CRITERIA FOR EVALUATION OF
THE HEALTH ASPECTS OF USING FLAVORING
SUBSTANCES AS FOOD INGREDIENTS

June 1976

Prepared for

BUREAU OF FOODS
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
WASHINGTON, D.C.

under

Contract Number 223-75-2002
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LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
9650 Rockville Pike
Bethesda, Maryland 20014
FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB) provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the opinions of knowledgeable investigators who are actively engaged in work in specific areas of biology and medicine.

This technical report was prepared for the Bureau of Foods, Food and Drug Administration (FDA) by the LSRO, FASEB, in accordance with provisions of contract number 223-75-2002. The report was written by the members of an ad hoc Select Committee on Flavor Evaluation Criteria (SCOFEC) with the assistance of the LSRO staff.

The Select Committee accepts the responsibility for the contents of the report. The members of SCOFEC are listed in Section VI, A. Other scientists provided useful information to the Select Committee; however, the listing of their names (Section VI, B) does not imply that they endorse the study conclusions. Special appreciation is expressed to Dr. Kenneth D. Fisher, Associate Director, LSRO for his valuable assistance to the Select Committee in the preparation of the numerous drafts of the report.

The report was approved by the Select Committee, the Director of LSRO, and subsequently by the LSRO Advisory Committee consisting of representatives of each constituent society of FASEB, under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the report was approved and transmitted to FDA by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

C. Jelleff Carr, Ph.D.
Director
Life Sciences Research Office
SUMMARY

In 1974, the Food and Drug Administration proposed changes in regulations concerning general recognition of safety and prior sanctions for food ingredients. These changes suggested more critical definition of what should be classed as Generally Recognized as Safe (GRAS) food ingredients. The Select Committee on Flavor Evaluation Criteria was convened by the Life Sciences Research Office, FASEB, to develop appropriate criteria for evaluation of the GRAS status of flavoring substances added to foods.

The Committee reviewed the history of the regulatory control of flavoring substances in the United States and other countries. They developed a plan for review of flavoring substances and have prepared background position statements that undergird recommended criteria and procedures for the comprehensive evaluation of the health effects of flavoring substances. The criteria and procedures described in this report suggest a three-tier evaluation process involving a review of existing information on flavoring substances and sequential value judgments based on the adequacy of evidence that biological effects are reversible, irreversible or equivocal. Where biological and toxicological data are adequate, a reasoned judgment can be made. In cases where data are inadequate or incomplete, further investigation of biological effects would be necessary before an evaluation opinion could be reached. This portion of the evaluation plan involves a modified tier-type progression of in vitro and in vivo testing. The report concludes with a recommendation of priorities based on data available, suspected toxic effects, and the extent of use of the flavoring substance.

The Committee conducted a preliminary evaluation of five flavoring substances to test the feasibility of the proposed criteria and procedures. They concluded that the proposed plan of review is workable.
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I. HISTORY OF THE REGULATORY APPROACH TO SAFETY OF FLAVORING SUBSTANCES

A. FEDERAL FOOD, DRUG AND COSMETIC ACT AND THE CONCEPT OF GENERALLY RECOGNIZED AS SAFE

The Federal Food, Drug and Cosmetic Act-1936 states that Generally Recognized as Safe (GRAS) substances are exempt from the premarketing clearance required for food additives. As stated in the Code of Federal Regulations (Office of the Federal Register, 1975a), GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. The Code also indicates that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimental data. The Food and Drug Administration (FDA) recognizes further (Office of the Federal Register, 1975b) that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Act did not identify all natural and synthetic flavoring substances used in foods. However, a number of naturally occurring spices, seasonings and related substances were added to the GRAS list during the period 1936 to 1958 (Office of the Federal Register, 1975c). In 1958, the FDA requested identification of other flavoring substances that should be GRAS from the Flavor Extract Manufacturers' Association of the United States (FEMA) (Hall and Oser, 1968).

B. FLAVOR EXTRACT MANUFACTURERS' ASSOCIATION APPROACH TO THE CONCEPT OF GENERALLY RECOGNIZED AS SAFE

In response to the FDA request, and because the statutes did not specifically state how general recognition of safety was to be established nor who the qualified experts were to be, the FEMA interpreted the statutes as allowing creation of expert panels outside the FDA or other agencies. On this basis, an Expert Panel was selected by the FEMA in 1958 to review flavoring materials used as food ingredients (Hall and Oser, 1968). 

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Since 1958, the FEMA Expert Panel has periodically surveyed manufacturers and users to obtain information on identity, quantities produced, levels of use, manner of use, and toxicological data related to natural and synthetic flavoring substances. The evolution of the criteria used for GRAS evaluation and the list of acceptable flavoring substances have been described in a series of publications by the FEMA Expert Panel (Hall and Oser, 1961, 1965, 1970; Oser and Ford, 1973a, 1973b, 1974, 1975; Oser and Hall, 1972). The FEMA/GRAS list is given in Appendix C.

A cogent point in the FEMA Expert Panel evaluation process was the relatively small quantity of flavoring substances added to foods (Tables I and II). For example, if the total amount of a given compound used was 1000 lbs (454 kg) per year, then the average daily consumption by adults would be approximately 0.1 µg per kg body weight per day. Relatively few flavoring agents are used in amounts greater than 10,000 lbs (4543 kg) per year and 71 percent are used in amounts less than 1000 lbs (454 kg) per year.

The distribution of flavoring substances by highest maximum use was also determined from surveys of manufacturers (Table II). The distribution of flavors noted in Table II illustrates the concentration of single substances in the foods in which they are used. Using estimates of maximum levels of use for all flavoring agents, and assuming maximum average consumption of foods containing flavoring substances, the total quantity of flavoring substances consumed was about 26 g per person per year (Hall and Oser, 1968). When this estimate was made, approximately 1100 flavor additives were used in foods, and of these, about 73 percent were used at less than 100 ppm as a maximum use level (Table II). It is important to emphasize that the FEMA/GRAS list identifies flavoring substances added to foods not those flavors naturally present in foods. However, most FEMA/GRAS substances also occur naturally.

The FEMA Expert Panel guidelines are similar to those developed by the Food Protection Committee in their review of substances used as flavoring materials. The 1969 report of the Food Protection Committee of the National Research Council expressed the opinion that:

"If a chemical has been in commercial production for a substantial period, e.g., 5 years or more, without evidence of toxicological hazard incident to its production or use, if it is not a heavy metal or a compound of a heavy metal, and if it is not intended for use because of its biological activity, it is consistent with sound toxicological judgment to conclude that a level of 0.1 ppm of the chemical in the diet of man is toxicologically insignificant."

