uterus)	5.0 percent females
6.	Cholangiocarcinomas X 2 (liver)	5.0 percent females

(e) Comments by OTA

Bladder cancers do not appear to be related to saccharin dosage. There was evidence of some toxicity (diarrhea, slower weight gain) in both males and females as well as earlier death in males on the 5-percent diet compared to controls.

The conclusion that the small number of bladder cancers is higher in the experimental animals than in the controls is difficult to accept. If the angiosarcoma in the control group is accepted as a bladder cancer, then there was one tumor in 113 control animals; in the larger number of experimental animals, there were four tumors in 429 animals. Even zero tumors in 113 animals would not be significantly different from four tumors in 429.

The leukemia and lymphoma data for males suggest an increase due to saccharin ingestion. However, the female data are quite different. The incidence in female controls is higher than in any experimental group. These differences may reflect sex differences or, alternatively, some sampling artifact. The authors attached no significance to these findings.

The argument that the experimentals had tumors not seen in control animals must be weighed against the occurrence of some tumors in the control population that were not seen in the experimentals (table 27).

Table 27.—Tumors in Control and Not in Saccharin-Fed Rats in 1974 Munro Study

	Tumors	Rats with tumors/Rats examined	
		Controls	Experimental
1.	Adenocarcinoma (large intestine)	1/1 13	0/429
2.	Fibrosarcoma (urethra)	1/1 13	0/429
3.	Hemangioendothelioma (skin)	1/1 13	0/429
4.	Fibrosarcoma (uterus)	1 /56	0/21 8
5.	Osteogenic sarcoma (rib)	1/1 13	0/429

This experiment has a number of features to recommend it: (1) it was published in a refereed journal; (2) the causal role of stones or bladder parasites was eliminated; and (3) reasonable numbers of animals were used.

The 1977 Canadian Study pathology records show that the mean time for appearance of bladder tumors is about 24 months. In Munro et al. (112), only about 10 animals remained alive in each group at 24 months (62). If more animals had lived that long, the incidence of bladder tumors might have been higher.

8. Summary: OTA Discussion of One-Generation Feeding Experiments

Certainly the best documented study (and one of only three published) is Munro et al. (112). The authors of that study concluded, "saccharin administration was not accompanied by an increase in tumor incidence" (in that experiment). The NAS committee and OTA agree with that conclusion. As mentioned in our discussion of this experiment, the small number of animals alive at 24 months may be considered a flaw in the experimental design. Had there been more animals, more tumors might have been detected. This same general criticism can be made for other experiments: too few animals were used, or too few survived 24 months.

The remainder of the experiments inspire less confidence. Different investigators concentrated on different pathologies. This fact may account for the relative absence of similar lesions in the reports of different studies. Although each of these experiments can be faulted, the investigators in all of them drew the conclusion that saccharin was not carcinogenic.

C. Cocarcinogeiss Experiments With Rats

Hicks and colleagues (69, 70) devised a model system for studying induction of bladder cancer in Wistar rats. A single dose of N-methyl-N-nitrosourea (MNU) was instilled through a urethral catheter into the bladder. 'Such a single dose of 2.0 mg MNU acts as an initiator, but it does not produce a carcinoma unless additional doses of MNU are administered. Three additional instillations at biweekly intervals resulted in all animals developing either transitional cell carcinomas or transitional plus squamous cell carcinomas of the bladder epitheliums.

Hicks et al. (69) added saccharin to the drinking water of animals that received a single dose of MNU. Appropriate controls were run (see table 28). MNU alone caused hyperplasia but no tumors. Saccharin produced two mildly hyperplastic responses. The combination of saccharin and MNU produced pathology in 11 of the 12 bladders examined, and tumors in 5. The dose of saccharin (2g/kg body weight/day) is equivalent to about 4-percent dietary saccharin.

Bladder stones were found in about 20 percent of the MNU animals, none of the saccharin animals, and 6/12 MNU + saccharin animals. Each of the five tumor-bearing MNU + saccharin rats had bladder stones.

At the time this experiment was published, about one-fourth of the rats had been examined. No followup paper detailing the results of the study of the remaining animals has been found.

The second paper by Hicks et al. (70) provides additional data about saccharin as a cocarcinogen, but it does not specify what data included in reference 57 were published in reference 56, nor does it say whether results in reference 57 were obtained on animals mentioned in reference 56.

These data show that spontaneous tumors occur rarely in Wistar Rats, and Hicks (68) states that none has been seen in 10-years observation of about 600 control rats. MNU alone produced no tumors. Saccharin was associated with a small number of late-appearing tumors. In contrast, the number and time of appearance of tumors were markedly changed when both MNU and saccharin were administered.

Table 28.—Cocarcinogenicity of Saccharin in 1973 Hicks Study

Group	Treatment	Total No. of Animals in Group	No. Killed so far for Examination	Time Killed	Condition of Bladder Epitheliums
A	None	50	12	Between 9 and 56 weeks	Normal
B	2.0 mg MNU by intra-vesicular instillation	50	12	Between 3 and 50 weeks	3/1 2 hyperplastic. All killed 12 wks. after dosing 9/1 2 normal. Killed between 12 and 50 wks after dosing
c	2.0 g kg^{-1} body weight d^{-1} saccharin in drinking water	50	12	Between 9 and 56 weeks	1 0/12 normal. 2/1 2 mildly hyperplastic. Killed after 36 wks.
D	2.0 mg MNU at week 6 plus 2.0 g kg^{-1} body weight d^{-1} saccharin in drinking water from week O	50	12	Between 9 and 56 weeks	1/1 2 normal.* 6/1 2 hyperplastic* 5/1 2 epithelial tumors.*

• **See Table 29.**

For a control, MNU-treated rats were also treated with cyclophosphamide, which causes urothelial necrosis followed by hyperplasia in both ‘animals and man. No tumors resulted from treatment with both of these agents.

The authors’ conclusion from these experiments was that the model will detect weak bladder carcinogens, and that saccharin is such a weak carcinogen. Furthermore, these experiments show that stones were associated with some but not all tumors, that some bladders had stones but no tumors, and that tumors occurred in animals free of the worm Trichosomoides *crassicauda.*

Mohr (108) is in the process of repeating Hicks’ experiments. Early results from his experiments have failed to demonstrate the earlier appearance of cancers in rats

treated with MNU + saccharin. Therefore, at the present time the published experiments show that saccharin is a cocarcinogen, and unpublished work, still in progress, show that it may not be a cocarcinogen.

Table 29.—Bladder Histology of Rats Treated With MNU and Saccharin in 1973 Hicks Study

Condition of Epitheliums	**No.** of Animals	Time Killed	**No.** of Animals with Calculi in Bladder
"Normal" (3 cell thick)	1	20 weeks	o
Mildly hyperplastic (up to 6 cell layers)	2	3 and 10 weeks	1
Grossly hyperplastic plus invasion of epitheliums by blood capillaries	4	12,13,16 and 30 weeks	1
Papillary outgrowths and/or polyploid nodular hyperplasia with solid downgrowths	2	8 and 24 weeks	2
Invasive carcinoma with both transitional and squamous cell elements	3	21, and 50 weeks	3

Table 30.—Incidence of Bladder Tumors in Cocarcinogenicity Experiments in 1975 Hicks Study

Treatment	**No.** of Rats	No. with Bladder Tumors	Percent Incidence of Tumors	No. of Weeks First Tumor Seen
None	98	o	o	—
MNU	124	0	0	—
Saccharin	253	4[b]	1.6	**95**
MNU + Saccharin[a]	**79**	46	58.0	8

[a]**Given at 4-8 percent dietary levels (2-4 g/kg body weight/day),**
[b]**Tumros observed macroscopically** in males; **histology underway.**

Reuber (141) reviewed these studies and concurred that saccharin was a cocarcinogen in these studies. He commented that the data in table 29 show that at 12 weeks following MNU + saccharin, there were severe hyperplastic lesions; at 24 weeks, there were nodules; and at 50 weeks, there were carcinomas.

The hyperplasia at 12 weeks may be a precursor to cancer. Hicks (70) pointed out, however, that while the combination of MNU + cyclophosphamide produced hyperplasia and hyperpolyploid cells in the epitheliums, no cancers resulted.

These studies provide an interesting model for possible exploration. They also provide data consistent with saccharin's being a carcinogenic agent.

TESTING OF SACCHARIN IN MICE

A. One-Generation Feeding Experiments

At least four one-generation saccharin feeding experiments have been carried out in mice. All of them were considered negative by their authors. Reuber (141) has selected some data from these experiments and presented them as positive. OTA concludes that some data from two studies may show saccharin's carcinogenicity, and that a third study is inconclusive or negative; the fourth study was not reviewed,

1. National Institute of Hygienic Sciences, Tokyo (124)

(a) Experimental Design

Genetically homogeneous mice of the dde strain were fed saccharin for 21 months. Each group contained 100 mice (50 M, 50 F), and saccharin was fed at O, 0.2, 1,0, or 5.0 percent of the diet.

(b) Results

Mortality: Ingestion of saccharin had no effect on mortality.

Body Weight: There were no significant effects of saccharin on body weight.

Tumor Incidence: Results are in table 31.

(c) Discussion

The authors' discussion was not available.

Table 31.—Incidence of Tumors in Necropsied Male Mice in Japanese Study (undated)

Dose (Percent)	Rats with tumors/Rats examined				
	Time of Necropsy				
	Unspecified	12 mo.	18 mo.	21 mo.	Total
o	1 /27	0/5	6/5	1 2/13	1 9/50
0.2.	1 /23	1/5	2/5	0/17	4/50
1.0.	3/22	“ 0/5	2/5	8/1 8	1 3/50
5.0.	1/1 9	1/5	4/5	20/21	26/50

(d) Comments by Others

Reuber (119) concluded that there was a significant increase in ovarian tumors in mice ingesting saccharin (see table 32).

Table 32.—incidence of Uterine Cancers in Necropsied Mice in Japanese Study (undated)

Dose (Percent)	Rats with tumors/Rats examined				
	Time of Necropsy				
	Unspecified	12 mo.	18 mo.	21 mo.	Total
0	**0/26**	**0/5**	1/5	**0/14**	**1 /50**
0.2	**0/22**	**0/5**	**0/5**	**3/18**	**3/50**
1.0	**0/29**	**0/5**	**0/5**	**7/11**	**7/50**
5.0	**0/28**	**0/5**	**0/5**	**6/12**	**6/50**

(e) Comments by OTA

Interpretation of this experiment is difficult. If the data for uterine cancers at 21 months are considered alone, cancer incidence appears to increase with saccharin dose (table 32). However, the data for females throughout the experiment do not support that conclusion. The data do not convincingly show that saccharin caused or did not cause cancer,

Data from animals examined at 21 months support Reuber's conclusion. While there is no clear-cut relationship between dose and incidence, the incidence in the treated animals is higher. Nevertheless, this effect may be an artifact of this experiment; the following experiment did not report an increase in uterine cancers in mice fed 5-percent saccharin.

2. Bio-Research Consultants (122)

(a) Experimental Design

Groups of 26 male and 26 female randomly bred mice were fed saccharin at 10,000 ppm (about 1 percent) or 50,000 ppm (about 5 percent) of their diet. All animals dying after 6 months were necropsied, and survivors at 2 years were sacrificed and necropsied. Two experiments were run in parallel with saccharin from different sources as the only variable between them.

(b) Results

Data from determining tumor incidence are shown in table 33.

(c) Discussion

The incidence of bladder cancer did not differ in the experimental and controls. None of the three bladder tumors was malignant. One group of treated male mice displayed an increase in incidence of vascular tumors, but the increase was neither statistically significant nor confirmed in the duplicate experiment.

Table 33.—incidence of Tumors, Bladder Tumors, and Vascular Tumors Found in Necropsied Mice in 1973 Bio-Research Consultants Study

Dose (Percent)	Mice with tumors/Mice examined					
	Males			Females		
	Total	Bladder	Vascular	Total	Bladder	Vascular
Controls						
0	7/1 9	**1/1 9**	**1/1 9**	14/17	0/17	1/17
Experiment 1						
1	16/1 4	0/14	1/14	7/14	0/14	1/14
5	1 3/15	0/15	2/15	17/18	0/18	4/18
Experiment 2						
1	1 3/15	0/15	1/15	15/14	0/14	4/14
5	1 7/19	2/19	8/19	16/18	0/18	3/18

(d) Comments by Others

Reuber (141) noted an increase in lung tumors in l-percent male mice (data not shown) and increased numbers of total and vascular tumors in male mice.

(e) Comments by OTA

These data support Reuber's contention concerning an association between saccharin and an increase in total and vascular tumors in males; furthermore, the number of vascular tumors was increased in saccharin-fed female mice. Because of the absence of an additional increase in lung tumors at 5 percent, however, OTA does not accept the conclusion from these data that lung cancer was caused by saccharin.

The other mice-feeding experiments have produced no results that support these findings. OTA interprets this experiment as being more positive than its authors proposed, but in need of verification.

3. Roe **et** al. **1970 (144)**

(a) Experimental Design

The general design of these experiments resembles the cocarcinogenesis experiments of Hicks (69,70). Female Swiss albino mice were treated with an oral dose of 50 mg benzo [a] pyrene (BP) in 0.2 ml polyethylene glycol (PEG). Seven days after this exposure, one group of 50 treated animals was started on a diet which contained 5-percent saccharin. Suitable controls, PEG alone, BP + PEG, PEG + saccharin were included and two other sweeteners, cyclamate and sucrose, were tested in parallel.

Animals were examined for "obvious tumor development" at weekly intervals and daily for general health, and sick animals were killed. All killed animals, those that died unobserved, and all those terminally sacrificed at 18 months were examined. All organs including the bladder were examined microscopically, but microscopic examinations were made only of identified or suspected neoplasms.

(b) Results

Treatment with BP or BP and saccharin did not affect longevity, although BP-treated saccharin-fed mice weighed less throughout the experiment than did non-BP-treated saccharin-fed mice. The authors had no explanation for this finding and considered it possibly spurious.

Microscopic examination for tumors produced the results shown in table 34.

Table 34.-Tumor Incidence (in Necropsied Animals) and Survival in 1970 Roe Study

Treatment	Dead Before 18 Mos.[a]	Tumors of Forestomach	Other Tumors
PEG	26/1 00	0	10
PEG & BP	30/1 00	0	14
PEG & Saccharin	6/50	o	0
PEG & BP & Saccharin	1 0/50	o	4
	Sacrificed at 18 Mos.[a]	**Tumors of Forestomach**	**Other Tumors**
PEG	65/1 00	2	24
PEG & BP	61/1 00	21	29
PEG & Saccharin	36/50	o	13
PEG & BP & Saccharin	32/50	11	12

[a]**Mice with** tumers/Mice examined.

(c) Discussion

Administration of BP resulted in an increase in forestomach tumors, but feeding of saccharin did not cause a further increase. No macroscopic bladder tumors were observed.

(d) Comments by Others.

None.

(e) Comments by OTA

The absence of microscopic pathological examinations from this experiment makes it impossible to compare these results to experiments that included such examinations.

Sacrifice of animals at 18 months, of course, eliminated any possibility of detecting tumors that would have developed later. Because of this fact, the results of this experiment cannot be compared to those of the other two experiments (122,124) in which mice were sacrificed at 21 or 24 months.

4. Verschuuren, et al. (143)

OTA was unable to obtain this report.

B. Implantation Experiments in Mice

Pellets of cholesterol containing saccharin were implanted into the urinary bladders of mice. Control animals received cholesterol pellets only. In both experiments (5,25) the incidence of tumors was significantly higher in the experimental.

Table 35.—Survival of Mice Living More Than 175 Days After Bladder Implantation and incidence of Changes in Mouse Bladders With Implants of Sodium Saccharin Suspended in Cholesterol in 1971 Bryan Study

Experiment no.	Mice Examined	Average Survival (days)	Squamous Metaplasia	Carcinomas		
				Total	Percentage	P Value
		Cholesterol alone				
1	63	378	1	8	13	
2	43	394	3	5	12	
		Saccharin & Cholesterol				
1	66	375	3	31	47	<.001
2	64	396	6	33	52	<.001

Positive results from implantation experiments were judged as warning signals of the possible carcinogenicity of saccharin (117). However, doubts were raised about how closely implantation mimics normal ingestion of saccharin, and the significance of tumor induction by implantation was questioned. The special concern about saccharin being a carcinogen of the bladder may have grown (at least partially) out of these studies. No regulatory action has been based on these studies.

TESTING OF SACCHARIN IN OTHER ANIMALS

A. Hamster Experiments (6)

1. Experimental Design

Groups of randomly bred Syrian golden hamsters were given 0.0-, 0.156-, 0.312-, 0.625-, or 1.25-percent saccharin in their drinking water for life. A group of 30 males and 30 females was fed at each level. Gross and histological examinations were performed on all hamsters.

2. Results

No tabular data were presented. The incidence of tumors was 10.1 percent in 169 controls and 14.9 percent in the 299 animals ingesting saccharin.

3. Discussion

The authors reported that the organ distribution and histological types of

neoplasms were within the range found for spontaneous tumors. They concluded that saccharin was not carcinogenic.

4. Comments by Others

None.

5. Comments by OTA

This experiment appears to have been well-executed. It produced no evidence for carcinogenicity. The levels of dietary saccharin given to the hamsters were less than those given to rats, and the experiment was of one generation duration. Therefore, while it is not positive, neither does it contradict the findings of the two-generation rat experiments.

B. Monkey Experiments (35)

1. Experimental Design

Aqueous sodium saccharin was administered by stomach tube to rhesus monkeys for 6.7 years. Doses of 20, 100, or 500 mg saccharin/kg/day (6 days a week) were given respectively, to 2, 2, and 3 monkeys. Three animals of each sex were used as controls. Routine hematological examinations and assays of serum components were carried out at 6-month intervals. During the sixth year of the test, urine was examined to determine whether long-term administration had caused any detectable metabolic adaptation.

2. Results

Three saccharin-fed monkeys died during the 6.7 years of testing. There were no tumors in their bladders. No metabolic adaptation was detected in the saccharin-fed animals, and no pathologies were associated with the sweetener.

3. Discussion

The authors concluded, “Our experience to date tends to reinforce the mounting evidence of noncarcinogenicity” (35).

4. Comments by Others

None.

5. Comments by OTA

The results of this experiment support the thesis that ingestion of saccharin by monkeys for 6.7 years does not induce new patterns of metabolism. This experiment may not have been suitable to test carcinogenicity because of the small numbers of animals involved and because the route of ingestion does not mimic human exposure. But it does show that ingestion of saccharin for 7 years produced no ill effects in monkeys.

SUMMARY STATEMENT ABOUT ANIMAL TESTING

In summary, the three most sensitive tests (49,67,165) have produced data showing saccharin is a carcinogen. While none of the three studies considered alone might have allowed a conclusion about the effect of saccharin on the bladder, the three taken together allow conclusions to be made.

The data from all three experiments show that: (1) saccharin-fed second generation rats had more bladder cancers than did control animals, (2) the bladder cancers were only weakly invasive and did not metastasize, (3) bladder cancers occurred in animals ingesting saccharin at 5 or 7,5 percent of their diet, and (4) male rats are more sensitive than females.

A statistical analysis of the results of the two-generation rat feeding experiments is shown in table 36. Results are presented as a fraction; the numerator is the number of bladder cancers, and the denominator is the number of animals (at least 18 months old) examined. In each experiment, the number of cancers found in the saccharin-fed animals exceeded the number found in the controls.

Table 36.—Results and a Statistical Analysis of the Two-Generation Rat Feeding Experiments

Study	Generation	Dose[a] (Percent)	Males[b]	Females[b]	Significance
Canada, 1977 (55)	Parental	0 5	**1 /36** **7/38**	0/38 0/40	p=0.075
Canada, 1 977(55)	Offspring	0 5	**0/42** **12/45**	0/47 2/49	p=0.003
FDA, 1973 (37)	Offspring	0 7.5	**1 /25** **7/23**	0/24 2/31	p=0.0l7
WARF, 1973 (135)	Offspring	0 5	**0/10** **8/15**	0/16 0/20	p=0.021

[a]Saccharin as a percent of the total diet. Five (5) percent is equal to about 2.5 g saccharin/kg body weight/day.
[b]Rats with bladder cancer/Rats examined.

To conclude, the two-generation experiments showed that saccharin caused an increase in bladder cancer and especially among males.

Two experiments (69,70) concerning the cocarcinogenic potential of saccharin are discussed in published reports. Instillation of single small doses of a chemical (methylnitrosourea, MNU) into the bladders of rats did not cause cancer; however, repeated instillations of the same chemical at 2-week intervals did cause cancer. The first dose is called an “initiator” dose, and the subsequent doses are “promotor” doses. Saccharin was shown to be a potent promotor. Ingestion of 2-percent or 4-percent saccharin before and after receiving one dose of MNU caused a significant increase in bladder cancers compared to animals receiving only MNU. Specific objections can be directed at efforts to apply these results to human conditions. It is

unlikely that humans encounter MNU, but it is equally clear that we are exposed to many other chemicals. The possibility exists that saccharin might be a cocarcinogen with other chemicals in our environment.

The results of implanting pellets of saccharin in mice bladders show that saccharin caused cancer in that animal (5,25). A number of reservations have been attached to extending the finding from implantation experiments to the human population. However, about 50 percent of animals exposed to saccharin in this way developed bladder cancers as compared to 13 percent in animals exposed to cholesterol only. Regardless of the interpretation placed on these experiments, it is important to note that saccharin has been shown to cause cancer in an animal other than the rat.

Data from one-generation rat feeding experiments have sometimes been judged to be positive and sometimes negative. Some of the studies do not support any conclusions. None of the negative experiments followed the protocols used in the two-generation experiments, and therefore no data contradict those positive results.

A committee of the NAS reported in 1970 that the two long-term rat feeding experiments available to them at that time were inadequate when judged against the current standards for animal testing (1 17). In 1974, seven additional studies were evaluated by a NAS committee (116). According to NAS, none of the studies provided positive evidence for saccharin's carcinogenicity. The design and execution of only one of those experiments, however, approaches the current guidelines for animal testing. It is more prudent to conclude that these studies were not conducted adequately than that they were negative.

Because of the deficiencies in the one-generation experiments, their data are viewed with skepticism. The data from the two-generation experiments lead to the conclusion that saccharin ingestion is associated with cancer. The lack of association in the one-generation experiments does not force any qualification of this conclusion.

At least four one-generation feeding experiments have been carried out in mice. Although none of them produced convincing evidence that saccharin causes cancer, neither are they convincingly negative. Experiments carried out with hamsters and monkeys showed that saccharin was not a carcinogen in those animals under the conditions of those experiments.

Biochemistry performed during the course of the monkey experiments showed that ingestion of saccharin for several years did not induce changes in metabolism. These results agree with others that show ingested saccharin is excreted unchanged, that is, without being metabolized (17).

This brief recapitulation of the history of saccharin illustrates how rapidly the nature of testing for carcinogenicity has evolved. Recently performed, more sensitive experiments have demonstrated the carcinogenicity of saccharin. Earlier, less sensitive experiments did not.

An important caution is attached to this part of the report. "Saccharin" is a mixture that contains the named substance and other chemicals. Although the saccharin used in the most recent experiment contains only about 20 parts per million impurities, it cannot be eliminated that an impurity is the carcinogen in saccharin. This possibility does not alter the conclusion that the mixture sold as "saccharin" is a carcinogen. Instead it may be that the chemical saccharin is safe, but the product consumed as "saccharin" may have a carcinogenic substance in it.

In summary, the three most sensitive tests have produced data showing saccharin is a carcinogen. Other experiments have not produced convincing data to support that conclusion. OTA has presented its reasons for considering some of the negative experiments to be inconclusive rather than convincing,

It was mentioned above that none of the negative experiments followed protocols comparable to the positive experiments. Therefore, there are no data from similar experiments that contradict the positive experiments. To date, none of the experiments (except implantation experiments) in species other than the rat have produced unequivocal evidence that saccharin is a carcinogen. These results may mean either that the experiments were negative or that they were not sufficiently sensitive. Whichever, it is prudent to take the results of experiments in the most sensitive species to determine carcinogenicity (121).

EXTRAPOLATIONS FROM LABORATORY DATA TO HUMANS

A. General Considerations

Several types of extrapolations can be applied to data from animal tests. Three types of extrapolations are listed below.

1. **From detected cancer incidence at high doses to projected incidence at low doses.** Usually, high doses of carcinogens are administered to produce a detectable number of cancers in small groups of animals; in the case of saccharin, effective doses are about 5 percent or more of the diet. A number of methods have been employed to extrapolate from incidence at high doses to predicted incidence at much lower doses.
2. From carcinogenicity **in animals to carcinogenicity in humans.** It is generally accepted that an animal carcinogen is also a human carcinogen. Extrapolation between cancer incidence in animals and expected incidence in humans is necessary to quantify the risk for human populations from exposure to a chemical.
3. **From mutagenesis frequency to carcinogenesis frequency.** Many carcinogens have been found to be mutagenic in short-term tests. This correlation serves as the basis for saying that agents shown to be mutagenic are probably carcinogenic.. Methods for quantitatively extrapolating from the mutagenic potency of a chemical to its expected carcinogenicity in laboratory animals have been developed.

The validity of conclusions reached by extrapolations depends on the accuracy and reliability of the experimental data as well as on the extrapolation procedures. Unfortunately, the saccharin data are not so good as to eliminate all misgivings about using them for quantitative estimates of human risk. The data are good enough, however, to allow the qualitative judgment that saccharin presents a potential risk to human health.

The interpretation of dose response curves depends on the model used for extrapolation. Acute toxicity testing produces dose response curves with “no-effect” or “threshold” levels. No toxicity is associated with doses below such levels. Mutagenicity testing, on the other hand, produces curves with no thresholds. Even

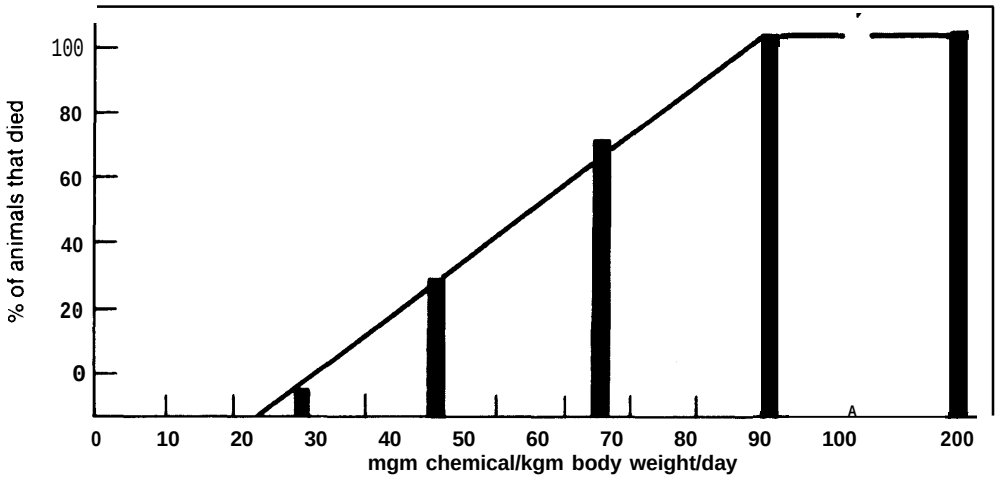
very small doses have an effect. There is a lack of agreement about which of these curves describes the incidence of cancers in a population exposed to a carcinogen, and no experiment designed to decide between the two models is accepted as definitive.

B. Dose-Response Relationship

1. Acute Toxicity Testing

Toxicity is usually tested in laboratory animals by dividing a population of genetically and nutritionally similar animals into subgroups. Each group is exposed to a particular amount of the substance in question, and the animals are observed over a period of time to detect possible deleterious effects of the substance. Figure 3 is a hypothetical example of the results for such a test to determine the dose of the substance that would be necessary to cause death in a population of animals.

Figure 3-Hypothetical Toxicity Curve



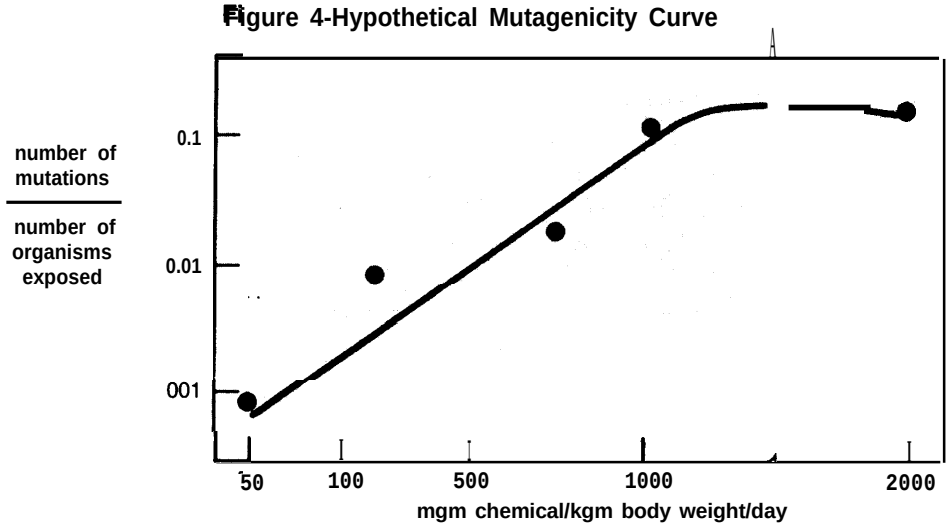
From the data, it can be concluded that doses of 100 and 200 mgm kill all animals, doses in the range from 30 to 100 mgm kill some fraction of animals, and doses of 10 to 20 mgm produce no deaths. Another conclusion to be drawn from these results is that at a dose somewhere between 20 and 30 mgm, there is a ‘‘no-ef feet level. ” Another term used to describe the no-effect level is “threshold.” Doses below the threshold level produce no effects. The line drawn in figure 3 is a “dose-response curve. ”

The outcome measured in the experiment in figure 3, death, concludes a complicated series of events. Death might result, for instance, if the substance kills cells. Increasing doses would kill more and more cells, but an organism would survive until the number of dead cells became intolerably large. Individual variations in the ability to tolerate dead cells would partially account for intermediate survival levels between doses of 30 and 100 mgm.

2. Mutagenicity Testing

A different type of dose-response curve describes the induction of mutations in a population. Such a curve is shown in figure 4.

Figure 4-Hypothetical Mutagenicity Curve



This curve has no threshold level, As the dosage is decreased, the frequency of mutations becomes lower and lower but does not reach zero. Instead, it levels out at the frequency equal to the spontaneous mutation rate. Practically, it becomes more and more difficult to measure low level mutagenesis, i.e., at the lowest dose in figure 4, only about 1 individual in 1,000 would be expected to harbor a mutation. For technical reasons, many such mutagenic dose-response experiments have been conducted with microorganisms in which 100 million organisms can be rapidly screened.

In contrast to toxic substances that may have to injure or destroy a large number of cells to produce death, a single interaction between an agent and DNA can produce a mutation. After the interaction, the normal cellular metabolism replicates and perpetuates the mutation, Death requires injury and subsequent pathology; mutation requires only an injury followed by normal cellular functions. These differences between the two measured events, death and mutation, may at least partially explain the differences between figure 3 and figure 4. Mutations also occur spontaneously; some number of mutations will appear even with no dose.

3. Carcinogenicity Testing

Examples such as figures 3 and 4 spark little controversy. Toxicity testing usually produces a curve with a threshold value, an area of increasing response to increased dose, and a plateau. Mutagenicity testing produces a curve with no threshold, an area of increasing response to increasing dose, and a plateau. In contrast, there is some controversy about whether a curve such as that in figure 3 or that in figure **4** describes the frequency of cancer as the dose of carcinogen decreases.

Some scientists suggest that an animals' immune system can protect it against cancer (27). If the immune system has a finite capacity to recognize and eliminate precancerous and cancerous cells, no carcinogen would cause a cancer until that capacity

is swamped. Such a mechanism could result in a threshold. However, there is little experimental support for this idea, and other scientists think that the immune system may promote tumor growth (138).

Many chemical carcinogens act after conversion of the administered compound to a highly reactive metabolize. This metabolize, in turn, chemically links to a vital component of the cell. In the case of some compounds that cause tissue damage, the cell contains protective agents, which inactivate the reactive metabolize. Cell damage occurs only after the supply of the protective substance has been exhausted. Additionally, other mechanisms may exist to detoxify or to excrete dangerous chemicals and metabolizes or to repair damaged DNA. If the efficiency of these protective systems is perfect until a finite limit is exceeded and if the system fails at that point, a threshold might result.

There are a number of problems involved in designing and executing an experiment to decide whether there is a threshold dose for a carcinogen. Large numbers of animals would be required, the experiment would be very expensive, and inspecting a large number of animals for cancers introduces many possibilities for human error. Even if such an experiment were adequately conducted, and a threshold obtained, this result would not necessarily mean that a threshold exists in human populations. Members of a laboratory animal population are inbred and genetically very similar. Humans, on the other hand, are outbred and genetically quite dissimilar. If all of the animals have the same capacity to detoxify small amounts of a chemical, this ability could account for the threshold. If humans have a similar capacity for detoxification, this capacity would be expected to vary widely among members of the population because of their genetic differences. Some people might be very sensitive; others less so. This wide variation makes it impossible to predict a threshold for human populations.

Carcinogenicity studies conducted with animals rely on doses many times higher than those to which humans are exposed. Such doses are necessary because of the limitations of animal experiments. Usually, groups of about 100 animals (50 male, 50 female) are exposed to a constant dose of a carcinogen. In the 1977 Canadian Study, 8.9 percent of animals exposed to 5-percent dietary saccharin from the time of weaning developed tumors. A tenfold reduction in the dose to 0.5 percent would be expected to result in a tenfold reduction in tumor frequency to about 0.9 percent. Of course, 0.9 percent of a group of 100 animals is less than one animal. Increasing the size of the exposed population would allow detection of the lower frequencies, but the cost of scaling up the experiments is very high. Those who accept the curve in figure 4 as a representation of carcinogenesis say that positive effects at high doses demonstrate a danger at any level. Those who believe that there is a threshold (figure 3) say that high dose experiments show only that high doses cause cancer, and that doses below the threshold may pose no danger. Determining a threshold level for any carcinogen has so far been impossible. Cancer, however, is a relatively common disease. If thresholds exist, they are frequently exceeded, and any additional exposure to a carcinogen might push more individuals across the threshold.

C. Extrapolation Methods

1. Extrapolation from detected incidence of cancer at high doses to expected incidence at lowest doses.

(a) The Question Of Thresholds. As already mentioned, an argument is sometimes made that there is a dose below which a carcinogen will not cause cancer. Craig

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and Miller (36), in a review of 151 dose-response curves, found none inconsistent with a no-threshold curve. Currently, the burden of proof is on those who espouse a threshold model. The subsequent discussion assumes that if a threshold exists, it has not been demonstrated, it has not been measured, and it cannot serve as a practical tool for making extrapolations.

(b) The Probit-Curve or the Marztel-Bryan (103) *Extrapolation.* This paper provides an easy-to-read introduction to the problem of establishing "absolute safety. " For example, what conclusions can be drawn if 100 animals are exposed to a dose of a suspected carcinogen, and no animal develops a tumor? At the 99-percent probability level, this experiment provides assurance that the true risk is less than or equal to 4.5 percent. Increasing the number of tested animals to 1,000 (assuming none develops cancer) reduces the risk to 0.45 percent, but it does not assure absolute safety.

This method estimates the risk of cancer by assuming that the risk decreases one standard deviation (one "probit") as the dose of carcinogen is decreased by 10. In the example of 100 cancer-free animals, the risk at that dose, D, is 4.5 percent. If it is decided that a risk of 1 cancer per 100 million exposed individuals represents "virtual safety" and is thus acceptable, this method calculates (using the one probit decrease for a tenfold dose-decrease relationship) that a dose of D/8,300 is acceptable. An attractive feature of this method is that it rewards "good testing. " For example, if the dose D is shown to be safe for 100 animals, the virtually safe acceptable dose would be D/8, 300: if 1,000 animals were used, the acceptable dose would be D/1,000; and if 50 were used, the acceptable dose would be D/18,000, Testing higher doses would result in acceptance of higher permissible levels. The paper also describes the application of this procedure to estimating risks when experiments show that cancers are caused by high doses.

In another readable paper Mantel and Schneiderman (105) argue for the general application of the Mantel-By ran model. However, this extrapolation procedure has been criticized because it leads to higher doses being associated with "virtually safe" incidence than does the single-event or one-hit hypothesis.

(c.) The Single-Event or One-Hit Hypothesis. This simple model proposes that the probability of a normal cell being transformed into a cancer cell varies directly with the dose. This relationship assumes that a single cell can be transformed into a cancer cell and can develop into a tumor. Evidence supports the idea that cancers result from single transformed cells (37).

Schneiderman (153) in testimony before the Congress used a no-threshold/linear model to estimate the number of bladder cancers to be expected in the United States if people areas sensitive to saccharin as are rats. Schneiderman used this model rather than one incorporating a probit or other relationship, although he has published (105) arguments for the use of probit relationships. Schneiderman (153) estimated that continual consumption of one 12 oz. diet soda per person per day would result in 600 to 1200 new cases of bladder cancer per year.

The one-hit models are most "conservative" in that they associate the highest risk with a given dose. Application of the one-hit model is recommended by Heel et al. (72) and the National Academy of Sciences (115).

The one-hit hypothesis fits some data available about humans. The incidence of leukemia among survivors of nuclear blasts, the incidence of various tumors follow-

ing occupational and therapeutic radiation, and the incidence of lung cancers in people who smoke cigarettes all vary directly with dose (quoted in 97 and see figure 1 for cigarette data).