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For organic chemicals meeting special structural requirements, but where toxicological data were deficient, the Food Protection Committee (1969) employed the following guideline:

"For many substances that are functionally effective in food at dietary concentrations above 0.1 ppm, but still much below any reasonable judgment as to their maximum safe level, as defined in the introduction, there is need to arrive at estimates of toxicologically insignificant levels. For these substances, it is justifiable to employ accumulated scientific experience, and to recognize their structural analogy to other chemicals whose metabolism or toxicity are known. Reasoning by analogy may be used to arrive at conclusions of toxicological insignificance. If a substance meets all the following criteria, it may be presumed to be toxicologically insignificant at a level of 1.0 ppm or less in the human diet.

1. The substance in question is of known structure and purity;
   2. It is structurally simple;  
   3. The structure suggests that the substance will be readily handled through known metabolic pathways; and
   4. It is a member of a closely related group of substances that, without known exception, are, or can be presumed to be, low in toxicity."

Examples that are intended to be illustrative, not limiting, are:
(a) Straight-chain or simply branched aliphatic alcohols, acids, and esters.
(b) Linear polymers of ethylene or ethylene oxide.
(c) Cellulose ethers.
(d) Monomeric aromatic compounds containing only carbon, hydrogen, and oxygen, and equipped with one or more functional groups that include hydroxyl, aldehyde, and keto.

'Closely related' shall be understood to mean:
(a) Near members of a homologous series.
(b) Geometric or positional isomers that would not be expected to present serious differences in chemical reactivity or steric effects.
(c) Substances of identical basic structure or differing only by (a) or (b) above, and possessing additional functional groups readily accommodated by known metabolic mechanisms.
(d) Compounds readily metabolized into substances meeting the other criteria here listed."
### TABLE I

TOTAL AMOUNTS OF GRAS SUBSTANCES USED IN FOOD FLAVORING

<table>
<thead>
<tr>
<th>Usage Category (lb./yr.)</th>
<th>Number of Substances</th>
<th>Percent of Total</th>
<th>Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>200</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>11-100</td>
<td>467</td>
<td>33</td>
<td>47</td>
</tr>
<tr>
<td>101-1,000</td>
<td>346</td>
<td>24</td>
<td>71</td>
</tr>
<tr>
<td>1,001-10,000</td>
<td>187</td>
<td>13</td>
<td>84</td>
</tr>
<tr>
<td>Over 10,000</td>
<td>222</td>
<td>16</td>
<td>100</td>
</tr>
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</table>

### TABLE II

DISTRIBUTION OF FLAVORING INGREDIENTS BY HIGHEST AVERAGE MAXIMUM USE LEVEL INTRODUCED INTO FOOD

<table>
<thead>
<tr>
<th>Use Level Category (ppm)</th>
<th>Number of Substances</th>
<th>Percent of Total</th>
<th>Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.99</td>
<td>27</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1-9.9</td>
<td>278</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>10-99</td>
<td>744</td>
<td>51</td>
<td>73</td>
</tr>
<tr>
<td>100-499</td>
<td>150</td>
<td>11</td>
<td>84</td>
</tr>
<tr>
<td>Over 500</td>
<td>223</td>
<td>16</td>
<td>100</td>
</tr>
</tbody>
</table>

The FEMA Expert Panel attached considerable weight to the natural occurrence of a substance in foods. While a long history of use in foods is not necessarily proof of safety, they concluded that the lack of evidence of a causal relation to human illness offers some justification for assuming a lack of acute toxicity. The more widely a substance has been found, the higher the levels of use, and the greater the volume of consumption of foods containing the substance, the more likely the presumption of safety (Hall and Oser, 1968).

It was also recognized that many flavors are present in foods as a result of methods of food processing and preservation such as cooking, drying, pickling, and canning. These flavors have been called "natural" and likely are composed of hundreds of individual chemical substances that impart the desired or usual flavor or taste. The Expert Panel addressed only the issue of flavors added to foods during processing. Processing, evaporation or other causes usually decrease the amount of the flavor substance in the food as it is consumed.

The FEMA Expert Panel accepted as a guide the fact that analogous chemically related compounds would have similar toxicities. Therefore, if the toxicity and metabolism of a substance were known, then closely related compounds would probably have similar metabolic fates and toxicities. The Panel frequently based its conclusions, at least in part, on such analogies and in a few cases entirely on analogy even though reasoning by analogy was recognized as a difficult procedure (Hall and Oser, 1961). This concept embodies the idea that the toxicity of a member of an homologous series can be predicted from the toxicity of the immediately adjacent congeners.

The criteria of key importance used by the FEMA Expert Panel in reaching a decision on each substance may be summarized as follows:

- analogies with chemical-related substances with known toxicity or metabolism;
- the nature, level and volume of use of the substance in foods;
- the toxicologic significance of the levels of use;
- available toxicity data;
- metabolic pattern of compound in the body; and,
- occurrence of the substance in natural foods.
C. FOOD AND DRUG ADMINISTRATION REGULATORY ACTIVITIES CONCERNING FLAVORING SUBSTANCES

The Code of Federal Regulations established that flavorings and extracts may be used safely in food under two conditions. First, they are to be used in the minimum quantity required to produce the intended effect and with regard to the principles of good manufacturing practice. Second, they are to be one or more of the substances generally recognized as safe (GRAS) that are published by the Office of the Federal Register (1975e, 1975f; See Appendix C).

Recognizing the possibility of potential hazard, the FDA has studied the toxicity of certain flavors and extracts (Hagan et al., 1967; Jenner et al., 1964). These studies involved about 200 substances of the 1100 flavoring substances in use at that time. Those selected for study were the flavoring agents that were used in greatest amounts, were most widely used, had structures similar to compounds of known toxicity, or were suspected to be toxic based on findings in the literature.

In these flavor and extract studies, oral LD₅₀ values were determined in one or more species (rat, mouse and/or guinea pig) and the substances were then fed in their diet for 90 to 120 days, usually at a maximal level of one percent of the diet. Chronic feeding studies on rats, and sometimes dogs, were conducted only on those compounds that demonstrated some toxicity in the short-term trials. Jones et al. (1962, 1971) noted that in conducting long-term toxicity studies where flavor substances are mixed in the animal diet, analyses of the diet as fed should be performed to monitor the stability of the compound because experience suggests considerable loss may occur over time.