(d) *Multiple stage models.* This family of theories proposes that more than one independent event is necessary to cause cancer. These models project curves that are concave upwards with increasing dose. Crump et al. (37) present arguments that at low doses such multihit models approach linearity.

(e.) Other models and discussions. Many are recommended only for the statistically sophisticated and are listed in the references of Heel et al. (72).

2. Extrapolations From Animal Experiments to Man

With appropriate experimental design and attention to detail, convincing results showing a relationship between cancer and exposure of an animal to a chemical can be obtained. Some discussion and extrapolation from those results allow a family of curves to be constructed that relate the observed incidence at high doses to the expected incidence at low doses.

The National Academy of Sciences has recommended that carcinogenicity testing be carried out in more than one species and that the results obtained with the most sensitive animals be applied to human populations (115). Adjustments must be made for differences in dose between animals and humans. Heel, et al. (51) recommended that doses be adjusted on the basis of relative surface areas, which are calculated as (man's weight/test animal's weight)$^{2/3}$. When chemicals are administered in the diet, doses expressed as percent dietary intake or parts per million (ppm) require no further adjustment.

Carcinogenicity testing in more than one species may be especially important because "laboratory animals are inbred. A particular strain may be very sensitive or very insensitive to a particular agent. Human populations contain individuals of widely differing sensitivities. Extrapolations from inbred animals to human populations can be better made with more data, but considerations of safety require that data from the most sensitive animal model be used for estimating human risk.

D. Relationship Between Short-Term Tests and Animal Tests

Meselson and Russell (107) have constructed a formula that relates the potency of a chemical in one short-term test, the Salmonella/Ames test, to its carcinogenicity in animals.

Fourteen chemicals that have been adequately studied in both animal systems and in the Salmonella/Ames test were included in the calculations. When carcinogenicity was plotted against mutagenicity, 10 of the chemicals fell on or near a straight line with a slope of 1. Therefore, mutagenicity correlates with carcinogenicity. Three nitrosoamines and N-nitrosomethylurea are not so mutagenic as expected. Refinements in the Salmonella/Ames test may improve the correlation between the mutagenicity and carcinogenicity of the nitroso compounds.

This demonstrated relationship is one of the first quantitative attempts to relate short-term mutagenicity testing to carcinogenicity. Combining this procedure with extrapolations from animals to humans could enable a person (a very brave one) to predict carcinogenicity of a chemical for humans on the basis of short-term test data.

No extrapolation from short-term testing of saccharin has been made. So far, short-term tests of saccharin have shown it to be nonmutagenic in the Salmonella/Ames test. A mixture of impurities extracted from saccharin is mutagenic, but the data are not yet firm enough to base extrapolations on them.

E. Extrapolation of Saccharin Data

The tools for extrapolation are available, and experiments have produced data with which extrapolations can be made. Even so, questions arise about whether the data are adequate for the extrapolations and whether the extrapolations make accurate predictions.

Table 37 presents a number of extrapolations that have been made to estimate human risk in a population that ingests one can of diet soft drink, containing 120 to 150 mg saccharin, per person per day. In all the extrapolations, calculations are based on data from the two-generation rat experiments (49, 67, 165). It is assumed that the population at risk is 200 million people, that life expectancy is 70 years, and that human sensitivity is the same as that of the male rat.

The data clearly show that the method chosen for dose adjustment has a sizable effect on the extrapolated figure. At present, there is no generally accepted choice among the adjustments, and this table is presented primarily to show the variety of figures possible.

Table 37.—Estimated Risks from Saccharin Consumption

Estimate 1
Dose adjusted to surface area by the expression mg/kg/day [human] = 5.6 mg/kg/day [rat]

Method of extrapolation	*Cases/year*
a. linear (71)	3,400
b. quadratic (a multistage model) (71)	15

Estimate 2
Dose adjusted to body weight by the expression mg/kg/day [human] = mg/kg/day [rat]

Method of extrapolation	*Cases/year*
a. linear (1 46)	600
b. linear (1 53)	600 to 1200

Estimate 3
Lifetime dose adjusted to body weight by the expression mg/kg/lifetime [human] = mg/kg/lifetime [rat]

Method of extrapolation	*Cases/year*
a. linear (23)	15,000

CONCLUSION

The animal studies on the possible carcinogenicity of saccharin have been analyzed, and reasons have been given for ascribing more confidence to some experiments than to others. The most reliable findings have been those from two-generation rat feeding experiments.

Several competing theories on extrapolating from the results of animal tests to humans have also been discussed, followed by application of some of the mathematical models to the available data on saccharin. Different models yield different results. There is no basis for judging which, if any, of these figures is accurate.

Appendix II

SHORT-TERM TESTS*

INTRODUCTION

A number of short-term tests have been developed to aid in evaluating the potential of substances to cause cancer. These tests can be conducted quickly, often requiring only a few weeks. Short-term tests examine the capacity of a substance to cause mutations or other genetic alterations. Most chemical carcinogens are either mutagens and/or can be shown to interact with DNA. Most short-term tests are designed to detect one or the other of these properties. The tests are in varying stages of development, and some have been more widely used than others.

Until this study, saccharin had not been systematically or extensively examined in short-term tests. About 20 studies of saccharin have been reported in the scientific literature, and they fall into three general types of short-term tests:

(1) Tests using Drosophila;

(2) In vitro and *in* vivo tests (in mammals) for induction of chromosome abnormalities; and

(3) Tests for the induction of dominant lethal mutations in mice.

The published results available through 1975 were reviewed by Kramers (85), who stated that an unequivocal conclusion about the mutagenicity of saccharin was not possible. Although some reports suggested that saccharin might have weak mutagenic activity, the reports contained limited or conflicting data, and positive results were of only borderline significance. In one study in which genetic effects were found (positive dominant lethal effects and chromosomal translocations in spermatocytes in mice), Kramers suggested that chemical impurities in the saccharin preparation, and not saccharin itself, could have been the responsible agent.

Reports subsequent to Kramers' review have not clarified the situation. Chinnici (29) did not detect crossing over in the X chromosome after growing Drosophila *melanogaster* (fruit flies) on culture medium containing 5-percent saccharin. It is unlikely that this dose level was achieved, however, because Drosophila do not ingest food containing saccharin at such high levels (172). Van Went-de Vries and Kragten (174) did not detect any chromosome abnormalities in bone marrow 'cells after oral administration of 1.5 g/kg/day of saccharin to Chinese hamsters for 3 days. The study was quite small, however, and only 50 metaphrases were examined for abnormalities. Machemer and Lorke (101) reported no increase in chromosome aberrations in spermatocytes of Chinese hamsters. This report is somewhat in contrast to the earlier

*Joyce C. McCann, a member of the advisory panel for this study, coordinated the OTA short-term test battery and was the principal author of this appendix.

positive results in mice (156); however, comparisons are difficult because species of test animals, doses, and routes of administration differed in the two experiments. Machemer and Lorke (100,101) reported orally administered saccharin to be negative in causing dominant lethal mutations in both male and female mice. The earlier positive study (156) used a different route of administration (intraperitoneal injection), making comparisons uncertain. Two other studies (126,150) reported some positive effect after oral administration of saccharin, but as discussed by Kramers, these results were questionable.

OTA SHORT-TERM TESTS

As part of this study, the Office of Technology Assessment commissioned a battery of 12 short-term tests to be conducted on saccharin, This battery marked the first time that saccharin had been tested by many of these methods. The purpose of conducting these short-term tests was to demonstrate to the Congress the nature of the tests, the speed with which they can be conducted, and their usefulness in making regulatory decisions. The test battery, which took about 3 months to complete, illustrates one way that short-term tests can be applied to a particular regulatory problem.

It also seemed possible that conducting a battery of short-term tests might help to clarify some of the uncertainties regarding the carcinogenesis of saccharin. Since saccharin causes cancer only at high doses in rats, there is controversy about whether saccharin is a carcinogen at the lower doses to which humans are exposed, or whether some secondary effect introduced by high doses causes cancer in rats. Similarly, questions have arisen about whether saccharin itself or impurities in saccharin caused the positive results in the carcinogenesis experiments. Positive results in short-term tests would add weight to the argument that saccharin is a carcinogen. The battery of tests was designed to determine, as definitively as possible within the time limits of this study, whether highly purified saccharin is mutagenic or causes other genetic alterations.

Of the 12 short-term tests commissioned by the OTA, 10 have been completed. The tests were conducted by their developers or recognized experts, who generously donated their time to this study. The tests conducted and the principal investigators are presented in table 38.

Table 38.—OTA Saccharin Short-Term Test Battery

	Collaborating Investigators
Positive Tests	
Sister Chromatid Exchange	Dr. Sheldon Wolff/Dr. Brita Rodin Laboratory of Radiobiology University of California San Francisco, Cal if. 94143
Mouse Lymphoma	Dr. Donald Clive Genetic Toxicology Laboratory Burroughs Wellcome Company Research Triangle Park, N.C. 27709

Table 38.—OTA Saccharin Short-Term Test Battery—Cont.

	Collaborating Investigators
Chromosome Aberration (CHO Cells)	Dr. Abraham Hsie/Dr. Juan San Sebastian Biological Division Oak Ridge National Laboratory P.O. Box Y Oak Ridge, Tenn. 37830
Negative Tests	
Salmonella/Ames	Dr. Bruce Ames/Dr. Joyce McCann Department of Biochemistry University of California Berkeley, Calif. 94720
Mitotic Recombination in yeast (D3)	Dr. Vincent Simmon Applied Microbiology Program Stanford Research Institute Menlo Park, Calif. 94025
Pol test (E. Coli)	Dr. Herbert Rosenkranz Department of Microbiology New York Medical College Valhalla, N.Y. 10595
Drosophila (sex-linked recessive lethal test)	Dr. Seymour Abrahamsen/Dr. Ruby Valencia Department of Zoology University of Wisconsin Madison, Wis. 53706
Unscheduled DNA synthesis (human fibroblasts)	Dr. Hans Stich Cancer Research Centre The University of British Columbia Vancouver, Canada V6T 1W5
In Vitro Transformation (hamster embryo cells)	Dr. Roman Pienta Frederick Cancer Research Center Frederick, Md. 21701
Induction of Plasminogen Activator (HeLa cells)	Dr. 1. B. Weinstein College of Physicians and Surgeons of Columbia University Institute of Cancer Research 99 Fort Washington Avenue New York, N. Y. 10032
Tests in Progress	
In Vitro Transformation (mouse C3H 10T1 /2 cells)	Dr. Charles Heidelberger/ Dr. Suktab Mondal University of Southern California Cancer Research Building 1303 North Mission Road Los Angeles, Calif. 90033
CHO/HGRPT	Dr. Abraham Hsie/Dr. Patrick O'Neill Biological Division Oak Ridge National Laboratory P.O. Box Y Oak Ridge, Tenn. 37830

The battery of tests included many of the most sensitive short-term tests currently available. Criteria for the inclusion of tests in the battery were: (1) sensitivity and validity for detecting carcinogens; (2) complementarily with the other tests in the battery and with test literature on saccharin; and (3) ability to be completed within the time constraints of the OTA study. Saccharin had been tested previously by only 2* of the 12 methods.

All tests were conducted using the same sample of saccharin that was used in the most recent Canadian carcinogenicity tests in rats. This material is referred to as "impure saccharin;" even though highly purified, it still contains very small amounts (about 20 ppm) of impurities. For this reason, all participating laboratories also received a sample of saccharin that had been specially purified to remove essentially all traces of impurities. This material is referred to as "pure saccharin."**

Results from three tests-sister chromatid exchange, mouse lymphoma, and chromosome aberration—were positive. Highly purified samples of saccharin were weakly active in these tests, and the results are clearly suggestive that saccharin itself has mutagenic properties. The results should be regarded with some caution, however. The responses were very weak in the three tests, even at the high dose levels tested. And the value of the sister chromatid exchange, mouse lymphoma, and chromosome aberration tests in predicting carcinogenicity has not yet been firmly established. However, validation of the tests has begun by testing a number of carcinogens and mutagens and a few noncarcinogens, with promising results.

The results of 7 of the 10 completed tests in the OTA test battery were negative; that is, saccharin did not cause mutagenic or other genetic alterations in the tests. These negative results, even in well-validated tests such as the Salmonella/Ames test, do not invalidate or cast suspicion upon the positive results for several reasons. Saccharin is detected as a carcinogen in rats only at high doses and is therefore called a "weak carcinogen. " Mutagenic effects were detected only at very high dose levels (5-10 mg/ml), and this fact, coupled with the results in the seven negative tests as well as the preponderance of negative results in the published literature, indicates that any mutagenic properties of saccharin are very weak. Thus, this property might not be detected in most short-term tests. Each of the short-term tests has its own set of limitations, both in sensitivity and in the range of chemical classes it can detect. Although it would be surprising if a potent carcinogen were negative in many different kinds of short-term tests, it is not surprising that a carcinogen such as saccharin might be detected in only a few systems.

Saccharin was detected only at very high dose levels in the three positive tests, In all but two of the seven negative tests, these dose levels either were not tested or could not be tested because lower doses were toxic. In the Pol test, the highest dose tested was about 0.02 mg/ml; in the plasminogen activator test, 0.05 mg/ml; and in the *Drosophila* sex-linked recessive lethal test, the highest concentration of saccharin that was well ingested was 2.5 mg/ml. In other tests, the highest nontoxic doses found were about 1 mg/ml in the Salmonella/Ames test; 3 mg/ml in the hamster embryo

*Several sex-linked recessive lethal tests in *Drosophila* have been published, with somewhat conflicting and uncertain results. Results obtained by Stoltz et al. (162) using the Salmonella/Ames test were independently confirmed for the battery.

**Both the "impure" and "pure" saccharin were generously provided by D. Stoltz and B. Stavric.

fibroblast *in vitro* transformation test; and 2 mg/ml in the 10T 1/2 in vitro transformation test. High doses (up to 50 mg/ml) were tested in the mitotic recombinant test in yeast, but the absence of any toxic effect even at such high dose levels suggests that saccharin may not have penetrated the yeast cell wall sufficiently. Only in the unscheduled DNA synthesis test were doses of 5-10 mg/ml tested.

It was somewhat surprising that saccharin was not positive in the unscheduled DNA synthesis test. This method is quite sensitive and measures changes that are likely to occur during the sister chromatid exchange and chromosome aberration processes. Additionally, the unscheduled DNA synthesis and sister chromatid exchange experiments were conducted in human cells (albeit different cell types) over similar dose ranges, *

Interpretations of the validity of the carcinogencity tests on saccharin are complicated by the presence of variable, and usually unspecified, amounts of impurities present in different batches of saccharin. The possibility that impurities might be responsible for the positive carcinogenicity test results on saccharin has long been debated. Even results from the most recent Canadian studies (in which saccharin containing only approximately 10 to 20 ppm impurities was tested) cannot unequivocally be considered as caused by saccharin itself, rather than by unknown carcinogenic impurities.

If carcinogenic impurities are present in preparations of saccharin, their detection and identification can be facilitated by demonstrating their mutagenicity in short-term tests. Uncertainties regarding impurities are characteristic of the published short-term data on saccharin, almost all of which are negative or of borderline validity.

Stoltz et al. (14,162) have demonstrated that the impurities found in the saccharin used in the Canadian study are mutagenic in the Salmonella/Ames test. The impurities are only weakly mutagenic, and it is not clear that they are sufficiently potent to have caused the positive carcinogenicity result. However, samples of saccharin used in the Canadian cancer tests contained far lower levels of impurities than commercial saccharin (to which humans are exposed), and it is possible that carcinogenic impurities in commercial saccharin pose a greater human health hazard than saccharin itself.

POSITIVE TESTS

1. **Sister Chromatid Exchange (SCE)**

The sister chromatid exchange (SCE) test (134,161) is similar to other cytogenetic procedures in that it measures changes in chromosomal structure. However, it employs a special staining technique to detect subtle changes that do not affect gross chromosomal structure. Classical cytogenetic techniques depend upon such gross changes in structure for detection. In many cases, SCEs have been shown to occur more frequently than gross chromosome aberrations after treatment of cells with chemical mutagens. For this reason, the SCE test may be a more sensitive method than standard cytogenetic procedures for detection of chemicals which have weak

*Similarly, the CHO/HGPRT test (still in progress) is closely related to the mouse lymphoma test, and it will be of interest to see if saccharin is detected in this system.

cytogenetic activity. The role of SCEs in the generation of mutagenic events in cells has not been proven. But considerable experimental evidence, consistent with the theoretical understanding of how mutations are likely to occur, indicates that events which cause SCEs also can cause mutations. A number of carcinogens and a few non-carcinogens have been tested using the SCE procedure (1,134,161), and the correlation looks promising. However, the method needs to be thoroughly validated to demonstrate its value as a predictor of carcinogenicity and mutagenicity.

The SCE data on saccharin (tables 39 and 40 and figure 5) are convincing, especially the data obtained on human cells (table 39), which shows a clear dose-response effect (figure 5). The effect is weak in both Chinese hamster and human cells, but the observation is well documented. * The fact that both impure and highly purified saccharin produced essentially the same results suggests that saccharin itself, and not impurities, caused the increase in SCEs. The possibility that either pH or ionic-strength effects is responsible for the increase is being considered in assessing the possible significance of these results. The medium was well buffered, there was no pH change in the saccharin-treated samples, and therefore pH changes were apparently not a factor (190). Control experiments in which the concentration of sodium chloride was varied suggest that ionic-strength effects are not likely to have been a factor (190).

Table 39.—induction of Sister Chromatid Exchanges (SCEs) by Saccharin in Human Lymphocytes *in vitro*[1]

	Impure Saccharin		Pure Saccharin	
Dose (Percent)	# SCEs per # Chromosomes	SCEs/cell	# SCEs per # Chromosomes	SCEs/cell
Experiment 1				
0	981/4588	9.81 ± 0.31	.....	.....
0.1 : : : : : :	1169/4592	11.69 ± 0.34[3]	.....	.....
-0.5[2]	1711/4594	17.11 ± 0.413	.....	.....
Experiment 2				
0	950/4592	9.50 ± 0.31	950/4592	9.50 ± 0.31
0.3 : : : : : :	1311/4595	13.11 ± 0.36[3]	1322/4590	13.22 ± 0.36[3]
0.5[2]	1607/4598	16.07 ± 0.40[3]	1745/4596	17.45 ± 0.42[3]

[1]Data from S. Wolff and B. Rodin. Procedures were as described by Perry and Evans (1 34). Cells cultured 72 hours in 20 µM bromodeoxyuridine (BUdR.). Saccharin was present during the entire incubation period.
[2]Higher doses were toxic.
[3]p < 0.001.

2. Mutagenesis Tests in Mammalian Cells in Culture

Several mutagenesis tests using mammalian cells in culture are in various stages of development and validation. The two used to test saccharin are the mouse lymphoma (31) and Chinese hamster ovary (CHO)/HGPRT (130) tests. These

*Abe and Sasaki (1), in independent experiments, reported similar results in Chinese hamster cells.

Table 40.—Induction of Sister Chromatid Exchanges (SCEs) by Saccharin in Chinese Hamster Ovary (CHO) Cells *in vitro*[1]

Dose (Percent)	# SCEs per # Chromosomes	SCEs per cell
Experiment 1 (impure saccharin)		
0	875/2027	*8.75±0.30*
0.1	953/2003	*9.53±0.30*
0.5	995/2027	*9.95±0.32*
1.0[2]	1246/2028	12.46±0.35[3]
Experiment 2 (impure saccharin)		
0	845/1 967	8.45±0.29
1.0[2]	1294/1 982	12.94±0.36[3]
Experiment 3 (pure saccharin)		
0	855/1 947	8.55±0.29
0.5	1021/1969	10.21±0.32[3]
1.0[2]	1121/2006	11.21±0.33[3]
Experiment 4 (pure saccharin)		
o	872/1979	8.72±0.30
0.8	1105/1987	11.05±0.33[3]
1.02	1196/1996	11.96±0.35[3]

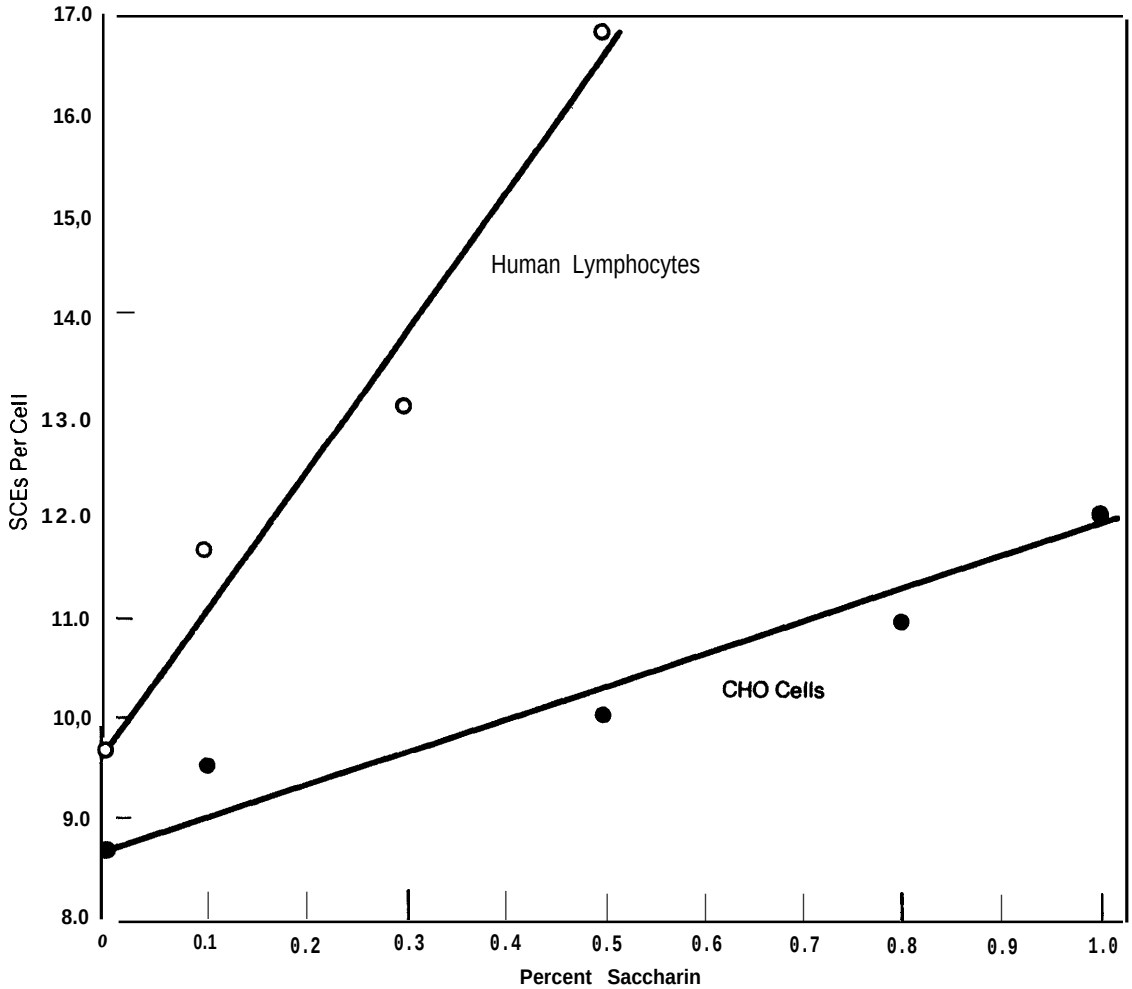
[1]Data from S. Wolff and B. Rodin. Cells cultured 24 hours in I0 µM. BUdr. I00 cells per point were examined.
[2]Higher doses were toxic.
[3]P < 0,001

methods are similar in that both measure mutations at a specific locus in either mouse lymphoma or CHO cells. Both select mutants that are resistant to either purine (HGPRT test) or pyrimidine (mouse lymphoma test) analogues which, if incorporated into DNA, are lethal to the cell. A mutation at the HGPRT genetic locus in CHO cells makes it impossible for a purine analogue to be incorporated into DNA, and a mutation at the TK genetic locus in mouse lymphoma cells prevents incorporation of certain pyrimidine analogues. Currently, the genetics of the CHO cell line is better defined, but the mouse lymphoma system has been more extensively used for mutagenesis testing. A validation study on the CHO/HGPRT system is in progress, and some results have been published (131). Although the mouse lymphoma test is being used by a number of laboratories and many chemicals have been tested, most of the results have not yet been published.

In tests with saccharin, a weak positive result was obtained in the mouse lymphoma test (tables 41 and 42).* The results are difficult to interpret as unequivocably positive because the effect is very weak, and there is no clearly reproducible dose-response. Also, the effect occurred only at doses that were quite toxic to the cells, as is shown by the percent survival. However, nearly all mutagens require such

*Final results of the CHO/HGPRT tests are not yet available. Several experiments have been completed and they did not detect any statistically significant mutagenic effect of saccharin. However, for technical reasons the results were not conclusive, and more experiments are in progress.

Figure 5– Induction of Sister Chromatid Exchanges by Saccharin



All data combined. For human lymphocytes the linear regression is given by Y = 9.64 (± 0.39) + 14.01 (±1.14)D where D is the % saccharin. **For** CHO cells the regression **is** given **by** Y = 8.66 (±0.21) + 3.37 (±0.32)D. **The coefficient of correlation** is **0.98 for human lymphocytes and 0.96 for CHO cells. (Figure 5** is **from Wolff and Rod in.)**

toxic doses for detection in this system *(30).* The results suggest, however, that both impure and highly purified forms of saccharin may be mutagenic. This result occurred at approximately the same dose levels as positive results obtained using the sister chromatid exchange and chromosome aberration test.

3. In *Vitro* Cytogenetic Tests for Chromosome Aberration in Mammalian Cells in Culture

Cytogenetic tests measure changes in the morphological structure of chromosomes. A number of different types of structural changes can be produced. Many of these changes are lethal to cells, but some can lead to stable mutagenic changes. Many cytogenetic methods are available, using a variety of cell types from

both mammals and humans and using both in vitro and *in* vitro procedures. For example, some of the most frequently used *in vitro* methods measure changes in chromosome structure in bone marrow cells, peripheral lymphocytes, or spermatocytes in animals. In vitro human peripheral lymphocytes are often used, as well as a wide variety of cell lines from both humans and animals.

Table 41 .—Induction of Mutations by Saccharin at the TK+/TK Locus in Mouse Lymphoma L5178Y Cells[1]

Concentration of Na Saccharin (mg/ml)	Impure Saccharin		Pure Saccharin	
	Percent survival	Mut. freq.[2]	Percent survival	Mut. freq.
o	100	o	o	o
10".0"	63	26	36	6
11.0	60	24	.....	.....
11.5	.....	.....	23	29
12.0	41	56	.....	.....
12.5	33	39	16	40
13.0	.....	.....	9.3	32
13.5	20	43	6.3	19
14.0	7.9	41	.....	
2-AAF[3](50pg/ml)	25	222	24	" "1 60" " "

[1]Data from D. Clive. Procedures were as described by Clive and Spector (1973). Procedures for use of the rat liver S-9 Mix are in preparation (30). Results shown for impure saccharin were replicated, and those for pure saccharin were from a single experiment. Aroclor-induced rat liver S-9 Mix was present.
[2]Number of mutants/1 0[5] survivors, after correcting for spontaneous background. The spontaneous background was about 40.
[3]2-AAF = 2-acetylaminofluorene is a known mutagen and was included as a positive control.

Table 42.—induction of Mutations by Saccharin at the TK+/TK Locus in Mouse Lymphoma L5178Y Cells[1]

Concentration of Impure Na Saccharin (mg/ml)	With Microsomes		Without Microsomes	
	Percent Survival	Mut. freq.	Percent Survival	Mut. freq.
0	100	0(Spont=58)	100	0(Spont=70)
7.6	55	20	.....	.....
8.5	35	32	.....	.....
10.0	22	45	.....	.....
11.5	6.9	76	.....	.....
13.0	2.1	99	.....	.....
14.5	.....	.....	25	17
16.0	.....	.....	66	30
17.5	.....	.....	9.6	45
19.0		.....	5.3	37
2-AAF(50µg/ml)	" "33 "	189	.....	
EMS[2](620µg/ml)	.....	.....	.....	1 0 9 8

[1]Data from D. Clive. Microsomes were Aroclor-induced rat liver S-9 Mix. For other details, see footnote to table 41.
[2]EMS = ethyl methane sulfonate, a known mutagen, which served as a positive control.

In the saccharin test battery, preliminary evidence for cytogenetic effects of highly purified saccharin has been obtained using CHO cells in the presence of a rat liver activation system (table 43). A variety of chromosome aberrations and an apparent dose response have been found. This cell line is also being used by the same investigators to test saccharin for its ability to induce point mutations (the CHO/HGPRT test, not yet concluded).

Table 43.—induction of Chromosome Aberrations by Saccharin in CHO Cells[1]

Concentration of Pure Na Saccharin (mM)	Chromosome Aberrations					
	Cytotoxicity (Percent of viable cells)	Chromatid Gaps	Chromosome Breaks	Translocations	Ring Formations	Abnormal Metaphrases per total metaphrases examined?
0	100	2	3	1	0	3/100 (3%)
25	98	3	6	4	0	9/120 (7.5%)
50	83	3	5	7	1	10/110 (91%)
100	45	8	14	24	9	25/128 (19.5%)
200[3]	<lo	6	18	15	6	23/110 (20.9%)
DMN (100µg/ml)	not tabulated -					23/140 (16.4%)

[1] Results are preliminary data from experiments still in progress (J San Sebastian J P O'Neill, and A W Hsie) All experiments were conducted in the presence of rat liver S-9 from Aroclor reduced animals (130,131) The sodium ion concentration was kept constant, at all doses of sodium saccharin, by varying the amount of NaCl added to the assay mixture Similar results have been Independently obtained and have been recently reported (Ishidate, M and Odashima, S., Mutation Res. 48, 337-354 (1977))

[2] The usual spontaneous background of abnormal metaphrases, in the absence of S-9 IS about 1 percent

[3] Nonspecific cytotoxic effects cannot be ruled out at this high dose level.

NEGATIVE TESTS

1. The *Salmonella/Ames* Test

This test is currently the most widely used of the short-term tests. A large number of known carcinogens have been tested and shown to be mutagens in this system (96,97,139,155,163). The method is very efficient for detection of organic chemical carcinogens (about 90 percent of those tested can be detected), but it does not detect all classes of carcinogens with equal efficiency. For example, metals, some chlorinated hydrocarbons, and the hydrazines are poorly detected.

The procedure uses several specially constructed strains of the bacterium *Salmonella typhimurium*. These strains contain different mutations that inactivate the genes necessary for the synthesis of the amino acid histidine, and as a result the bacteria cannot grow unless this amino acid is added to the growth medium. The test is carried out by exposing the bacteria to the chemical to be tested and measuring the number of bacterial colonies that are able to grow in the absence of histidine. Each such bacterial colony is the product of a mutational event. A correlation between increasing dosage of a chemical and increasing numbers of colonies shows the chemical to be mutagenic. The method also incorporates rodent (or human) liver extracts into the assay mixture to provide "activating enzymes, " which are necessary to metabolize some carcinogens to their active forms.

Saccharin was tested in *Salmonella* over a wide dose range by Stoltz, et al. (162) and found to be negative. These results are independently confirmed by the data in table 44, Impure saccharin was tested on the five standard tester strains (TA100, TA1535, TA1537, TA1538, TA98) over a wide dose range (.001 to 100 mg per petri plate) and in the presence of activating enzymes from either aroclor or phenobarbital induced rat liver homogenate.

Table 44.—Negative Assay of Saccharin in the Salmonella/Ames Test[1]

Dose (mg/plate)	TA100			TA1535			TA1537			TA1538			TA98		
	S-9	PB[2]	PCB[3]	S-9	PB	PCB	S-9	PB	PCB	S-9	PB	PCB	S-9	PB	PCB
0	155	149	141	29	21	19	6	9	11	8	20	25	33	35	39
.001	174	173	148	24	19	18	6	9	14	17	21	31	41	57	56
.01	128	160	124	28	15	14	7	12	12	11	25	34	30	49	56
.1	163	148	141	31	14	18	15	18	8	17	14	25	34	36	49
.5	149	132	140	18	14	23	13	11	15	9	16	33	28	43	48
1.0.	141	138	137	27	15	14	6	19	10	8	21	24	39	38	48
10.0	116	153	124	20	18	7	10	8	9	14	22	44	51	32	43
20.0.	163	135	126	19	15	11	10	11	8	9	15	25	55	41	48
50.04	108	120	108	19	14	13	6	10	13	14	15	23	33	39	55
100	89	82	91	6	5	10	3	4	4	10	6	8	29	29	27
MMS (2µl)	(+)														
MNNG (lµg)				(+)											
9-AA(50µg)							(+)	•	•						
2-AA(10µg)											5009				
B(a) P(5µg)															827

[1]Data from Yamasaki, J. McCann and B N Ames The standard Plate assay was used, as described in Ames, McCann, and Yamasaki, 1975 MMS = methyl methane sulfonate, MNNG = N-methyl -N-nitronitrosoguanidine, 9-AA = 9-aminoacridine, 2-aminoanthracene, B(a)P = benzo(a)pyrene.
[2]PB = phenobarbital reduced S-9 Mix (100 µl/plate)
[3]PCB = aroclor reduced S-9 Mix (20 µl/plate)
[4]The lower number of revertants at the 50 and 100 mg/ml dose levels indicate toxicity
+ = positive in a spot test

The Salmonella/Ames test was also used to test impurities in saccharin, and these results are discussed in a separate section.

2. **Mitotic Recombination in Yeast**

A number of different types of genetic events can be assayed in yeast. The most widely used assays for testing carcinogens and mutagens are those that measure mitotic recombination (194). This process involves breaking and rejoining parts of homologous chromosomes and can lead to changes in the genetic characteristics of the organisms. Mitotic recombination can result in chromosomal mutations that affect large numbers of genes, as compared to point mutations, which affect single genes. The basic molecular processes involved in these two mutagenic events are most likely related because chemicals that induce mitotic recombination, almost without exception, also cause point mutations. The yeast that has been most commonly used to detect mitotic recombination events is *Saccharomyces cerevisiae* D3. Over 100 carcinogens and noncarcinogens have been tested in this strain as part of an NCI-sponsored contract (154) to evaluate short-term tests. The method does detect a number of carcinogens and is useful, but it is not as sensitive as many of the other short-term tests.

Mitotic recombination tests using the D3 system with saccharin up to 5-percent dose levels, both with and without aroclor-induced rat liver activation, were negative. Both the impure and pure samples were tested, and each test was conducted three times, The results are given in table 45.

3. **Tests for DNA Repair Activity (Unscheduled DNA Synthesis and the Pol Test)**

These tests measure the ability of chemicals to interact with DNA in a way that causes DNA repair to occur. The unscheduled DNA synthesis test (149) measures DNA repair directly in cultured human fibroblasts (cells derived from human skin) after treatment with chemicals. This method is quite sensitive and has been shown to

Table 45.—Negative Assay of Saccharin for Mitotic Recombination in *Saccharomyces cerevisiae* D3[1]

Concentration (Percent)	Mitotic Recombinants per 10^5 Survivors[2]					
	Experiments Without S-9[3]			Experiments With S-9		
	1	2	3		2	3
Saccharin (impure)						
0	<2.3	9.6	5.7	6.1	12.2	14.6
0.1	11.6	3.1	21.6	6.3	2.9	16.7
0.5	13.5	1.8	9.7	2.4	7.3	15.8
1.0	11.1	3.1	6.4	22.9	1.7	37.0
2.5		3.6			1.8	
5.0	10.0		5.3	8.0		9.8
Control, Diepoxybutane	.....	1429	2000	.....	763	2440
Saccharin (pure)						
0	<2.3	9.6	5.7	6.1	12.1	14.6
0.1	9.8	2.1	8.1	7.3	12.0	15.4
0.5	9.1	2.4	33.3	4.2	6.4	27.3
1.0	3.0	3.3	13.3	6.5	2.0	22.7
2.5		1.9			1.7	
5.0	2.6		17.2	2.3		6.3
Control Diepoxybutane	.....	1429	2000	.....	763	2440

[1]Data from V. Simmon. Procedures were as described bv Zimmerman (1941).
[2]Saccharin had no significant toxic effect.
[3]S-9 Mix rat liver homogenate, activating enzymes, from Aroclor induced rats.

detect a wide range of chemical carcinogens and mutagens. The Pol test is used to detect chemicals that cause particular types of damage in DNA. This type of damage can be repaired by an enzyme present in the bacteria. The Pol test determines the toxicity of the test chemical in mutants that lack the DNA repair enzyme. A positive result is inferred if the chemical under test is more toxic to the bacteria lacking the repair enzyme than it is to the parent strain that contains the enzyme. The Pol test, though useful, is not applicable to detection of a wide range of carcinogens and mutagens. The method has recently been improved (145), and the modified as well as the standard procedures were used in testing saccharin.

Saccharin was negative in both the unscheduled DNA synthesis and the Pol tests. Unscheduled DNA synthesis was measured in bacteria exposed to a dose range from 0.002- to 2-percent saccharin, Neither the pure nor the impure sweetener was positive at any dose. An additional experiment to determine whether saccharin (over the same dose range) would interfere with ultraviolet radiation-induced repair of DNA was also negative. Results of the Pol tests are given in table 46. A positive result would have produced a larger zone of inhibition in the Pol A^- test in the upper half and a reduction in Pol A^- viable bacteria in the lower half when compared to the Pol A^+ data. In these experiments, saccharin was tested at only one dose level (0.5 mg/ml) which, considering the weak activity saccharin has shown in other systems, is not sufficiently high to constitute a definitive test. Urine from rats treated with impure saccharin was also tested in the Pol test, with negative results (table 47).