Based on these studies, the absence of any evidence for a possible carcinogenic effect or other untoward reactions, a 2000-fold margin of safety was considered as indicative of permitted use. If all criteria were not met, continued use on an interim basis was permitted pending further toxicity testing. Although numerous flavoring ingredients have been tested in this manner, many more substances have been approved by FDA as regulated food additives (Office of the Federal Register, 1975d). In many cases, FDA sanction was made without such testing, but was based on reasoned judgment from knowledge of natural occurrence, data on metabolism, and low self-limited use. The studies of acute oral toxicity (Jenner et al., 1964) and subacute and chronic toxicity (Hagan et al., 1967) were initiated by FDA because toxicological data on food flavoring substances were limited, despite long history of use and known natural occurrence.
An arbitrary, but widely accepted, rule for extrapolating animal toxicity data to man is to determine the highest dose that produces no observable adverse effects and accepting 1/100th or 1/1000th of that dose as "safe" for human consumption. This concept includes recognition of the inherent limitations that are involved in animal studies. However, the extent of testing depends on the type of food additive and the degree of human exposure. In addition, specific methods for carcinogenicity, teratogenicity and mutagenicity testing have been developed (Food and Drug Administration, Advisory Committee on Protocols for Safety Evaluations, 1970, 1971).

In 1964, some of the FEMA/GRAS lists of flavors were accepted by FDA as recognized flavors and extracts. Portions of the FEMA approved lists were reviewed by the FDA and published. Approximately 75 percent of the 1509 substances on current FEMA/GRAS lists are FDA approved flavors and extracts published in the Office of the Federal Register (1975c, 1975e, 1975f). The FDA lists of flavoring substances published in the Code of Federal Regulations has remained essentially unchanged since 1965. However, in 1974, the FDA proposed to clarify and redefine the status of all GRAS substances including the natural and synthetic flavorings (Anonymous, September 23, 1974).
II. CHARGE TO THE SELECT COMMITTEE ON FLAVOR EVALUATION CRITERIA

The GRAS concept embodies the principle that GRAS substances are generally considered safe because there is no evidence of harmful effects in their long history of use in food. The validity of the concept has been debated because the absence of adverse information is only presumptive evidence that the consumption of a substance is safe. However, in developing criteria for evaluation of a significant risk to the public health, it is recognized that a reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited.

On September 23, 1974 the FDA published a notice of proposed rule changes concerning General Recognition of Safety and Prior Sanctions for Food Ingredients (Anonymous, September 23, 1974). The proposed changes were suggested to more critically define what should be classed as GRAS. The entire question of what criteria and parameters for GRAS are to be used by FDA is under review and essentially this is the charge to the Select Committee on Flavor Evaluation Criteria (SCOFEC) with respect to flavor ingredients added to foods.

An ad hoc Select Committee on Flavor Evaluation Criteria (SCOFEC) was convened by LSRO, FASEB to develop appropriate criteria for the evaluation of safety of flavoring substances used in foods. Members of the SCOFEC are listed in Section VI. These qualified scientists, selected to review and appraise the available information on the criteria used to evaluate the health aspects of using various flavoring agents in foods were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines.

The Committee was to examine the GRAS concept as related to food in general and flavoring substances in particular. They were to review the needs of FDA as reflected in the Federal Food, Drug and Cosmetic Act (1936), as amended, for the evaluation of the safety of substances in foods. In addition the Committee was to specifically examine the FEMA Expert Panel's criteria employed in their selection of the so-called FEMA/GRAS list of flavors. The Committee was to review other studies made in assessing the safety of natural and artificial flavoring substances by similar scientific bodies. These latter reports constituted basic reference materials for the Committee. The criteria employed in the toxicological evaluation of chemical compounds added to or naturally present in foods were of prime concern to the Committee. It was understood that a major responsibility of the Committee was to recommend those criteria most timely and appropriate to evaluate the health aspects of using flavoring substances as food ingredients.
III. PROCEDURES OF THE SELECT COMMITTEE ON FLAVOR EVALUATION CRITERIA

The panel selected for the evaluation process was composed of qualified scientists representing the fields of nutrition, biochemistry, pharmacology, environmental toxicology, odor and taste physiology, chemical carcinogenesis, teratology, and behavioral pharmacology. Ad hoc consultants and professional LSRO staff assisted in the review and analysis.

SCOFEC members were expected to exercise judgment and draw upon their experience to assess the validity of conflicting reports, to weigh scientific data and opinion, and to select priorities for arriving at appropriate evaluation criteria. In fulfilling their charge, the Committee was well aware of the guidelines imposed by the GRAS concept and of the issue of benefit versus risk. They recognized that an ultraconservative approach to the selection of criteria would require detailed toxicologic evaluation of each one of the 1500 flavoring substances used in foods was unacceptable.

At the first of their five executive sessions, they considered the genesis of the FDA review of all GRAS substances, the charge to the Committee, their requirement to sign a confidential statement of employment and financial interests, and received a general review of work of the FEMA Expert Panel and reports by other organizations with their recommendations for safety evaluation of food flavoring substances.

At the second meeting they discussed the validity of rapid screening tests for carcinogenicity and mutagenicity, toxicity testing programs of the flavor industry, the factors considered by the food flavor industry in their development of suitable flavors, safety testing in general, consumer acceptance of flavors, and the extent of flavor use. The Committee also met with representatives of consumer interest groups to hear comments on the safety, extent of use, and need for food flavors, as well as the necessity to evaluate the health aspects of flavoring substances. An outline of their report was prepared and they began a review of the extensive reports of other scientific panels on toxicological testing of food substances (See Appendix B).

A draft report was prepared and reviewed by SCOFEC at their third meeting. The Committee believed that various facets of their deliberations should be documented to support their recommended criteria. These are presented in Appendix A of this report entitled "Background Information". Based on detailed discussions of the several parts of the report at this two-day meeting, the committee outlined a plan for selection of priorities for evaluation. It was agreed that the final report would include a critique of the FEMA Expert Panel criteria, proposed minimal criteria, and priorities for evaluation of the numerous flavoring substances.
The fourth meeting of the Committee was devoted to preparing the final report. The Committee reviewed the proposed criteria and the process that might be followed in future studies employing these criteria to assess the safety of the numerous flavoring substances.

The Committee proceeded to test their criteria with examples of flavor substances. Members of the SCOFEC reviewed each of five compounds selected from the monograph entitled, "Scientific Literature Review of Aliphatic Primary Alcohols, Esters, and Acids in Flavor Usage" (FEMA, 1974-1975). The final draft of this report incorporates the SCOFEC analysis of this application of the proposed criteria.
IV. RECOMMENDED SAFETY EVALUATION OF FLAVORING SUBSTANCES

A. REVIEW OF FLAVOR EXTRACT MANUFACTURERS' ASSOCIATION CRITERIA

Faced with the difficult task of evaluating the safety of the numerous food flavoring agents, the FEMA Expert Panel established evaluative criteria from reasonable generalizations of accepted toxicological principles. These criteria (noted on page 13) included background information needed in the toxicological evaluation of any chemical. The FEMA Expert Panel considered this information as minimal for the determination of the safety of flavoring substances and for their decision on FEMA/GRAS status of flavoring substances (Hall and Oser, 1968).

The decisions of the FEMA Expert Panel on specific flavoring substances during the past 12 years were made on the basis of available data; however, the quality and quantity of scientific evidence needed to meet their criteria involved a value judgment on the part of the Panel. It is recognized that other scientists may apply the criteria differently in terms of the amount of scientific evidence available or necessary. Fulfilling the criteria for safety evaluation may require more or less information depending upon the interpretation of minimal data needed. For example, it is implicit in the FEMA criteria that available toxicity data would be used in evaluation of flavoring agents; however, toxicological effects such as carcinogenesis, mutagenesis, and teratogenesis were not available at the time of their evaluations.

Guidelines developed by the FEMA Panel for safety evaluation of flavoring substances were first published by Hall and Oser (1961) and were further developed by Hall and Oser (1968). The most explicit description of the criteria and rationale utilized in developing the guidelines has been outlined by Gerarde (1972). The following paragraphs paraphrase the cardinal tenets and include SCOFEC comments on each.

1. **The Proof of Toxicologic Insignificance of Food Flavors is Their Ingestion By Man Through the Years Without any Apparent Harm or Adverse Effects.**

While this statement appears reasonable, it does not take into account the concept of establishing proof scientifically; establishing the absence of harmful effects requires valid investigations and cannot be presumed from the apparent absence of observable harmful effects. No practical prospective or retrospective monitoring systems exist that could detect
potential adverse effects of flavoring agents resulting from human consumption. Such effects would be reported only on the chance that an astute observer could establish a cause and effect relationship. However, such observations are unlikely, for the labeling of foods does not disclose the specific chemical nature of each flavoring agent. Many putative adverse effects, such as birth defects, allergy, or cancer would be expected to occur infrequently and have a long latency period. In addition, adverse effects may mimic a common disease entity and thus go unnoticed, as dietary history is not a part of the information usually obtained by attending physicians. The FEMA Expert Panel:

"took the position that flavor ingredients used at average maximum levels of less than 10 p.p.m. in foods, and at an annual volume of less than 1,000 pounds, for at least 10 years of common use, can indeed be said to be toxicologically inconsequential, provided the chemical structure is compatible with a reasonable presumption of the capacity of the body to dispose of trace quantities safely through known metabolic pathways." (Hall and Oser, 1968)

This statement is in contrast with the recommendations of the Food Protection Committee of the National Research Council (1969) (see page 11) that 1.0 ppm of a chemical in the diet of man would be considered toxicologically insignificant depending upon the extent of toxicological data available and the structure of the substance. The SCOFEC concludes that the FEMA criterion as well as the statement of the Food Protection Committee are, by themselves, not acceptable (See Appendix A, page 55).

2. **The Simple Fact that a Flavoring Agent is Present in Food in Low Concentration Should Suggest its Safety . . . Only the Dose Makes a Poison.**

SCOFEC does not concur with this tenet. There is no scientific basis to assume relative harmlessness, at least with regard to chronic toxicity, of a chemical if it is ingested at a dose level far below its known toxic dosage. A few chemicals can exert possibly irreversible toxic effects at extremely low levels of exposure. A further objection to this guideline is the problem of cumulative toxicity associated with multiple low-level exposures over long periods of time (See Appendix A, page 66). In addition, current concern with carcinogenicity, teratogenicity, mutagenicity, and long-term chronic effects make this FEMA qualification inadequate for the establishment of GRAS status (See Appendix A, page 84).

3. **Substances Present in the Tissues of Normal, Healthy Individuals are Presumed to Be Safe at Concentrations Found in the Tissues.**
If this guideline refers to substances elaborated by the normal metabolic pathways of the body, the SCOFEC agrees. However, it should be recognized that some toxic substances not natural to cells of healthy individuals may be metabolized by normal metabolic pathways in these tissues. Persistent environmental contaminants inhaled, ingested, or absorbed dermally are examples of such substances. Most synthetic and natural flavoring substances are not likely to fall into this category. While tentative decisions might be reached on the safe use of a substance, individual exceptions are sufficiently numerous to require scrutiny of each substance. The possible formation of hazardous derivatives in the gastrointestinal tract, the fate of the compound in the body in the trace quantities consumed, and the analogy to closely related substances warrant individual review. Foreign compounds such as flavoring substances, should be evaluated from the standpoint of physical and chemical properties, the quantity consumed, the alterations induced by digestion and the amount and quality of toxicological information about such derivatives.

4. **Toxicity is the Capacity of a Substance to Cause Injury. It is an Inherent, Unalterable Molecular Property, Dependent Upon Chemical Structure. However, Toxicity and Hazard are not Synonomous.**

SCOFEC agrees with these definitions, but it should be pointed out that toxicity can be measured only in biological systems. Toxicity is an intrinsic capacity of a substance to cause injury; hazard is the likelihood that the substance will produce injury under the circumstances of exposure (Coon, 1973). Thus, the assessment of hazard requires knowledge of the probability and extent of exposure to the specific chemical (See Appendix A, page 58).

5. **Compounds With a Low Order of Toxicity, Usually Producing Central Nervous System Depression, Cause "Physical Toxicity"; the Effect Depends Upon the Concentration of Molecules in Tissues, Rather than Molecular Nature or Chemical Composition.**

This statement is a paraphrase of Ferguson's principle of thermodynamic activity. It is generally true of the narcotic effect of low-molecular weight aliphatic and nonnitrogenous aromatic compounds. However, it ignores the extraneuronal effects of such chemicals. For example, blood levels of alcohol which produce unconsciousness are severalfold higher than blood levels that produce alcoholic cirrhosis of the liver. Similarly, carbon tetrachloride is hepatotoxic at a much lower dose than that required for anesthesia (See Appendix A, page 69).

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6. The Toxicity of a Member of a Homologous Series Can Be Predicted From Toxicity of Immediately Adjacent Cogensers.

The SCOFEC considers this guideline of structural analogy one of the most difficult to assess. Prediction of toxicity of a flavor which is an ester (or acetal) of a monofunctional alcohol and a straight-chain fatty acid (or aldehyde) has been successful for many simple compounds because the properties of metabolic products of digestion are well known and provide some basis for the predictions within these series. It is impossible to judge that a flavoring substance such as a polyfunctional isomer is without significant hazard on the basis of analogy to a compound or compounds of similar chemical structure even when their metabolic fate and toxicity are known. The metabolism of such a compound is only partially predictable at present, and may result in formation of toxic metabolites although an analogous compound may be harmless. Therefore, SCOFEC concludes that the hazard involved in incorporating a flavor in food cannot be reliably predicted solely from the chemical and physical properties and the metabolism of a closely related compound (See Appendix A, page 58).