Table 46.—Negative Assay of Saccharin in the Pol Test[1]

	S-9[2]	Zone of Inhibition (mm)	
		Pol A+ (wild type bacteria)	Pol A- (mutant bacteria)
Disc Diffusion Assay			
Saccharin (impure)			
500 µg.	-	0	0
	+	0	0
Positive control			
(MMS,[4] 0.13 µmol)	-	41	68
Negative control			
(CAP,[5] 30 µg).	-	29	29
Modified Suspension Assay[3]		Viable Bacteria per ml	
Dose (µg/ml	s-9	Pol A+	Pol A-
0	-	7×10^8	7.4×10^8
	+	8×10^8	7.6×10^8
500.	-	6.3×10^8	6.9×10^8
	+	7.1×10^8	8.3×10^8

[1]**Data from H.** Rosenkranz. Procedures were as described by Rosenkranz, et al. (1 45)
[2]S-9 Mix rat liver homogenate from Aroclor induced rats.
[3]Cells were exposed for 24 hours, 37°C.
[4]**MMS = methyl methanesulfonate**
[5]**CAP = chloramphenicol**

Table 47.—Negative Assay of Urine from Rats Treated with Saccharin in the Pol Test[1]

Sample	Viable Cells/ml	
	Pol A+	Pol A-
Solvent Control.	3.6×10^8	1.6×10^8
Solvent Control + ß-glucuronidase.	4.3×10^8	1.8×10^8
Solvent Control + S-9 + ß-glucuronidase	4.3×10^8	2.2×10^8
Pre-treatment Urine + ß-glucuronidase	4.2×10^8	1.7×10^8
Pre-treatment Urine + ß-glucuronidase + S-9. . . .	3.6×10^8	1.7×10^8
6 hour Urine + ß-glucuronidase	3.8×10^8	1.8×10^8
6 hour Urine + ß-glucuronidase + S-9.	3.5×10^8	1.7×10^8
24 Hour Urine + ß-glucuronidase.	4.5×10^8	1.8×10^8
24 Hour Urine + ß-glucuronidase + S-9	3.8×10^8	1.8×10^8

[1]Data **from H. Rosenkranz. Procedures were as described by Rosenkranz, et al. (1 45) and by Durston and Ames (1975). Rats (ea. 150 grams each) each received 1 to 2.5** grams saccharin by gavage. Pre-treatment as well as **pooled 6 to 24 hour urines were assayed** in both the disc diffusion assay (results not shown) and modified suspension assay.

4. Drosophila Sex-Linked Recessive Lethal Test

The fruit fly, Drosophila, can be used to detect a variety of mutagenic and chromosomal breakage events (2). The test used here employs the whole animal and is an *in vitro* test in contrast to most of the other, *in vitro,* short-term tests. *Drosophila* has enzymes that are capable of carrying out many of the same reactions that activate chemical carcinogens in mammalian systems. The most sensitive assay using *Drosophila,* which detects the broadest range of mutagens at the lowest concentrations, is generally considered to be the sex-linked recessive lethal test (2,176), which detects lethal mutagenic changes in the X chromosome. Usually, in this method, male flies are treated with the chemical to be tested (in this case saccharin was added to the nutrient medium), and they are then mated to female flies that have not been treated. Female progeny receive one X chromosome from the female parent and one X chromosome from the male parent; male progeny receive one X from the female parent and one Y from the male parent. Thus, all males carry one X and one Y chromosome, and all females carry two X chromosomes. Even if the one X chromosome carried by the parental male flies has suffered lethal damage from the chemical treatment (due probably to chromosome breakage caused by the chemical), this damage will not affect the viability of the first generation progeny. A "lethal X" will, in the progeny females, have another healthy X to carry out the needed functions of the X chromosome. A "lethal X" will not be present in any of the male progeny because the one X chromosome in each male progeny is always donated by the female parent, which was not treated with the chemical. The next step in the assay is to mate the first generation daughters to normal untreated males. Now, if one of the X chromosomes carried by the females is the "lethal X", this chromosome will be passed on to some of the male offspring of this second mating. Because males have only one X chromosome, the males receiving the "lethal X" chromosome will die, and in fact such offspring are never hatched. Since so much is known about the distribution of chromosomes among progeny, the experiment can be designed so that a normal mating will result in two phentoypic classes of male offspring. If the class of male offspring that would have received the "lethal X" is missing, then this fact is evidence that the initial chemical treatment, two generations previous, caused lethal chromosome damage in the X chromosome of the treated males.

Results of the sex-linked recessive lethal tests in *Drosophila* are shown in table 48 and were substantially negative. Although there was a statistically significant increase in recessive lethals in brood 1, if multiples are not included in the calculation, the result is not significant. The normal procedure in *Drosophila* assays is to remove multiples from the calculation because they often result from spontaneous mutations, and for this reason these results must be considered negative. However, multiples occurred at doses of saccharin when only some of the flies ingested saccharin (see footnote in table 48 for details), and an effect due to saccharin, although it appears unlikely, cannot be ruled out.

Any positive effect of saccharin might be expected to be very weak, In order to detect a doubling of the spontaneous rate, about 8,000 flies must be examined in a sex-linked recessive lethal test. Since flies did not efficiently ingest saccharin at dose levels greater than 0.25 percent (table 48), the number of flies receiving a significant dose of saccharin (the group treated with 0.25-percent saccharin) was large enough to detect only about a quadrupling of the spontaneous rate. Thus, if saccharin caused less than a quadrupling of the spontaneous rate, it would not have been detected in this test.

Table 48.—Negative Assay of Saccharin for the Induction of Sex-1 inked Recessive Lethals in Drosophila [1]

Experiment	Cone. (Percent)	Survivors (Percent)	Number of Tests (lethals/non-lethals) Brood 1[3]	Brood 2[3]	Total Tests	No of Lethals	Lethals (Percent)
Treated							
1	0	54[4]	32260	0/1923	4186	3 singles	0.072
	1	96	3/1 841	0/1629	3473	3 singles	0.086
2	1	100	14 / 2228	9/2175	4427	5 singles 1 double 1 multiple of 16	0.512(0.159)[5]
3	0 5	100	13 2439	0 /1205	3657	4 singles 1 double 1 multiple of 7	0.355(0.160)[5]
	025	100	2$1730	1/1449	3182	3 singles	0.094
Totals from exp 1,2,3			35/10498	113/8381	18924	45	0.238
Percent lethals per brood			0 332'	0119			
Controls							
1		100	O 1604	1 1518	3123	1 single	0.032
2		100	52013	8 1896	3922	5 singles 4 doubles	0.331
3		100	2 1589	2860	2453	2 singles 1 double	0.163
Total Controls			715206	11 4274	9498	18	0.190
Percent lethals per brood			0134	0257			

[1]Data from R Valencia and S Abrahamsen. Procedures were as described by Abrahamsen and Lewis (2) Canton-S wild type males were treated for 72 hours with saccharin (Impure) and mated with FM6 females Files were treated im petri dishes m experiments 1 and 2, and to improve ingestion of saccharin in closed vials in experiment 3

[2]Ingestion of saccharin at doses greater than 0.25 percent was incomplete About half the files ingested some saccharin at the O 5-percent level there was only occasional Ingest [on at the 1 -percent level and there was no ingestion at the 10-percent level

[3]Brood 1 mainly assays for effects on mature sperm and Brood 2 also assays for effects on spermatids and younger sperm

[4]Mortality IS most likely not due to saccharin toxicity, but to starvation

[5]In parentheses percent lethals have been calculated after removal of doubles and multiples, which are often due to spontaneous mutations

[6]Statistically significant (Chi. square 5 25) Includes doubles and multiples

5. *In Vitro* Transformation

It is by no means clear that the molecular changes that lead to the altered growth potential associated with transformation of cells *in vitro* are the same as the changes that occur during carcinogenesis *in vitro.* However, several lines of evidence strongly suggest that there is a relationship between the processes and that mutagenesis may be the crucial molecular event. *In vitro* transformation tests measure the ability of a chemical to change a cell from a form that cannot cause a tumor in an animal to a form that will result in a tumor if the treated cells are re-injected into the animal.

Several *in vitro* transformation systems are under development. The hamster embryo (135) and C3H mouse 10T 1/2 (143) systems used to test saccharin have both been used to detect a number of carcinogens and mutagens. The National Cancer Institute has recently completed a validation study on the hamster embryo system (135) in which 54 carcinogens and 21 noncarcinogens were tested. The method was quite efficient and was able to detect transforming activity for all but eight of the carcinogens. It did not detect activity for any of the noncarcinogens. The cells used in *in vitro* transformation assays can activate some carcinogens to their active forms. But in order to detect a broad range of carcinogens, these tests have to be coupled to a metabolic activation system. Work is in progress on this alteration in a number of laboratories.

Saccharin, both the impure and purified samples, was negative when tested in the hamster embryo system at doses up to 10 mg/ml (table 49). Preliminary results in the 10T 1/2 system at 1 and 2 mg/ml were also negative (table 50), and a more extensive experiment is in progress.

Table 49.—Negative Assay of Saccharin for *in vitro* Transformation in Hamster Embryo Fibroblasts[1]

Dose (µg/ml)	Transformed colonies per survivor	
	Impure Saccharin	Pure Saccharin
0	0/781	0/781
1	0/888	0/865
3.2	0/912	0/893
10	0/863	0/912
32	–	0/920
100	0/876	0/881
316	0/938	0/1013
1,000	0/860	0/1018
3,160	0/839	0/971
10,000	0/71	0/228
Positive control: 3-methylcholanthrene (1 µg/ml)	3/629	3/629

[1]Data from R. Pienta. Procedures were as described by Pienta, et al. (135). Rat liver homogenate (activating enzymes) was not present. Plates were scored for transformants after 8 days incubation with saccharin.

Table 50.—Preliminary Negative Assay of Saccharin for in vitro Transformation in C3H Mouse 10T½ Cells[1]

Dose (mg/ml)	Transformed Colonies per Number of Dishes Scored	
	Impure Saccaharin	Pure Saccharin
0	0/5[2]	0/5
1	0/7	0/5
2	0/5	0/9

[1]Data from S. Mondal and C. Heidelberger, Procedures were as described by Reznikoff, et al. (1 43). A more extensive experiment is in progress. Cells were incubated 24 hours with saccharin, about 2,000 cells per dish were plated, and plates were scored for transformants after 6 weeks growth. The doses of saccharin used were non-toxic.

[2]A type 3 (malignant) focus of growth has not been observed in previous control experiments involving a total of over 5000 dishes.

6. Induction of Plasminogen Activator

Plasminogen is an enzyme which, when activated, degrades fibrin, a substance involved in the blood clotting process. Many mammalian cell lines, after transformation with viruses or chemical carcinogens, produce a substance that will activate plasminogen. Recently it has been shown that a number of tumor promoters are very potent inducers of plasminogen activator (187). As discussed earlier in this report, some evidence suggests that saccharin has tumor-promoting activity. For this reason, the plasminogen activator assay was included in the saccharin test battery. The plasminogen activator system has thus far been used only for detecting the plant diterpene tumor promoters, and whether it is capable of detecting other types of promoters is not known.

Both the impure and pure samples of saccharin were negative in this assay when tested from 1-50 µg/ml (table 51). Rather low doses of saccharin were tested in this

system compared to the other in vitro methods. However, the levels were more than 1,500 times higher than active doses of the potent promoter 12-O-tetradecanoylphorbol-13-acetate.

Table 51 .—Negative Assay of Saccharin for Induction of Plasminogen Activator

Dose (μg/ml)	Percent Fibrinolysis			
	Impure Saccharin		Pure Saccharin	
	3 hr	*6* hr	*3* hr	**6** hr
A. In the absence of TPA[2]				
o	**0.6**	**5.8**	**0.6**	**5.8**
1	**0.6**	**6.9**	**1.4**	**5.9**
5	0.5	**6.5**	**0.7**	**5.2**
10	1.8	**7.6**	**1.4**	**6.3**
50	0.1	**6.0**	1.7	10.3
B. In the presence of TPA (30 ng/ml)				
o	**56**	**67**	**56**	67
1	**56.2**	**62**	**57.6**	64.6
5	**54.5**	**61.5**	**57.9**	62.6
10	**58.0**	**60.2**	**64.5**	68.7
50	**46.2**	**55.0**	**56.4**	64.9

[1]Data from I. B. Weinstein. HeLa Cells (human cell line) were exposed to saccharin and TPA for 24 hours. Experiments exposing cells for 48 hours yielded similar results (data not shown). Lysates of exposed cells were prepared, and incubated with ^{125}I-fibrin plates in the presence of purified human plasminogen for either 3 or 6 hours, and the percent digestion of ^{125}I was determined (187).
[2]TPA = 12-0-tetradecanoyl-phorbol-13-acetate, a potent promoter of carcinogenesis, and inducer of plasminogen activator.

RESULTS OF TESTS ON MUTAGENIC IMPURITIES IN SACCHARIN

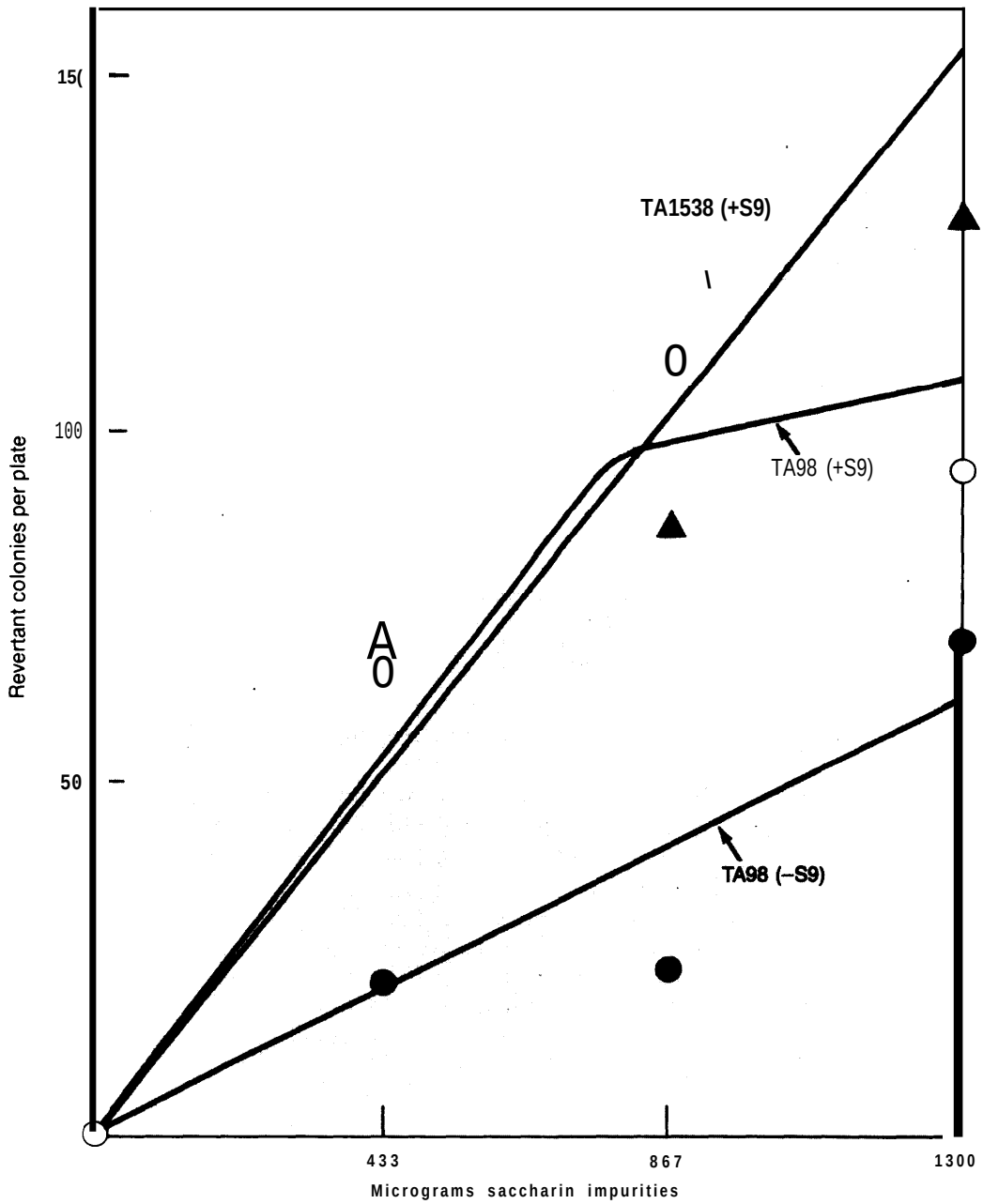
Saccharin used in the recent Canadian carcinogenicity tests, even though highly purified (and much purer than commercial saccharin), still contained a very low level (10 to 20 ppm) of impurities. A number of impurities are present, but as yet none has been chemically identified. Stoltz et al. (162) extracted 1 kg of this saccharin with organic solvents (e.g., chloroform) and recovered about 13 mg of impurities. When tested in the Salmonella/Ames test, this material was weakly mutagenic. These results have been independently confirmed by Yamasaki and Ames (figure 6) using a sample of the chloroform extract (kindly supplied by D. Stoltz).

It is not unreasonable that the weak activity of these impurities was not detected when saccharin itself was tested in the Salmonella/Ames test. About 400/μg of impurities were required in order to detect any mutagenic activity (figure 6). The impurities are present in such small amounts in unpurified saccharin that over 30 g of saccharin would have had to be added to a petri plate in order to expose the bacteria to a mutagenic dose of impurities. In testing saccharin, the highest possible dose was 0.1 g, which produced some toxicity in the bacteria.

More recent, as yet unpublished, work appears to support the conclusion that impure saccharin (and commercial samples of saccharin) contains impurities that are mutagenic in the Salmonella/Ames test. Mutagenic activity was detected in urine of mice after oral administration of several different samples of saccharin. The degree of

mutagenic activity detected varied for the different samples and was least mutagenic for highly purified saccharin (26).

Figure 6-Assay of Saccharin Impurities in the Salmonella/Ames Test



Impurities were from a chloroform extract of saccharin used in the Canadian carcinogenicity test prepared and kindly provided by D. Stoltz. The extract (about 13 mg) was dissolved in 3 ml DMSO. Mutagenesis testing was in the standard plate assay (10) in the presence (20 µl **per plate) or absence of aroclor induced S-9 Mix. For the bacterial tester strains TA98 and TA1538 spontaneous revertants were about 40.**

Appendix III

FEDERAL REGULATION OF CARCINOGENIC SUBSTANCES

INTRODUCTION

Federal authorities to regulate carcinogenic substances are usually contained within statutory provisions for regulating toxicity in general. With two major exceptions, the relevant statutes do not specifically mention carcinogenicity or cancer. These exceptions are the Federal Food, Drug, and Cosmetic Act and the Toxic Substances Control Act. Both Acts contain provisions that relate directly to carcinogens, and both specify procedures for regulating carcinogenicity that are distinct from those for general toxicity.