7. The Functional Group or Groups in a Molecule Determine the Nature or Quality of the Toxic Action Inherent in a Chemical. The Size of the Molecule Determines the Intensity or Potency, and Increasing the Molecular Weight of a Chemical in a Homologous Series Tends to Decrease the Toxicity.

Taken together, these two statements would predict that in the series of aliphatic acids one would find similar toxic effects among all members of the series and that the longer the carbon chain, the less toxicity would be observed. This effect applies to a relatively limited series of compounds in which the biological effect relates to a particular biological property (See Appendix A, page 58).

8. The Chronic Toxicity of a Chemical Varies Inversely with Rate of Conversion of the Chemical to Less Toxic Products and Their Rate of Elimination From the Body.

While detoxification is typical, there are notable examples of enhancement of toxicity during chronic exposure as the result of biotransformation. Many of the known carcinogens require metabolic activation to their proximate carcinogenic metabolites in order to interact with cell growth regulatory factors. Thus, the FEMA statement tends to obscure the often minor, but sometimes significant, toxicologic enhancement associated with in vivo biotransformations. Microorganisms and mucosal cells can play a role in altering the residues of compounds from digestion and absorption. Bacteria have a large range of metabolic capabilities far in
excess of mammalian cells but both systems can produce significant changes in food components such as flavoring compounds (See Appendix A, page 69). Other chemical interactions between flavor chemicals and nutrient or non-nutrient chemicals present in food may occur during food processing or within the gastrointestinal tract. The potential for forming toxic products or altering biological availability of essential nutrients should also be considered in evaluation of safety of food flavors (See Appendix A, page 76).

9. **The Number of Enzymes in the Body is Limited. Therefore, the Number of Metabolic Reactions that Can Occur in the Body is Limited.**

There is evidence that hepatic, microsomal enzymes can be induced when the liver is challenged by a wide diversity of foreign substances. It seems reasonable to assume that flavoring substances would be metabolized by existing enzyme systems or would be metabolized by induced hepatic or extra-hepatic microsomal enzymes. The toxicologic consequences of microsomal enzyme induction under chronic dosing are under investigation, but their role in detoxification mechanisms is not fully elucidated (See Appendix A, page 72).

10. **The Usual or Preferred Metabolic Pathway Operates at Low Dose Levels of a Chemical and Alternative Pathways May Be Activated When Large Doses of Chemicals Enter the Body.**

If multiple dose-related metabolic pathways exist, the proportion of molecules of a substance being processed by each pathway will depend on the relative affinity and velocity constants of the chemical with the enzymes involved. Unless these factors are known, it is impossible to predict the preferential pathway at any dose, in any tissue, or in any species. Therefore, while different metabolic pathways for a chemical are likely to be active at different dose levels, it does not follow that relevant toxicity testing can be performed in animals only at those levels approximating the calculated human intake levels (See Appendix A, page 79).

11. **Chemicals Not Normally Present in Food and Having a Marked Difference in Chemical Structure From the Types of Chemicals Found in Food Should Be Regarded as Foreign Chemicals.**

SCOFEC agrees but notes that the examples cited in support of this guideline do not include the numerous chemicals naturally found in food that are known to have significant toxicity. Safrole is an example of a naturally occurring animal carcinogen found in some foods. Moreover, many foods may contain microbial toxins such as aflatoxin. Thus, the presence of a chemical in the natural ecosystem and normally present in certain foods does not insure lack of hazard.
The FEMA Expert Panel used these guidelines in their deliberation; however, no combination of such presumptions can adequately replace reliable data derived from well-designed toxicity testing. Experience has shown that, if adequately investigated, some chemicals may exhibit unique toxicologic properties, unpredictable from previous knowledge of related compounds. Such differences may appear minor in toxicity testing of a few animals of a given species, but may be far more significant in millions of people (See Appendix A, page 86).

B. PROPOSED CRITERIA

1. Basic Information

The assessment of safety requires adequate, reliable, and appropriate scientific data as a basis for rational value judgments. In theory, categories of required basic information can be developed prior to application of criteria; in practice, it is impossible to specify because of the non-uniformity of available information on each flavoring substance. For this reason, SCOFEC has specified an ideal situation, and then utilized this in developing an evaluation process. In the ideal situation, the following are the categories of information that should be included in a monograph on flavoring substances such as the first Scientific Literature Review of Aliphatic Primary Alcohols, Esters, and Acids in Flavor Usage (FEMA, 1974-1975):

<table>
<thead>
<tr>
<th>Basic Information Required - Ideal Situation</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Chemical and physical properties</td>
</tr>
<tr>
<td>1) common name and synonyms</td>
</tr>
<tr>
<td>2) source</td>
</tr>
<tr>
<td>3) trade names and manufacturers</td>
</tr>
<tr>
<td>4) chemical identity and names, including empirical and structural formulas</td>
</tr>
<tr>
<td>5) physical description, including characteristics and properties</td>
</tr>
<tr>
<td>6) chemical properties, including stability and reactivity</td>
</tr>
<tr>
<td>7) analytical techniques, including accuracy, precision and reliability</td>
</tr>
<tr>
<td>8) production processes or techniques</td>
</tr>
<tr>
<td>9) specifications for commercial and food grades</td>
</tr>
<tr>
<td>b) Manner and extent of use - human exposure</td>
</tr>
<tr>
<td>1) history of use in foods</td>
</tr>
<tr>
<td>2) qualitative and quantitative aspects of natural occurrence</td>
</tr>
</tbody>
</table>
3) quantity produced (manufactured and imported) for use as a flavoring agent annually
4) qualitative and quantitative aspects of use as a food ingredient
5) per capita use (range and average in terms of mg per kg body weight per year)
6) quantitative and qualitative aspects of consumption by age groups and special subgroups in the population, if applicable

c) Animal tests with pathological examinations (two mammalian species: one rodent, one nonrodent)
   1) acute toxicity
   2) short-term feeding
   3) long-term feeding
   4) special studies, such as carcinogenicity, mutagenicity, and fetotoxicity (may be combined with multigeneration, long-term feeding trials)

d) Physiological effects in man
   1) absorption, transport, distribution, excretion
   2) metabolic biotransformations
   3) biochemical actions
   4) interactions with drugs
   5) interactions with nutrients
   6) interactions with other food additives

e) Human experiential data
   1) experimental observations
   2) inadvertent exposures
   3) clinical reports
   4) anecdotal reports
   5) special studies, such as allergenicity

f) Literature references
   1) complete copies or equivalent of studies cited
   2) bibliographic citations for all data

A multiplicity of interrelated factors will affect the availability and adequacy of appropriate scientific data. The SCOFEC concludes that some latitude must exist in determining what basic information is required for evaluation of any given food flavoring substance. However, this issue should
be resolved by the scientists actually involved in the evaluation process. Certain minimal data should be available and assessing the adequacy of such data is the responsibility of the evaluators. The minimal acceptable basic information available should conform as closely as possible to that listed under Basic Information Required - Ideal Situation. The extent to which fewer data would be considered adequate must be evaluated on a substance by substance basis.

2. **Evaluation Procedures**

The SCOFEC has outlined a three level evaluation procedure that involves review of existing information and sequential decisions based on adequacy of data. The SCOFEC believes that evaluation of flavoring substances with the proposed procedures will lead to one of six evaluation opinions concerning the future status of these substances (Table III).

The first level evaluation procedures are outlined in Figure 1. A judgment of the adequacy of existing information would be made from scientific literature in the monographic compilations. An incomplete monograph, or an insufficient data base, would defer evaluation until specified deficiencies in essential information were corrected. If the initial review suggests that sufficient data are available, then evaluation proceeds to second level (Figure 2) involving analysis of the adequacy of data.

To obviate delayed evaluation of substances suspected to be hazardous, initial screening of each monograph should include selection of those flavoring substances that have been shown or suspected to have toxic effects in short or long term animal studies, or in tests for mutagenic, teratogenic or carcinogenic effects.

The second level evaluation would involve more critical scrutiny of available data, particularly information from human and animal exposures (Figure 2). In cases where studies of toxicological properties and data from human exposures exhibit no evidence of adverse health effects at any level of exposure, the flavoring substance would be cleared (See 3-1, Table IV). Where toxicological data provide evidence for, or reasonable grounds to suspect, adverse health effects, the reversibility of the observed biological responses and their relation to dosage levels should be determined. As indicated in Figure 2, evidence that the substance can produce irreversible effects *in vivo* would result in withdrawal. Evidence of reversible effects would suggest limited clearance. In this case, current usage might continue, but further evaluation would be necessary for new or increased levels of usage. Additional biological data would be required for this reevaluation process.
TABLE III
EVALUATION OPINIONS

1. Clearance:

The evidence in the available information on ____________ is adequate to demonstrate that there are no reasonable grounds to suspect a hazard to the public when it is used at current levels or those that might reasonably be expected in the future.

2. Limited Clearance:

The evidence in the available information on ____________ is adequate to demonstrate that there are no reasonable grounds to suspect any hazard to the public when it is used in the manner now practiced and in the amounts currently used. However, there is insufficient information on the possibility of health hazards from new uses or increased levels of ____________ as a flavoring agent.

3. Temporary Clearance:

While no evidence in the available information on ____________ demonstrates a hazard to the public when it is used in the manner now practiced and at levels that are currently used, uncertainties exist. Additional studies should be undertaken as indicated.

4. Interim Withdrawal:

Evidence in the available information on ____________ is equivocal but suggests that there may be a hazard to the public when it is used in the manner now practiced and at levels that are currently used. Additional studies should be undertaken as indicated and use of ____________ should be discontinued until reevaluation can be completed.

5. Withdrawal:

The evidence on ____________ in the available information is adequate to suggest that the reported adverse health effects may be deleterious to the public when ____________ is utilized in the current manner and levels of use.

6. No Opinion, Required Tests Specified:

There is little or no evidence on the biological effects of ____________ . Accordingly, an evaluative decision cannot be made and therefore certain studies must be conducted to provide an adequate basis for assessing the existence or absence of adverse health effects of ____________ when it is used in the manner now practiced and at levels now current.
Based on a review of the first Scientific Literature Review of Aliphatic Primary Alcohols, Esters, and Acids in Flavor Usage (FEMA, 1974-1975), the SCOFEC believes that the available toxicological data on a majority of the flavoring substances will be either equivocal or incomplete. For this reason, the third level evaluation procedure involves first an interim value judgment and second, suggested test procedures to provide definitive information on toxicological properties.

The third level evaluation procedures (Figure 3) assume that sufficient basic information is available to reach a conclusion suggesting temporary clearance or interim withdrawal. If temporarily cleared, the studies needed for evaluation would be indicated in the initial review. Interim withdrawal would be indicated if available toxicological data suggests irreversible effects or if reversible effects are, in the opinion of the evaluating committee, sufficient to merit discontinuance of use until adequate data have been collected from other studies specified in the review.

SCOFEC considers the decision on temporary clearance versus interim withdrawal as an expedient conclusion due to inadequate toxicological data for complete evaluation. After specified data were supplied, or following a finite period of time, the flavoring substance would be reevaluated and a conclusion of clearance, limited clearance or withdrawal would be reached.

In either case, the SCOFEC concludes that at this level in the evaluation procedure, the absence of credible toxicological data requires further investigation of biological effects.

Essentially, SCOFEC proposes a modified tier-type progression of in vitro and in vivo testing that is analogous to evaluation schemes proposed by other organizations (Bridges, 1973; Council of Europe, 1974; McCann et al., 1975; Stoltz et al., 1974). The tests included in the third level evaluation process (Figure 3) include:

a) In vivo testing
   1) acute oral LD₅₀ studies
   2) subchronic toxicity study

b) In vitro testing
   1) mutagenesis; microbial indicators with and without mammalian metabolic activation
   2) carcinogenesis; mammalian cells in culture with positive or negative induced malignant transformations

The in vivo toxicity studies should include an acute oral LD₅₀ in one rodent and one nonrodent species. These values are useful for comparative purposes and to provide a basis for doses to be tested in additional studies.
Subchronic toxicological data would be obtained by repeated administration of the test material (in the diet or the drinking water) in a rodent and nonrodent species. The duration of exposure should be at least 90 days (13 weeks). Effects on growth and development, behavior, blood and urine, and appropriate organ function would be determined at the onset and at intervals during exposure.

The rodents used in this subchronic toxicity study should be the F/1a generation of parents exposed from weaning. Both sexes should be fed at least 3 dosage levels with appropriate controls. Some survivors should be sacrificed and necropsied (all moribund animals to be sacrificed and necropsied; all animals found dead to be necropsied). Appropriate tissues would be submitted for histopathological examination. Some animals withdrawn from the test material at the end of the exposure period would be placed on a control diet for evaluation of reversibility of the observed effects. Observations would be continued during the next 4 weeks with the survivors sacrificed and necropsied and appropriate tissues submitted to a histopathological examination.

The proposed third level tests include in vitro mutagenicity and carcinogenicity testing with microbial indicators and mammalian cells in culture, respectively. These procedures are relatively inexpensive, rapid and reasonably predictive of possible irreversible toxicity. SCOFEC recommends these two in vitro systems solely as prescreening tests because currently, none of the available in vitro test systems can be used to establish that a substance will or will not be mutagenic or carcinogenic in experimental animals or man. Such conclusions can only be made from multigeneration lifetime feeding trials and associated studies in experimental animals.