Nine statutes are important in the regulation of carcinogenic substances. One of these, the Food, Drug, and Cosmetic Act, generally takes precedence over other Federal laws for the carcinogenicity of substances that may be ingested. Health hazards in the workplace are covered by the Occupational Safety and Health Act. A third class of substances, those to which consumers are likely to be exposed (other than foods, drugs, cosmetics, and other excluded substances), is regulated by the Consumer Product Safety Act and the Federal Hazardous Substances Act. Four statutes administered by the Environmental Protection Agency (EPA) cover specific areas of the physical environment: the Clean Air Act; the Water Pollution Control Act; the Safe Drinking Water Act; and the Federal Insecticide, Fungicide, and Rodenticide Act. The Environmental Protection Agency also administers the Toxic Substances Control Act, a law designed to fill in gaps in the regulatory coverage of toxic substances in the environment.

Of these laws, only the Food, Drug, and Cosmetic Act contains provisions such as the “Delaney clause” that allow no regulatory discretion. When a substance regulated by one of these provisions is found to be a carinogen, it must be banned. No other law, not even that for hazards to consumers, mandates such specific obligatory action. Thus the laws are not consistent with each other.

The various laws also differ in their approaches to risk/benefit analysis. Some, such as the Toxic Substances Control Act, explicitly require the balancing of health risks against economic and other public impacts of regulation. Others permit such analysis, but do not require it. The Food, Drug, and Cosmetic Act requires it in some cases and forbids it in others, depending on the types of substances in question.

Summaries and discussion of the statutory authorities for the four classes of substances (ingested, workplace, consumer products, and environmental) follow.

FDA REGULATION OF INGESTED SUBSTANCES

Humans ingest a variety of substances that are under the control of the Food and Drug Administration (FDA): as foods, food additives, color additives, drugs, vitamins, and minerals; as residues from animal feed, animal drugs, and pesticides; and even as cosmetics. The statutes and regulations used by FDA to control these substances vary with the form of ingestion.

Statutes referred to are from the Federal Food, Drug, and Cosmetic Act, as amended; Title 21, United States Code (copy dated October 1976). Regulations are quoted from Title 21, Code of Federal Regulations,* chapter 1. Unless otherwise noted, "section" references are to the statutes.

A. Food and Substances in Foods

(1) Definitions

(a) The term "food" means

> **(1)** articles used for food or drink for manor other animals, (2) chewing gum, and (3) articles used for components of any such article [section 201 (f)].

(b) A Food Additive is any substance

> **the** intended use of which results or may reasonably be expected to result, directly or indirectly, **in its becoming a component or otherwise affecting the characteristics of any food [section 201(s)].**

(i) A Food Additive under law is thus not simply anything added to food. Certain substances that are added to food are exempted from the statutory provisions relating to Food Additives but are still subject to the other provisions of the Act (such as section 402, the section on adulterated foods), The definition of Food Additive is restricted to substances "not generally recognized . . . to be safe under the conditions of its intended use." [This qualification is the basis for the Generally Recognized As Safe (GRAS) List.] The section then continues: "except that such term [Food Additive] does not include:

> (1) **a pesticide chemical in or on a raw agricultural commodity; or**
> **(2) a pesticide chemical to the extent that it is intended for use or is used in the production, storage, or transportation of any raw agricultural commodity; or**
> **(3) a color additive; or**
> **(4) any substance used in accordance with a sanction or approval granted prior to the enactment of this paragraph pursuant to this Act . . . or [other Acts]. [This last phrase is the so-called "prior sanction" clause.]**
> **(5) a new animal drug [section 201(s)].**

(ii) Thus, any substance that fits the definition of a Food Additive given above and which is not on the GRAS list, for which "prior sanction" has not been granted, or which does not fit any of the other excluded

*Numbers of sections are those in use as of February 1977.

categories is a Food Additive and is specifically subject to regulation under section 409 of the Act.

(iii) Any substance that is a Food Additive is also a Food and subject to other provisions of the Act.

(c) A Color Additive is a material that

> **(A) is a dye,** pigment, or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived, with or without intermediate or final change of identity, from a vegetable, animal, mineral, or other source, and
>
> **(B) when added or applied to food, drug, or cosmetic, or to the human body or any part thereof; is capable (alone or through reaction with other substances) of imparting color thereto; except that such term does not include any material which the Secretary, by regulation, determines is used (or intended to be used) solely for a purpose or purposes other than coloring [section 201 (t)].**

(2) Regulation of Food Additives

(a) [409(a)]: Once a substance is classified as a Food Additive under the strict meaning given above, it is to be deemed unsafe for the purposes of section 409(c) (3) (a) unless it has been exempted for investigational use [section 409(i)], or,

> there **is in effect, and it and its use or intended use are in conformity with, a regulation issued under this section prescribing the conditions under which such additive may be safely used [section 409(a) (2)].**

In **either of these cases, the Food Additive is not in violation of section** 402(a), the food adulteration section, which serves as the basis for prohibiting use.

The regulation is not to be issued if a fair evaluation of the data

> **fails to establish that the proposed use of the food additive, under the conditions of use to be specified in the regulation, will be safe:** *Provided,* That no additive shall be deemed to be safe if it is found to induce cancer **when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal, except that this proviso shall not apply with respect to the use of a substance as an ingredient of feed for animals which are raised for food production, if the Secretary finds (i) that, under the conditions of use and feeding specified in proposed labeling and reasonably certain to be followed in practice, such additive will not adversely affect the animals for which such feed is intended, and (ii) that no residue of the additive will be found (by methods of examination prescribed or approved by the Secretary by regulations, which regulations shall not be subject to subsections (f) and (g)) in any edible portion of such animal after slaughter or in any food yielded by or derived from the living animal, [section 409(c)(3)(A)]**

If a regulation is issued, FDA may set tolerance limits, specify the foods in which the Food Additive may be used and in what amounts, labeling instructions, etc.

In determining whether a regulation shall be issued, the following factors (as well as any others that are relevant) shall be considered:

> **(a) the probable consumption of the additive and of any substance formed in or on food because of the use of the additive;**
> **(b) the cumulative effect of such additive in the diet of man or** animals, taking into account any chemically or pharmacologically related substance or substances in such diet; and
> (c) safety factors which in the opinion of experts qualified by scientific training and experience to evaluate the safety of food additives are generally recognized as appropriate for the use of animal experimentation data. [section 409(c)(5)]

(b) Before a Food Additive is marketed, the petitioner has the burden of **proof to show that the proposed Food Additive is safe and performs as claimed. However, once a Food Additive is on the market, with an approved regulation, a change occurs. While the** *burden of proof* remains with the original petitioner, the burden of "going forward" with the *evidence* shifts to FDA. That is, FDA has the responsibility for presenting evidence that will lead to a reconsideration of a Food Additive's safety. Under the "Delaney clause," FDA's responsibility is satisfied as soon as it finds that a Food Additive is carcinogenic. When FDA proceeds under the general safety clause, it must present evidence that the Food Additive has been shown to have certain effects (e.g., toxicity) and that these effects lead to harm. The general safety clause is the portion of 409(c) (3) (A) that precedes the "Delaney clause. "

(c) Action against Food Additives deemed unsafe is taken on the basis of section 402(a) (2) (c), the adulterated food section (to be described later).

(d) Other sections of the Code of Federal Regulations that are especially pertinent to food additives are excerpted below.

(i) 21 CFR 121.1*—Definitions and Interpretations

> **(f) 'Common use in food' means a substantial history of consumption of a substance by a significant number of consumers in the United States. . .**
>
> * * * * * * *
>
> **(h) 'Scientific procedures' include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.**
> **(i) 'Safe' or 'safety' means that there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance. Safety may be determined by scientific procedures or by general recognition of safety. In determining safety, the following factors shall be considered:**
>
> > **(1) The probable consumption of the substance and of any substance formed in or on food because of its use.**

***21** CFR 121.1 refers to Title 21 of the Code of Federal Regulations, Section 121.1. Other citations will follow this format.

(2) The cumulative effect of the substance **in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet.**
(3) Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate. . .

* * * * * * *

(k) 'General recognition of safety' shall be determined in accordance with $121.3.

§121.3 Classification of a food ingredient as generally recognized as safe (GRAS).
(a) General recognition of safety maybe based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food.

(ii) 121.5—Safety factors to be considered:

In accordance with section 409(c) (5) (C) of the act, the following safety factors will be applied in determining whether the proposed use of a food additive will be safe: Except where evidence is submitted which justifies use of a different safety factor, a safety factor in applying animal experimentation data to man of 100 to 1, will be used: that is, a food additive for use by man will not be granted a tolerance that will exceed l/100th of the maximum amount demonstrated to be without harm to experimental animals.

(iii) 121.6-General principles for evaluating the safety of food additives:

(a) In reaching a decision on any petition filed under section 409 of the act, the Commissioner will give full consideration to the specific biological properties of the compound and the adequacy of the methods employed to demonstrate safety for the proposed use, and the Commissioner will be guided by the principles and procedures for establishing the safety of food additives stated in current publications of the National Academy of Sciences-National Research Council. A petition will not be denied, however, by reason of the petitioner's having followed procedures other than those outlined in the publication of the National Academy of Sciences—National Research Council if, from available evidence, the Commissioner finds that the procedures used give results as reliable as, or more reliable than, those reasonably to be expected from the use of the outlined procedures. In reaching a decision, the Commissioner will give due weight to the anticipated levels and patterns of consumption of the additive specified or reasonably inferable. For the purposes of this section, the principles for evaluating safety of additives set forth in the above-mentioned publications will apply to any substance that may properly be classified as a food additive as defined in section 201 (s) of the act.
(b) Upon written request describing the proposed use of an additive and the proposed experiments to determine its safety, the Commissioner will advise a person who wishes to establish the safety of a

food additive whether he believes the experiments planned will yield data adequate for an evaluation of the safety of the additive.

(iv) 121.4001—Saccharin, ammonium saccharin, saccharin, calcium saccharin, and sodium saccharin ("Interim Regulation" on saccharin):

The food additives saccharin, ammonium saccharin, calcium saccharin, and sodium saccharin may be safely used as sweetening agents in food in accordance with the following conditions, if the substitution for nutritive sweeteners is for a valid special dietary purpose and is in accord with current special dietary food regulations and policies or if the use or intended use is for an authorized technological purpose other than calorie reduction:

(a) Saccharin is the chemical, 1, 2-benzisothiazolin-3-one-1, 1-dioxide, ($C_7H_5NO_3S$). The named salts of saccharin are produced by the additional neutralization of saccharin with the proper base to yield the desired salt.

(b) The food additives meet the specifications of the 'Food Chemicals Codex. '

(c) Authority for such use shall expire when the Commissioner receives a final report and recommendations from the National Academy of Science Committee on Saccharin and publishes an order based on this report.

(d) The additives are used or intended for use as a sweetening agent only in special dietary foods, as follows:

(1) In beverages, fruit juices, drinks, and bases or mixes when prepared for consumption in accordance with directions, in amounts not to exceed 12 milligrams of the additive, calculated as saccharin, per fluid ounce,

(2) As a sugar substitute for cooking or table use, in amounts not to exceed 20 milligrams of the additive, calculated as saccharin, for each expressed teaspoon full of sugar sweetening equivalency.

(3) In processed foods, in amounts not to exceed 30 milligrams of the additive, calculated as saccharin, per serving of designated size.

(e) The additives are used or intended for use only for the following technological purposes:

(1) To reduce bulk and enhance flavors in chewable vitamin tablets, chewable mineral tablets, or combinations thereof.

(2) To retain flavor and physical properties of chewing gum.

(3) To enhance flavor of flavor chips used in nonstandardized bakery products.

(f) To assure safe use of the additives, in addition to the other information required by the act:

(1) The label of the additive and any intermediate mixes of the additive for manufacturing purposes shall bear:

(i) The name of the additive.

(ii) A statement of the concentration of the additive, expressed as saccharin, in any intermediate mix,

(iii) Adequate directions for use to provide a final food product that complies with the limitations prescribed in paragraphs (d) and (e) of this section.

(2) The label of-any finished food product containing the additive shall bear:

(i) The name of the additive.
(ii) The amount of the additive, calculated as saccharin, as follows :
(a) For beverages, in milligrams per fluid ounce;
(b) For cooking or table use products, in milligrams per dispensing unit.
(c) For processed foods, in terms of the weight or size of a serving dish which shall be that quantity of the food containing 30 milligrams or less of the additive.
(iii) When the additive is used for calorie reduction, such other labeling as is required by part 125 or §3.72 of this chapter.

(e) Listing of other pertinent Regulation sections:

Section #	*Title*
121.3	Eligibility for classification as GRAS
121.4	Tolerances for related food additives
121.8	Food additives in standardized foods
121.40	Affirmation of GRAS status
121.41	Determination of food additive status
125.1	Definitions and interpretations of terms [for dietary uses]
125.7	Label statements relating to nonnutritive constituents [of dietary foods]

(3) **Regulation of Color Additives**

(a) There are many similarities between the regulation of color additives and food additives. Any substance meeting the definition of "Color Additive" given above is to be deemed unsafe unless the Secretary of HEW has issued a regulation specifying its safe conditions of use [section 706(a)]. Without such a regulation, a color additive is to be regarded as adulterated under sections 402(c) when in foods, 501 (a) in drugs, and 601 (e) in cosmetics.

(b) The Secretary shall issue the regulation only when

the data before him establish that such use . . will be safe. . *.Provided, however,* **that** *a* color **additive shall be deemed to be suitable and safe for the purpose of listing under this subsection for use generally in or on food, while there is in effect a published finding of the Secretary declaring such substance exempt from the term 'food additive' because of its being generally recognized by qualified experts as safe for its intended use, as provided in section** 201 (s). [section 706(b) (4)].

(c) In determining safety the following factors, among others, are to be considered:

> **(i) the probable consumption of, or other relevant exposure from, the additive and of any substance formed in or on food, drugs, devices, or cosmetics because of the use of the additive;**
> **(ii) the cumulative effect, if any, of such additive in the diet of man or animals, taking into account the same or any chemically or pharmacologically related substance or substances in such diet;**
> **(iii) safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of color additives for the use or uses for which the additive is proposed to be listed, are generally recognized as appropriate for the use of animal experimentation data; and**
> **(iv) the availability of any needed practicable methods of analysis for determining the identity and quantity of (I) the pure dye and all intermediates and other impurities contained in such color additive, (II) such additive in or on any article of food, drug, or cosmetic, and (III) any substance formed** in or on such article because of the use of such additive, [section 706(b)(5)(A)]

(d) However, the color additive section of the Act has its own Delaney-type clause that takes precedence over the above criteria:

> **(B) A color additive (i) shall be deemed unsafe, and shall not be listed, for any use which will or may result in ingestion of all or part of such additive, if the additive is found by the Secretary to induce cancer when ingested by man or animal, or if it is found by the Secretary, after tests which are appropriate for the evaluation of the safety of additives for use in food, to induce cancer in man or animal, and (ii) shall be deemed unsafe, and shall not be listed, for any use which will not result in ingestion of any part of such additive, if, after tests which are appropriate for the evaluation of the safety of additives for such use, or after other relevant exposure of man or animal to such additive, it is found by the Secretary to induce cancer in man or animal**: *Provided,* **that clause (i) of the subparagraph (B) shall not apply with respect to the use of a color additive as an ingredient of feed for animals which are raised for food production, if the Secretary finds that, under the conditions of use and feeding specified in proposed labeling and reasonably certain to be followed in practice, such additive will not adversely affect the animals for which such feed is intended, and that no residue of the additive will be found (by methods of examination prescribed or approved by the Secretary by regulations, which regulations shall not be subject to subsection (d)) in any edible portion of such animals after slaughter or in any food yielded by or derived from the living animal. [section 706 (b)(5)(B)]**

(e) The Act also allows exemptions for the investigatory use of color additives section 706(f) and for the provisional listing of commercially established color additives, pending further investigation as to their safety [section 203, Title II, of Public Law 86-618],

(f) Action against color additives found to be unsafe by the provisions of section 706 is taken on the basis of adulteration. That is, the substance of which a color additive is a component will be classified as adulterated.

(i) A drug is adulterated if it bears, contains, or is itself an unsafe color additive. [section 501 (a) (4)]

(ii) A food is adulterated if it is, or it bears or contains, an unsafe color additive. [section 402(c)]

(iii) A cosmetic is adulterated if it is not a hair dye and it is, or it bears or contains, an unsafe color additive. [section 601(c)]

(4) Regulation **of Vitamins and Minerals**

(a) Vitamins and minerals, and components of such substances, are regulated as foods unless therapeutic or other medical claims are made for the **vitamin or mineral by its sponsor. If** such claims are made, these substances are to be considered drugs and must go through the New Drug Application (NDA) process (unless they fall under the "grandfather clause" described above).

(b) Specific statutory language for vitamins and minerals deemed to be foods covers potency levels, labeling, and the like. Their safety is to be assessed by provisions relating to foods in general, not by provisions in section 411 ("Vitamins and Minerals"). For example, such vitamins and minerals are subject to section 402, adulterated foods.

(5) **Regulation of Foods in General**

(a) Section 301 of the Act prohibits the introduction of any adulterated or misbranded food into interstate commerce. It also prohibits the adulteration or misbranding of foods already in interstate commerce.

(b) Section 402 lists the criteria by which a food is to be deemed adulterated. The following excerpts are of particular interest for this report:

> **(a)(1) If it bears or contains any poisonous or deleterious substance which may render it injurious to health; but in case the substance is not an added substance such food shall not be considered adulterated under this clause if the quantity of such substance in such food does not ordinarily render it injurious to health; or**
> **(2)(A) If it bears or contains any added poisonous or added deleterious substance (other than one which is (i) a pesticide chemical in or on a raw agricultural commodity; (ii) a food additive; (iii) a color additive; or (iv) a new animal drug which is unsafe within the meaning of section 406, or (B) if it is a raw agricultural commodity and it bears or contains a pesticide chemical which is unsafe within the meaning of section 408(a); or (C), if it is, or it bears or contains, any food additive which is unsafe within the meaning of section 409: Provided, that where a pesticide chemical has been used in or on a raw agricultural commodity in conformity with an exemption granted or a tolerance prescribed under section 408 and such raw agricultural commodity has been subjected to processing such as canning, cooking, freezing, dehydrating, or milling, the residue of such pesticide chemical remaining in or on such processed food shall, notwithstanding the provisions of section 406 and 409, not be**

deemed unsafe if such residue in or on the raw agricultural commodity has been removed to the extent possible in good manufacturing practice and the concentration of such residue in the processed food when ready to eat is not greater than the tolerance prescribed for the raw agricultural commodity; or (D) if it is, or it bears or contains, a new animal drug (or conversion product thereof) which is unsafe within the meaning of section 512; or. . .

* * * * * * *

(c) If it is, or it bears or contains, a color additive which is unsafe within the meaning of section 706(a).

(d) If it is confectionery, and—

(1) has partially or completely imbedded therein any nonnutritive object: *Provided,* **that this clause shall not apply in the case of any nonnutritive object if, in the judgment of the Secretary as provided by regulations, such object is of practical functional value to the confectionery product and would not render the product injurious or hazardous to health;**

(2) bears or contains any alcohol other than alcohol not in excess of one-half of 1 per centum by volume derived solely from the use of flavoring extracts; or

(3) bears or contains any nonnutritive substance: *Provided,* **that this clause shall not apply to a safe nonnutritive substance which is in or on confectionery by reason of its use for some practical functional purpose in the manufacture, packaging, or storage of such confectionery if the use of the substances does not promote deception of the consumer or otherwise result in adulteration or misbranding in violation of any provision of this Act:** *And provided further,* **That the Secretary may, for the purpose of avoiding or resolving uncertainty as to the application of this clause, issue regulations allowing or prohibiting the use of particular nonnutritive substance. "**

If section 409 and, therefore 402(a) (2) (C) were deleted, 402(a) (2)(A)(ii) would also be eliminated. Food additives, like other foods, would then be covered by the general provision on food adulteration (section 402).

(c) Section 406, Tolerances for Poisonous Ingredients, has been mentioned by various individuals in regard to saccharin and other sweeteners. However, the wording of the clause indicates that if the use of a poisonous ingredient can be *avoided (by* good manufacturing practice or because it is not required by production), then the ingredient itself and foods that contain it are to be deemed unsafe, adulterated as per section 402(a) (2) (A). This section, which is primarily used for environmental contaminants that may get into food, states:

Any poisonous or deleterious substance added to any food, except where such substance is required in the production thereof or cannot be avoided by good manufacturing practice shall be deemed to be unsafe for purposes of the application of clause (2) (A) of section 402(a); but when such substance is so required or cannot be so avoided, the Secretary shall promulgate regulations limiting the quantity therein or thereon to such

> extent **as he finds necessary for the protection of public health, and any quantity exceeding the limits so fixed shall also be deemed to be unsafe for purposes of the application of clause (2) (A) of section 402(a). While such a regulation is in effect limiting the quantity of any such substance in the case of any food, such food shall not, by reason of bearing or containing any added amount of such substance, be considered to be adulterated within the meaning of clause** (1) **of section 402(a). In determining the quantity of such added substance to be tolerated in or on different articles of food the Secretary shall take into account the extent to which the use of such substance is required or cannot be avoided in the production of each such article, and the other ways in which the consumer may be affected by the same or other poisonous or deleterious substances.**

(d) Of course, a great many other sections in the Act pertain to food regulation. Those sections relevant to the purposes of this report have, however, been described.

B. Drugs and Substances in Drugs

(1) Definitions

(a) The term "drug" means

> **(A) articles recognized in the official United States Pharmacopoeia, official Homeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them; and (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (C) articles (other than food) intended to affect the structure or any function of the body of man or other animals; and (D) articles intended for use as a component of any articles specified in clause (A), (B), or (C); but does not include devices or their components, parts, or accessories. [section 201 (g)(l)].**

(b) The term "new drug" means:

> **(i) Any drug (except a new animal drug or an animal feed bearing or containing a new animal drug) the composition of which is such that such drug is not generally recognized by scientific experts] as safe and effective for use. . .**

except that such a drug is not considered to be a "new drug" if it was in use under the conditions of the 1906 Pure Food and Drug Act (that is, it was permitted to be on the market before the enactment of the present (1938) Act) and is still labeled for the same conditions of use; or

> **(ii) Any drug recognized, as a result of scientific investigations, as safe and effective, but which has not been used "to a material extent or for a material time under such conditions [for which it is shown to be safe and effective] [section 201 (p)].**

(2) The Drug Approval Process

(a) The Act, as amended, requires that no new drug may be marketed unless an application for marketing has been approved by the Secretary of HEW

[section 505(a)]. The FDA is the agency that has been assigned the responsibility for implementing this Act. The approval of an application to market a new drug is based in large measure on a demonstration of its safety and efficacy. The requirement that efficacy be demonstrated was added by the 1962 amendments to the Act. The FDA approves or disapproves a New Drug Application (or allows an existing drug to stay in the market) when it judges that the total biochemical action of the drug yields positive results that outweigh the risks and when the individual ingredients are either safe or provide benefits outweighing their risks.

(b) Section 505(i) and its implementing regulations permit exemptions for the investigational (research) use of new drugs. Any person or organization which wishes to do research on a new drug **in human beings must file a "notice or claimed exemption for investigational new drug" (IND) and then wait at least 30 days. If FDA does not prohibit commencement during the 30-day period, human trials may begin. Decisions to permit research under INDs are based on criteria ensuring that human subjects are not exposed to unjustified and unnecessary safety risks.**

(c) Following IND-approved research (or during it), a New Drug Application (NDA) is submitted to FDA by the organization developing the drug.

When an NDA is submitted, FDA (on the basis of criteria of safety and efficacy specified in the Act) must within 180 days approve or disapprove the application. This time limit may be extended by mutual agreement.

Section 505(d) of the Federal Food, Drug, and Cosmetic Act sets forth the six criteria to be used in not approving an application to market a new drug. Four of these criteria deal with safety and purity [section 505(d) (1) through 505(d) (4)], one deals with labeling requirements [section 505(d) (6)], and one deals with efficacy [section 505(d) (5)].

(d) Relevant parts of section 505(d):

> If the Secretary finds . . . that **(1) the investigations, reports of which are required to be submitted to the Secretary pursuant to subsection (b), do not include adequate tests by all methods reasonably applicable to show whether or not such drug is safe for use under the conditions prescribed, recommended, or suggested in the proposed labeling thereof; (2) the results of such tests show that such drug is unsafe for use under such conditions or do not show that such drug is safe for use under such conditions; (3) the methods used in, and the facilities and controls used for, the manufacture, processing, and packing of such drug are inadequate to preserve its identity, strength, quality, and purity; (4) upon the basis of the information submitted to him as part of the application, or upon the basis of any other information before him with respect to such drug, he has insufficient information to determine whether such drug is safe for use under such conditions; or (5) evaluated on the basis of the information submitted to him as part of the application and any other information before him with respect to such drug, there is a lack of substantial evidence that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the proposed labeling thereof; or (6) based on a fair evaluation of all material facts, such labeling is false or misleading in any particular.**

If **any of these conditions hold, the Secretary shall not approve the NDA.**

(e) The term "substantial evidence" refers, in tests for efficacy, to

> **evidence consisting of adequate and well-controlled investigations, including clinical investigations, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved, on the basis of which it could fairly and responsibly be concluded by such experts that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling or proposed labeling thereof, [section 505(d)]**

Safety is to be determined by "adequate tests by al1 methods reasonably applicable." [section 505(d)(l) and 505(d)(2)]

(f) Because safety and efficacy are separate criteria, FDA must weigh the advantages (the benefits) of the drug against the dangers (safety, risks) in deciding whether to approve an NDA or to allow approval to stand. Thus, drugs that would not meet the criteria of safety for foods may be approved because their benefits outweigh the risks. This approval is possible because the statutes for regulating drugs contain no Delaney-type clause; safety is implicitly recognized as a relative concept. A drug containing a substance, such as saccharin, would therefore, not be automatically unapproved.

(3) New Information on Risks

(a) If new information is developed or learned about the risks of a drug or a substance in a drug, FDA can take several actions. If the agency believes information to be substantial, it could issue a regulation proposing to classify the previously approved drug as a "new drug. " This action requires that the evidence on safety and efficacy be reexamined and the new information taken into account. FDA may also require the sponsor of the drug to perform additional tests of its safety or efficacy.

Section 505(e) specifies that:

> **The Secretary shall, after due notice and opportunity for hearing to the applicant, withdraw approval of an application with respect to any drug under this section if the Secretary finds (1) that clinical or other experience, tests, or other scientific data show that such drug is unsafe for use under the conditions of use upon the basis of which the application was approved; (2) the new evidence of clinical experience, not contained in such application or not available to the Secretary until after such application was approved, or tests by new methods, or tests by methods not deemed reasonably applicable when such application was approved, evaluated together with the evidence available to the Secretary when the application was approved, shows that such drug is not shown to be safe for use under the conditions of use upon the basis of which the application was approved; or (3) on the basis of new information before him with respect to such drug, evaluated together with the evidence available** to him when the application was approved, that there is a lack of sub**stantial evidence that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling thereof.**

Withdrawing approval automatically classifies the drug as a "new drug."

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(b) When a substance that is a component of a number of drugs becomes suspected of posing risks to health, all drugs containing it can be classified as "new drugs" (after appropriate notice) as specified above. The regulations issued by FDA contain several relevant sections relating to the above points. For example,

> (a) **A new drug may not be approved for marketing unless it has been shown to be safe and effective for its intended use(s). After approval, the applicant is required to establish and maintain records and make reports related to clinical experience or other data or information necessary to make or facilitate a determination of whether there are or may be grounds under section 505(e) of the act for suspending or withdrawing approval of the application. Some drugs, because of the nature of the condition for which they are intended, must be used for long periods of time—even a lifetime, To acquire necessary data for determining the safety and effectiveness of long-term use of such drugs, extensive animal and clinical tests are required as a condition of approval. Nonetheless, the therapeutic or prophylactic usefulness of such drugs may make it inadvisable in the public interest to delay the availability of the drugs for widespread clinical use pending completion of such long-term studies. In such cases, the Food and Drug Administration may approve the new-drug application on condition that the necessary long-term studies will be conducted and the results recorded and reported in an organized fashion. The procedures required by paragraph (b) of this section will be followed in order to list such a drug in 5310.304.**
>
> **(b) A proposal to require additional or continued studies with a drug for which a new-drug application has been approved may be made by the Commissioner on his own initiative or on behalf of any interested person. Prior to issuance of such a proposal, the applicant will be provided an opportunity for a conference with representatives of the Food and Drug Administration. When appropriate, investigators or other individuals may be invited to participate in the conference. Such proposal and a summary of the grounds upon which it is proposed will be published in the** *Federal Register* **acting on the proposal. Proposals submitted by interested persons may be refused by written notice from the Commissioner if the proposal is not supported by reasonable grounds. Upon final determination that special studies, records, and reports are required for a drug, such requirements will be published in $310.304. [section 310.303]**

(c) Similar provisions apply to over-the-counter drug products, many of which contain saccharin:

> **An over-the-counter (OTC) drug listed in this subchapter is generally recognized as safe and effective and is not misbranded if it meets each of the conditions contained in this part and each of the conditions contained in any applicable monograph. Any product which fails to conform to each of the conditions contained in this part and in an applicable monograph is liable to regulator action.**

(a) The product is manufactured in compliance with current good manufacturing practices, as established by Parts 210, 211, 225, 266 and 229 of this chapter. . .

* * * * * * *

(e) The product contains only suitable inactive ingredients which are safe in the amounts administered and do not interfere with the effectiveness of the preparation of with suitable tests or assays to determine if the product meets its professed standards of identity, strength, quality, and purity. Color additives may be used only in accordance with section 706 of the act and Parts 8 and 9 of this chapter.

Regulations pertaining specifically to drugs and new drugs for humans appear in 21 CFR, Parts 300-499.

(4) Regulatory Action

As stated above, no new drug may be marketed or remain on the market unless an approved NDA is in effect for that drug [section 505(a) of the Act]. Approval, once given, can be withdrawn in accord with the provisions of section 505(e), as described above. According to section 301 (d), any organization or individual that markets or continues to market an unapproved drug is in violation of section 505.

C. Regulation of Cosmetics

(1) Definitions

(a) The term "cosmetic" means:

(1) articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and (2) articles intended for use as a component of any such articles; except that such term shall not include soap. [section 201 (i)].

(b) A cosmetic shall be deemed to be adulterated:

(a) **If it bears or contains any poisonous or deleterious substance which may render it injurious to users under the conditions of use prescribed in the labeling thereof, or, under such conditions of use as are customary or usual [an exception is made for coal-tar hair dyes that are properly labeled]. . .**

*** * * * * * ***

(d) If its container is composed, in whole or part, of any poisonous or deleterious substance which may render the contents injurious to health.
(e) If it is not a hair dye and it is, or it bears or contains, a color additive which is unsafe within the meaning of section 706(a). [section 601].

(2) Regulation of Cosmetics

(a) Regulation of cosmetics is relevant to this study because certain cosmetics, such as lipstick or toothpaste, and substances in cosmetics may be ingested by the consumer.

(b) Action is taken against an adulterated cosmetic under the provisions of Section 301, "Prohibited Acts." The specific clauses are the same ones as those used to ban adulterated foods and drugs: Sections 301(a), (b), and (c).

D. Residues of Certain Substances

(1) Definitions

(a) Certain substances that are not deliberately ingested by humans are ingested as residues from their use in animal feeds, animal drugs, and pesticide chemicals. Animal feeds are considered to be "foods" by the Act and thus subject to the applicable portions of its chapter IV, "Foods." However, animal feeds may also contain animal drugs that could remain as a residue and thus be ingested by humans. In these cases, the residues of such animal drugs are regulated by several other sections of the statutes.

(b) The term "pesticide chemical" means

> **any substance which, alone, in chemical combination or in formulation with one or more other substances, is an 'economic poison' within the meaning of the Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. secs. 135-135K) as now in force or as hereafter amended, and which is used in the production, storage, or transportation of raw agricultural commodities. [section 201 (9)]**

(c) The term "new animal drug" means

> **any drug intended for use for animals other than man, including any drug intended for use in animal feed but not including such animal feed—**
>
> **(1) the composition of which is such that such drug is not generally recognized. . .as safe and effective for use under the conditions prescribed, recommended, or suggested in the labeling thereof; [except that any animal drug that was in the market before June 25, 1938 and subject to the 1906 Pure Food and Drug Act (and is being represented for the same indications) is not to be deemed a "new animal drug;"] or**
> **(2) the composition of which is such that such drug, as a result of investigations to determine safety and effectiveness for use under such conditions, has become so recognized but which has not. . been used to a material extent or for a material time under such conditions. [section 201 (w)].**

(d) The term "animal feed" means

> **an article which is intended for use for food for animals other than man and which is intended for use as a substantial source of nutrients in the diet of the animal, and is not limited to a mixture intended to be the sole ration of the animal. [section 201 (x)]**

(2) Regulation of Animal Drugs

The definition of "drug" given in the Act also applies to drugs used in animals. The term "new drug" does not. Instead, the term "new animal drug" is used. Treatment of new animal drugs is very similar to that for new drugs. An application for a

new animal drug follows a procedure parallel to the NDA process for new human drugs, except that the new animal drug process contains a Delaney-type clause. Section 512(d) (1) lists the criteria used in approving or not approving an application to market a new animal drug. According to clause (H) of the section, an application must be refused if it is found that

> **such drug induces cancer when ingested by man or animal or, after tests which are appropriate for the evaluation of the safety of such drug, induces cancer in man or animal, except that the foregoing provisions of this subparagraph shall not apply with respect to such drug if the Secretary finds that, under the conditions of use specified in proposed labeling and reasonably certain to be followed in practice (i) such drug will not adversely affect the animals for which it is intended, and (ii) no residue of such drug will be found (by methods of examination prescribed or approved by the Secretary by regulations, which regulations shall not be subject to subsections (c), (d), and (h)), in any edible portion of such animals after slaughter or in any food yielded by or derived from the living animals.**

Thus, if humans will ingest any residue of a new animal drug that induces cancer in man or animal, then the new animal drug application cannot be approved. Action is taken against the drug under the provisions for an unsafe new animal drug or on animal feed containing an unsafe new animal drug [sections 501 (a) (5) and sections 501 (a) (6)]. Similarly, if an approved animal drug is found later to meet the above conditions, its approval will be rescinded under the provisions of section 512(e) (1) (B).

When a new animal drug has been approved, foods containing residues of such drug are not considered adulterated. However, if approval has not been given, such foods are deemed to be adulterated by the terms of section 402(a) (2) (D). According to this section, a food is adulterated

> **if it is, or it bears or contains, a new animal drug (or conversion product thereof) which is unsafe within the meaning of section 512.**

In short, if no potentially carcinogenic residues (as determined by assay methods that meet the criteria of FDA) of an animal drug (or a conversion product thereof) will be ingested by humans, its safety for humans is not an issue. If noncarcinogenic residues are likely to be ingested, the animal drug (in the allowable amounts) must meet the conditions of safety of the Act. If the animal drug has been shown to be carcinogenic, no residues identifiable by prescribed assay methods are permitted,

(3) Regulation of Pesticide Residues

A food is deemed to be adulterated "if it is a raw agricultural commodity and it bears or contains a pesticide chemical which is unsafe within the meaning of section 408(a)." [section 402(a) (2) (B)] section 408 discusses "Tolerances for Pesticide Chemicals in or on Raw Agricultural Commodities":

> **Any poisonous or deleterious pesticide chemical, or any pesticide chemical which is not generally recognized. . .as safe for use, added to a raw agricultural commodity, shall be deemed unsafe for the purposes of the application of clause (2) of section 402(a) unless—**
>
> > **(1) a tolerance for such pesticide chemical in or on the raw agricultural commodity has been prescribed by the Secretary of Health, Education,**

> **and Welfare under this section and the quantity of such pesticide chemical in or on the raw agricultural commodity is within the limits of the tolerance so prescribed; or**
> **(2) with respect to use in or on such raw agricultural commodity, the pesticide chemical has been exempted from the requirement of a tolerance by the Secretary under this section [when a tolerance is not necessary to protect the public health]. [section 408(a)]**

The responsibility for this section has been delegated to the Environmental Protection Agency, but enforcement according to section 402 remains with FDA. The Act specifies certain factors (other than general safety of the chemical) for the Secretary of HEW to consider in issuing the regulations referred to above:

> **The Secretary shall give appropriate consideration, among other relevant factors, (1) to the necessity for the production of an adequate, wholesome, and economical food supply; (2) to the other ways in which the consumer may be affected by the same pesticide chemical or by other related substances that are poisonous or deleterious; and (3) to the opinion of the Secretary of Agriculture as submitted with a certification of usefulness under subsection** (1) of **this section. [section 408(b)]**

Although there is no Delaney-type clause for pesticide residues, the Act does state that:

> **In carrying out the provisions of this section relating to the establishment of tolerances, the Secretary may establish the tolerance applicable with respect to the use of any pesticide chemical in or on any raw agricultural commodity at zero level if the scientific data before the Secretary does not justify the establishment of a greater tolerance. [section 408(b)]**

Tolerance levels relate to the amounts of residues permitted in foods that will be ingested by humans.

(4) Other Residues

(a) Color Additives have been discussed above. Section 706(b)(5)(B) indicates that if a color additive used in animal feed were shown to be carcinogenic, its use is prohibited *unless no residue* of the color additive found its way into the human diet.

(b) Food additives in animal feed are also subject to section 409(c)(3)(A) [the "Delaney clause"].

REGULATION OF CARCINOGENIC SUBSTANCES IN THE WORKPLACE

The purpose of the Occupational Safety and Health Act, Public Law 91-596, is to ensure working conditions as safe and healthy as possible for every working person. It is administered primarily by the Occupational Safety and Health Administration of the Department of Labor. Certain functions related to scientific research are the responsibility of the National Institute of Occupational Safety and Health of HEW.