Available data suggest that there is a high correlation between carcinogenesis produced in long-term testing in animals and results obtained using bacterial assay systems that measure mutagenic potential and cell culture systems that detect neoplastic transformation (Committee 17, Council of the Environmental Mutagen Society, 1975; McCann et al., 1975). Recent estimates of agreement are as high as 90% (McCann et al., 1975). If microbial and cell transformation tests are used together, the risk of "false negatives" getting through the screening procedure is minimized. However, it must be pointed out that negative results in such a screening procedure do not provide an adequate basis to conclude that a chemical is not carcinogenic; long-term animal tests would be required to demonstrate lack of carcinogenicity. False positives remain a problem "with highly sensitive" systems; however, a positive response in these tests would raise concerns and a decision would be made either to withdraw a flavoring substance that is of marginal importance or to conduct expensive long-term tests on animals.

A suggested scheme for disposition of the presence or absence of adverse health effects in the third level evaluation is given in Table IV.
If tests are uniformly negative, clearance would be suggested. If *in vivo* tests are equivocal, they should be repeated and modified to address the apparent inadequacies of the protocol for the subchronic toxicity study. If *in vivo* tests do provide evidence of toxicity, but *in vitro* tests are negative, an interim decision prior to completion of lifetime ingestion, multigeneration tests would be in order. In instances where one or more of the *in vitro* screening tests provide evidence of potential mutagenicity or carcinogenicity, the substance would be withdrawn until further evaluation tests could be completed.

The SCOFEC is fully aware of the investment that is required in chronic toxicity evaluation tests involving two mammalian species; however, there is at this time no feasible alternative for supplying adequate data for evaluation of the safety of substances used as food ingredients. The subchronic toxicity study is the logical basis for the lifetime feeding studies and these can be coupled with a three-generation reproduction study. Such tests are costly and time-consuming. They should be required only when fully justified on the basis of evidence available and the extent of use of the flavoring substance in foods.

Ideally, lifetime studies are conducted in two mammalian species. One study, usually in the rat, should begin with exposure at time of conception. Termination of the study should be according to survival and not be arbitrary at two years. Currently lifetime is defined as the point when only 20 percent of the starting group is still alive. To establish negative findings as valid, more than half the starting rats should have survived at least 18 months. Animals should be observed carefully; clinical and complete gross and microscopic pathology studies should be conducted. When indicated, specific tests may be required for certain compounds, such as cataractogenesis and neurological effects.

Reproduction studies usually in the rat over three filial generations should be combined with the lifetime study in order to derive the *in utero* exposed animals for the chronic phase. All reproductive indices should be recorded. Some of the pregnant females, usually of an F₀ and F₂ group should be sacrificed before term to assess teratologic or other effects such as fetal death and resorptions, and to make counts of *corpora lutea*.

3. Other Considerations

Protocols for acute and subchronic toxicity studies are numerous and the details depend upon the substances under test. For example, "experimental" animals implies the "most suitable species." "Adequate number" means that the results can be subjected to appropriate statistical analysis. The "most suitable species" is the one in which the disposition of the test material most closely approximates that in the human. Such knowledge presumes comparative experimental study of the test material in the suitable species and in man. Obviously, availability of testing facilities, fiscal constraints, and other factors will influence choice of the most suitable species.
The SCOFEC notes that the criteria for the evaluation of the safety of flavoring substances and their effects on animals and man may differ from those employed for foods, other food additives, or drugs. These differences relate to the unique character and function of flavoring substances. The following facts warrant consideration:

- Flavoring substances can initiate food intake and influence the digestive processes.
- The intended mode of action of flavoring substances is via the gustatory and olfactory system.
- The intake of flavoring substances is self-limiting.
- There is enormous variation among individuals in what they perceive with any flavoring material. These differences in response to flavor are chemical-specific and concentration dependent.
- Each species lives in its own sensory world. Man, with his senses cannot predict what will appeal to or offend a particular animal species. These differences must be considered in the design of toxicity tests of flavoring substances in animals.
FIGURE 1

FIRST LEVEL EVALUATION PROCEDURES

BASIC INFORMATION FROM SURVEY OF EXISTING SCIENTIFIC LITERATURE
A) Chemical and Physical Properties
B) Manner and Extent of Use
C) Toxicological Data
D) Human Experiential Data

Data Adequate for Evaluation

Data Inadequate for Evaluation

Proceed to Second Level Evaluation Procedures (Figure 2)

Complete Parts A, B, C, D as Required
FIGURE 2
SECOND LEVEL EVALUATION PROCEDURES

Data Adequate for Evaluation

No Evidence of Adverse Health Effects (at any level)

Clearance

Evidence of Adverse Health Effects (at any level)

Effects Reversible (either dose or not dose dependent)

Limited Clearance*

Data on Reversible Effects Equivocal or Incomplete

Proceed to Third Level Evaluation (Figure 3)

Effects Irreversible

Withdrawal

*See Table III
FIGURE 3

THIRD LEVEL EVALUATION PROCEDURES

Data on Reversible Effects Equivocal or Incomplete

Temporary Clearance

Interim Withdrawal

Acute Oral and Sub-chronic Toxicity Study in Mammalian Species

In vitro Mutagenesis Test

In vitro Carcinogenesis Test

With "Activators"

Without "Activators"

Disposition Based on the Presence or Absence of Adverse Health Effects*

*See text for discussion; see also Table IV
TABLE IV
SUGGESTED SCHEME FOR DISPOSITION BASED ON THE PRESENCE (+) OR ABSENCE (-)
OF ADVERSE HEALTH EFFECTS

<table>
<thead>
<tr>
<th>Major Categories of Test Results(^1)</th>
<th>Third Level Test Procedures</th>
<th>Proposed Disposition(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subchronic Toxicity</td>
<td>In vitro Mutagenesis Activators -</td>
</tr>
<tr>
<td>3-1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-2</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>3-3</td>
<td>+</td>
<td>-</td>
</tr>
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<td>3-4</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3-5</td>
<td>-</td>
<td>±</td>
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<td>3-6</td>
<td>-</td>
<td>±</td>
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<td>3-7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3-8</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>3-9</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>

\(^1\) Not a complete list of possible combinations of categories, see text for discussion
\(^2\) Refer to Table X for evaluation opinions
\(^3\) If subchronic toxicity tests are equivocal, repeat prior to lifetime studies; see text for further discussion
\(^4\) Value judgment necessary for continued use during period of further testing
\(^5\) Multigeneration lifetime feeding, mammalian carcinogenesis, and reproduction studies would be needed before disposition would be reevaluated, see text for discussion
C. EVALUATION PRIORITIES

The SCOFEC recognizes that review and evaluation of the material assembled in the several monographs on flavoring substances will be a difficult task. Application of the suggested criteria to all flavoring substances at one time is not possible, because monograph preparation, which began in 1974, will not be completed for several years. In addition, ongoing research can be expected to supplement material already collected in the monographs.