The Act does not address carcinogenicity specifically, but rather toxicity in general. The Secretary of Labor, by promulgating a regulation, can set occupational safety and health standards for toxic substances.

Section 6(b) (5) of the Act specifies that:

> **The Secretary, in promulgating standards dealing with toxic materials or harmful physical agents under this subsection, shall set the standard which most adequately assures, to the extent feasible, on the basis of the best available evidence, that no** employee will suffer material impairment of health or functional capacity even if such employee has regular exposure to the hazard dealt with **by such standard for the period of his working life.**

A **standard can specify the conditions of use "reasonably"** necessary or appropriate to provide safe and healthful employment. Standards are developed on the basis of research, demonstrations, experiments, and such other information as may be appropriate. In addition to safety and health goals, other factors are considered in setting standards: feasibility, state of scientific knowledge, and experience gained under this Act and other health laws.

The Department of Labor does, however, issue regulations specifically on carcinogenic substances in the workplace. A draft of proposed regulations on exposure of workers to cancer-causing chemicals was made public in January 1977. As of July 20, 1977, this proposal remains a draft; current procedures still apply. That is, each substance suspected of or confirmed as being carcinogenic is considered individually and, depending on the available evidence, a standard specifying allowable conditions of use is issued.

Under the draft proposal, setting standards case by case would be replaced by the use of three uniform job-health standards. Each carcinogen or suspected carcinogen would be placed into one of three categories. Each category has its corresponding uniform standard (allowable exposure levels may vary depending on the substance, even within the same category).

The proposed categories are:

Category I Toxic *Materials*

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

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

Category II Toxic Materials

A substance will be classified as Category II Toxic Material ("suspect" carcinogen) if the evidence of carcinogenicity in humans or one or more mammalian species is found by OSHA to be only "suggestive" as opposed to confirming. Such a distinction would be based on generally accepted standards of review for such scientific studies.

Category III Toxic Materials

A substance for which the evidence of carcinogenicity is found inadequate to classify as Category I or 11 will be classified as Category 111.

When a substance is classified into category I, the Secretary of Labor is required immediately to issue an emergency standard by a regulation setting forth the allowable conditions of use. At the same time that the emergency standard is issued, a process to develop a permanent standard begins. The use of such a classified substance can be prohibited altogether. When a less dangerous alternative to the substance is available, the substance must be banned.

Placing a substance in category II initiates a process that will result in a permanent standard setting forth allowable safe uses of the substance. The Occupational Safety and Health Administration also is required to notify HEW (especially the National Cancer Institute and the National Institute of Occupational Safety and Health), EPA, and other applicable agencies that the evidence of the substance's carcinogenicity is only "suggestive" and request that the agencies engage in or stimulate further research.

The OSHA statutes do not contain any Delaney-type clause. The agency (and thus the Secretary of Labor) can set limits of exposure greater than zero for substances shown to cause cancer. Further, the draft proposal clearly states that the safety aspects of prohibition are to be balanced against technological feasibility and economic consequences.

While the Federal Food, Drug, and Cosmetic Act specifically addresses "cancer," the OSHA proposed regulations refer to malignant *and* benign neoplasms and tumors. If a substance meets the other criteria (e.g., testing with positive results in two mammalian species), it is placed into category I even if all tumors formed are benign.

REGULATION OF CONSUMER PRODUCT SAFETY

The Consumer Product Safety Commission (CPSC) administers two laws that are applicable to carcinogenic substances, the Consumer Product Safety Act (CPS Act) and the Federal Hazardous Substances Act (FHS Act).

The FHS Act speaks of toxicity and health hazards and the CPS Act of "risk of death, personal injury, or serious or frequent illness," but neither speaks of carcinogenicity. The CPS Act is concerned with substances ("products") used by consumers—in the home, for recreation, etc. The prime intent of the FHS Act is to cover household substances. Foods, drugs, cosmetics, tobacco, pesticides, and many types of radiation are excluded from the jurisdiction of both Acts.

Carcinogens that fall under the coverage of either Act can be banned, restricted, or required to be properly labeled. Such actions arise from the "toxicity" of carcinogenic substances. The CPS Act explicitly requires balancing frequency and severity of risk against the effects of regulatory actions on cost, utility, and availability of the product. The FHS Act does not include provisions for taking into account the benefits (or the "costs" of regulating—for example, economic impact), but in making rulings under this Act the Commission takes such considerations into account to a limited extent.

The Commission has not developed its own procedures for identifying and classifying carcinogens. It relies primarily on other organizations for information. The National Cancer Institute and the National Academy of Sciences are its two prime sources.

One interesting quirk of the statutes could pose some problems for the Commission's regulation of carcinogens. Seventeen years ago, when the FHS Act was signed into law, there was much less concern about regulating carcinogenic substances. The FHS Act contains explicit guidelines for the type of animal testing that is considered sufficient to ban or restrict a substance, but only for *acute* toxicity. There are no guidelines for chronic toxicity testing and thus no mention of or guidance for testing of carcinogens. This gap has led critics to argue, according to the CPSC, that the authority of the Commission as defined by the FHS Act is very weak in the area of regulating carcinogens.

LAWS OF THE ENVIRONMENTAL PROTECTION AGENCY

The Environmental Protection Agency (EPA) administers eight separate statutes; five of these have provisions that relate to the identification and regulation of environmental carcinogens. Four of these statutes are concerned with specific environmental areas: Clean Air; Clean Water; Safe Drinking Water; and Insecticides, Fungicides, and Rodenticides ("pesticides"). The fifth, the Toxic Substances Control Act, is an umbrella statute designed to cover gaps in the regulatory coverage of environmental carcinogens (and other toxic substances).

None of the EPA statutes establishes regulatory criteria or actions to be taken when a substance is identified as a carcinogen as opposed to its identification as a toxic substance in general. Thus, in general, carcinogens are regulated in the same manner as other pollutants.

There is no Delaney-type clause in any of these statutes, although an argument could be made that a type of "partial-Delaney clause" exists in single sections of two of the Acts (see below). The Administrator of EPA must weigh the risks to health from exposure to a carcinogen against the costs of controlling its use and the benefits of allowing continued use. The use of such a benefit-risk analysis permits EPA to take into account technological feasibility (e.g., technical ability of an industry to remove the carcinogen from its waste products or final products), economic impact, and ability to enforce or monitor regulatory standards effectively. The EPA can set discharge or emission levels (exposure limits) at zero for a known carcinogen, but it is not required to do so.

A possible exception to the above statement is contained in section 307 of the Federal Water Pollution Control Act and in section 112 of the Clean Air Act. These sections relate to substances that have been identified as definitely hazardous to the public health. The Environmental Protection Agency is required to establish emissions standards for substances so identified and covered by the appropriate act. Some people have viewed these sections as "partial-Delaney clauses." Although EPA is not required to set zero-exposure levels, at the same time it is not specifically directed or allowed to apply benefit-risk analysis. The only criterion identified is hazard to health. Thus, once a substance is identified as a carcinogen (or otherwise very dangerous), the immediate and serious public health hazard would be eliminated by setting a zero exposure limit.

Despite the general lack of specific references to carcinogenicity in the EPA statutes, the EPA Administrator takes this risk into account as a matter of policy. That is, the benefits of allowing the carcinogenic product to be used must be great enough

to offset the greater health hazard posed by a carcinogenic substance. Thus, the "cutoff" point in the benefit/risk weighing is shifted to favor public health and becomes more conservative because of the serious long-range health danger of carcinogens.

CASE STUDY: THE "BANNING OF TRIS"

Contrary to popular belief, the chemical "tris" has not been banned entirely. The Consumer Product Safety Commission (CPSC) has banned only tris intended for use in children's apparel. Although many of the circumstances surrounding tris and saccharin are similar, the cases contrast. A description of the tris case highlights an important difference in the regulatory authorities of the two agencies involved, FDA and CPSC.

The chemical tris was used in children's apparel, especially sleepwear, in order to meet safety standards for flame retardation issued by the CPSC under the authority of the Flammable Fabrics Act. As with saccharin, some indication of the carcinogenicity of tris existed for some time prior to the decision to ban, but CPSC did not consider it convincing. In March 1976, the Environmental Defense Fund (EDF) petitioned the CPSC for a review of the health danger of tris. The EDF did not believe that there was enough evidence of carcinogenicity to ban tris at that time, but requested that CPSC require labeling of tris-treated apparel that would indicate the potential health risk. Several months after the petition was received, CPSC announced it would await the results of a National Cancer Institute rodent-feeding study before making any decision. The results were made available in February 1977, and CPSC decided that the positive findings of those tests, combined with the other evidence available, provided sufficient reason for banning tris-treated children's apparel. The ban took effect on April 8, 1977. Tris-treated adult apparel was not banned because: (1) several of the specific pieces of evidence (for example, the rate of ingestion when children suck on garments) apply only to children, and (2) since no safety standard **requires flame retardant adult apparel, then tris or other flame retarding chemicals are rarely used in that apparel.**

In deciding to institute the ban, CPSC did consider the economic impact of the ban, the availability of alternate chemicals (there are some), and the benefits of the use of tris-as well as the overall risks to health from its use. This balancing contrasts to the banning of saccharin, where none of the above factors are allowed to enter the analysis. The demonstrated carcinogenicity of saccharin was the only allowable factor in the decision by FDA. Thus, the regulatory discretion of CPSC in this matter was greater than that of FDA in the case of saccharin.

On June 23, 1977, the district court of South Carolina overturned the ban on tris for procedural reasons. The ruling is being appealed and does not affect the present use of the ban as an example of regulatory discretion,

Appendix IV

CHRONOLOGY OF EVENTS LEADING TO THE STUDY

Saccharin is a nonnutritive sweetener that was discovered in 1879 and has been in use since the turn of the century. Prior to 1972, it was classified as "generally recognized as safe" (GRAS), a classification meaning it was not considered a "food additive" for the purposes of the FDA law and therefore did not need FDA approval.

The increasing use of nonnutritive sweeteners and the widely publicized 1969 ban on cyclamates led to investigations of the carcinogenic potential of saccharin. Preliminary results of a long-term feeding study indicated formation of bladder tumors. On February 1, 1972, FDA removed saccharin from GRAS status and issued an interim food additive regulation limiting the use of saccharin in foods. FDA extended the interim regulation while awaiting a National Academy of Sciences review of the experimental data, including the two long-term feeding studies that showed bladder tumors in rats with diets of 5- and 7.5-percent saccharin. The Academy's December 1974 report stated that saccharin itself could not be identified as the cause of the tumors because of possible impurities as well as problems with experimental design and procedures. The FDA therefore continued the interim regulations while awaiting results of tests being conducted in Canada.

The Canadian study was designed to answer the objections raised in the Academy report, principally that impurities in the saccharin (specifically, a byproduct of the manufacturing process, ortho-toluenesulfonamide, or OTS) might have been the carcinogen. The Canadian study separated rats into control, saccharin, and OTS populations. The results showed that the saccharin group had an increased incidence of bladder tumors, while OTS group did not.

On March 9, 1977, FDA announced the results of the Canadian study and stated that the law required the removal of saccharin from the food supply, citing the "Delaney clause" of the Federal Food, Drug, and Cosmetic Act.

On April 14, 1977, FDA Commissioner Donald Kennedy announced the intention to propose a ban on saccharin, which was published in the Federal Register on April 15, 1977. The proposed ban would:

(1) Revoke the interim food additive regulation under which saccharin and its salts are currently permitted as ingredients in foods, thereby banning its use in foods and beverages.

(2) Allow the marketing of saccharin as a single ingredient, over-the-counter (OTC) drug.

(3) Remove saccharin from cosmetics that are likely to be ingested, such as toothpastes, mouthwashes, and lipstick.

(4) Remove saccharin as a nonmedical ingredient in drugs, e.g., when it is used to make drugs taste better.

(5) Prohibit saccharin in animal drugs and animal feeds.

On March 18, 1977, Senator Edward M. Kennedy (D-Mass.), Chairman of the Subcommittee on Health and Scientific Research of the Senate Committee on Human Resources, and three other members of the subcommittee suggested that the Office of Technology Assessment convene a panel of scientists and medical specialists to study the technological basis for the FDA ruling, and to report their findings in 60 days. He also invited OTA and a group of scientists to participate in an open executive session of his subcommittee on March 24, 1977, to discuss the usefulness and feasibility of such a study, and to identify the technical and scientific issues about which more information was needed.

On March 21 and 22, 1977, the Subcommittee on Health and the Environment of the House Committee on Interstate and Foreign Commerce held oversight hearings on the FDA's proposed ban.

The Senate Subcommittee on Health and Scientific Research held the open executive session on March 24, 1977, and the invited scientists* agreed that such a study would be feasible and useful. On March 29, 1977, Senator Kennedy, Chairman of the Subcommittee, and Senator Richard S. Schweiker (R-Pa.), its ranking Republican, requested the Office of Technology Assessment to conduct the study. On March 30, 1977, the Technology Assessment Board, the Congressional body that governs OTA, approved request.

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* John J. Burns, Vice President for Research, Hoffman La-Roche, Inc.
Emilio Q. Daddario, former Director, Office of Technology Assessment.
Cyrus Levinthal, Professor of Biology, Columbia University.
Matthew Meselson, Chairman of the Department of Biochemistry and Molecular Biology, Harvard University.
David P. Rall, Director of the National Institute of Environmental Health Sciences.
Frank J. Rauscher, former Director, National Cancer Institute, and currently Vice President for Research, American Cancer Society.
Frederick C. Robbins, Dean, Case Western Reserve Medical School, and Chairman, Health Advisory Committee, Office of Technology Assessment.

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Addendum

EPIDEMIOLOGICAL STUDIES OF SACCHARIN

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EPIDEMIOLOGICAL STUDIES OF SACCHARIN

Shortly after a draft of this report was transmitted to Congress on June 7, 1977, OTA learned of a Canadian epidemiological study that showed a positive correlation between consumption of artificial sweeteners and bladder cancer in human males (74). At almost the same time, an American epidemiological study found no correlation between bladder cancer and consumption of artificial sweeteners (192). In order to allow full examination and public discussion of these studies, FDA Commissioner Donald Kennedy announced on June 27, 1977, that the comment period on the proposal to ban saccharin would be extended to August 31, 1977 (77). Summaries of these two studies have recently been made available to OTA.

THE (POSITIVE) CANADIAN EXPERIMENT

Experimental Design

A total of 821 newly diagnosed cases of primary bladder cancer were identified in three Canadian provinces between April 1974 and June 1977. Of these cases, 632 people (480 males and 152 females) were personally interviewed in their homes and asked questions about their use of artificial sweetener drops or tablets. The information from these interviews was compared to information obtained from interviewing an equal number of controls. Each case was matched with a control of the same sex and same age (within 5 years) who lived in the same neighborhood.

Results

The average ages for all bladder cancer cases were: males, 67.7 years; male controls, 67.2; females, 69.1; female controls, 68.4. For males, 69 cases (compared to 43 controls) had ever used artificial sweeteners; and for females, 18 cases (compared to 30 controls) had ever used artificial sweeteners. The conclusions drawn from these data are that artificial sweetener use increases the risk of bladder cancer in males by a factor of 1.6 (i.e., 69/43=1.6) and that there is no association between sweetener use and bladder cancer in females (i.e., 18/30=0.6). However, too few cases of female bladder cancer were found to conclude with any statistical assurance that sweeteners had any effect on cancer incidence in women.

The authors made an effort to separate saccharin users from users of other sweeteners. Although they have reservations about the accuracy of separating these groups (some cases and controls did not recall whether they had used saccharin or cyclamates, or both), the males identified as saccharin users had a risk of 1.7, and female users had no increased risk. Males who used more than seven tablets or drops of saccharin a day for more than 3 years were at a greater risk than less frequent users. The authors concluded that these data showed a dose-response relationship.

Male diabetics were at greater risk from the use of sweeteners, including saccharin, than male diabetics who did not use sweeteners. This conclusion was complicated because male diabetics who never used sweeteners were at a reduced risk (0.8 as compared to nondiabetic nonusers), and diabetic users had essentially the same risk as nondiabetic users (1.9).

The cases and control populations differed from one another in a number of ways: educational levels, occupations, and infection histories. The authors state that these differences were taken into consideration and did not alter the conclusion that sweetener use was associated with higher risk.

Summary

Male **users of sweeteners were more frequently found among bladder cancer cases than among controls. Male users of sweeteners and male diabetic users of saccharin were both at increased risk; females were not.**

THE (NEGATIVE) AMERICAN HEALTH FOUNDATION STUDY

Experimental Design

Over a **15-year span, bladder cancer patients were identified** in 17 hospitals in six U.S. cities. Each bladder cancer case was matched with a control on the basis of age, race, and sex. Because bladder cancer had been associated with tobacco use, the controls were selected from people hospitalized for cancers that are not associated with tobacco use.

Beginning in 1973, each case-control pair was questioned about artificial sweetener consumption. Although 574 males and 158 females were included in the 15-year study, a smaller number (132 males and 31 females) were questioned about sweetener consumption.

Results

The average age of male cases was 61; female cases 62. Thirteen of 132 male cases and 5 of 31 female cases had ever used sweeteners. These numbers do not differ significantly from 16 of 124 male controls and 5 of 29 female controls who had used sweeteners. The conclusion drawn from these data is that sweetener consumption was not found to be associated with increased risk. There were no statistically significant differences in marital status, education, or places of residence between the cases and controls.

Diabetics may consume more sweeteners than do nondiabetics in the general population. In this study, 40 male and 11 female cases were found to be diabetic. This frequency was not statistically different from the 38 male and 8 female diabetics found in the control population. Thus, diabetics were not overrepresented in cases compared with controls.

Summary

No correlation was found between sweetener consumption and occurrence of bladder cancer.

COMPARISON OF THE TWO STUDIES

The Canadian paper is in press in the journal, Lancet, and the American Health Foundation paper will appear in Cancer. The most striking differences between the two methods were the origins of the cases and the choice of controls. In the Canadian study, all cases from three provinces were included and compared to controls who

resided in the same neighborhood. In the American study, cases were obtained in selected hospitals and compared to controls who were sick with other cancers in the same hospitals.

The Canadians found that cases and controls differed in educational levels. No such difference was found in the American study, probably because the cases and controls were of "high social class, " based on hospital selection. These differences in selection of cases and controls may account for the difference in results. Ernest Wynder (191) has informed FDA Commissioner Kennedy that he will have data on more than 400 users and 4,000 controls by the end of September 1977.

If more extensive data and evaluation support the Canadian conclusions, the saccharin experience would be an additional example of animal testing predicting a human risk. Specifically, the results of the Canadian epidemiology study, if confirmed, show that saccharin causes bladder cancer in males. This finding parallels the rat experiments, which also showed that only males "are affected. Furthermore, the risk estimated by assuming 1) a mg/kg dose relationship between rats and humans, and 2) a linear extrapolation between rats and humans was 600 to 1,200 cases per year. This estimate from rat studies agrees with the 1,000 to 2,000 cases estimated from human studies.

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