For these reasons, the SCOFEC suggests that review and evaluation of the health aspects of flavoring substances can be undertaken with the following priorities:

1) the availability of monographic compilations on chemical, physical, and biological properties of flavoring substances will determine which substances can be evaluated;

2) as noted previously (page 28), initial screening of each monograph should include selection of those flavoring substances that have been shown or are suspected to have toxic effects in short or long term animal studies, or in tests for mutagenic, teratogenic, or carcinogenic effects. These substances should be evaluated first;

3) with this exception, it seems that extent of use is the most logical prioritizing guideline. On this basis, the evaluation (Figures 1, 2, and 3) should be conducted on the basis of quantities used in foods in the following order:
   a) extent of use in excess of 5000 kg per year,
   b) extent of use in excess of 500 kg per year,
   c) extent of use in excess of 50 kg per year,
   d) extent of use less than 50 kg per year.

D. APPLICABILITY OF PROPOSED CRITERIA AND EVALUATION PRIORITIES

The SCOFEC has utilized the proposed minimal criteria in the evaluation of several substances contained in the first FEMA/GRAS review monograph (FEMA, 1974-1975). This exercise was considered a necessary step in reaching the conclusion that the basic information requirements and proposed schema for flavor evaluation do provide a workable method for evaluation of the safety of flavoring substances.
The Committee selected five substances at random and evaluated only the information contained in the FEMA/GRAS monograph. Because no additional information was sought or included in this evaluation test, the opinions reached by the SCOFEC must be considered an exercise of applying the criteria and not a complete evaluation of the five flavoring substances _per se_. The SCOFEC realizes that another evaluation committee might reach different conclusions particularly if the review and evaluation process were to include other information supplementing the FEMA/GRAS monograph.

The substances and information available in the FEMA/GRAS monograph used in this trial evaluation are indicated in Table V. Table entries refer to availability of information, not to adequacy of data except in the entry for "Pharmacological and Toxicological Studies." In this case "limited" refers to existence of data in the monograph and references cited, but the number of studies, experimental protocols, or types of toxicity testing were, in the considered judgment of the SCOFEC, incomplete or inadequate in terms of the proposed minimal criteria.

The SCOFEC examined the available information in relation to the proposed minimal information required. They discussed these data for each substance, and using the proposed scheme (see Figures 1, 2, and 3, pages 34, 35, and 36) reached a consensus on an evaluation opinion. On the basis of this evaluation and reflecting the quantities used in foods as flavoring substances, the SCOFEC developed the following opinions on the five substances:

(#13) **Valeric Acid** - temporary clearance based upon studies that suggest little evidence of adverse health effects. Additional studies required (see Figure 3) but a low priority for testing because of the quantity used in foods.

(#29) **Undecyl Alcohol** - temporary clearance for reasons similar to those noted above; however, a high priority for further testing despite low quantity of usage because related substances have been identified as possible cocarcinogens.

(#99) **Citral** - temporary clearance based on a need for additional long-term studies; a high priority for testing because more than 40,000 kg are used annually in the United States.

(#160) **Allyl Butyrate** - limited clearance because of some dose-related evidence of adverse health effects at high doses. However, temporary clearance might also be indicated if additional data were available to determine if the reported adverse health effects are either equivocal or reversible. A low priority for testing because of the quantity used in foods.
<table>
<thead>
<tr>
<th>FEMA #</th>
<th>Substance</th>
<th>Chemical &amp; Physical Properties</th>
<th>Occurrence in Foods</th>
<th>Usage in Foods</th>
<th>Pharmacological &amp; Toxicological Studies</th>
<th>Metabolic Studies</th>
<th>Reference Cited</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Valeric Acid</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>29</td>
<td>Undecyl Alcohol</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>limited</td>
<td>none</td>
<td>yes</td>
</tr>
<tr>
<td>99</td>
<td>Citral</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>limited</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>160</td>
<td>Allyl Butyrate</td>
<td>yes</td>
<td>not known to occur naturally</td>
<td>yes</td>
<td>limited</td>
<td>none</td>
<td>yes</td>
</tr>
<tr>
<td>168</td>
<td>Methyl Valerate</td>
<td>yes</td>
<td>yes</td>
<td>limited</td>
<td>none</td>
<td>none</td>
<td>no</td>
</tr>
</tbody>
</table>

*FEMA, 1974-1975.*
(#168) Methyl Valerate - interim withdrawal if no additional information on usage in foods and quantities consumed could be obtained; if such data are available, temporary or limited clearance possibly based upon natural occurrence of the substance; low priority for further testing based on quantity used in foods.

Based on this trial evaluation, the SCOFEC has concluded that the plan of review with the criteria developed by the Committee could be workable. The primary difficulty will be in establishing priorities for additional studies on any compound. Any future review and evaluating group must work out their own system of priorities for each of the FEMA/GRAS substances as noted by the SCOFEC. These will be developed best after considering the total number of compounds, not just those substances in the first FEMA monograph submitted to FDA.
V. LITERATURE CITED


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The members of the SCOFEC Committee wish to express their appreciation to Lee C. Rogers and C. Grace Gurtowski, LSRO, for technical and bibliographic assistance in the preparation of this report.
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BACKGROUND INFORMATION

I. IMPORTANT CONSIDERATIONS FOR EVALUATION OF FLAVORING SUBSTANCES

These reviews of various issues related to the health hazards of flavoring substances were prepared by the SCOFEC. The discussions should be viewed as discourses on issues that the Committee considered in the evolution of appropriate criteria for evaluation of the safety of food flavoring agents. They are included primarily as support for the positions taken by SCOFEC regarding the recommended safety evaluations of flavoring substances (Section IV).

A. THE SIGNIFICANCE OF LONG-TERM USE OF FLAVORING SUBSTANCES IN FOODS

Widespread consumption of a chemical substance in the human diet without producing any overt harmful effects does not provide scientific proof that the substance has not in fact produced some unrecognized subtle, insidious, harmful effect. It is difficult to ascertain specific causal relationships from continued or intermittent consumption of a substance that produces adverse effects only after years of ingestion. For example, the recognition of the dietary causes of goiter, lathyrisim, favism and ergotism occurred long after the diseases were first known. Suspicions of hazards related to chronic ingestion of substances continue to arise based on experimental results in animals or uncontrolled epidemiologic observations. For example, it is currently suspected, though not proved, that sodium (Meneely, 1973) and cadmium (Underwood, 1973) in the diet may affect the pathogenesis of hypertension. Saccharin has been used worldwide as an artificial sweetener for three quarters of a century. Despite the absence of known harmful effects of saccharin ingestion by man, and after many chronic toxicity studies in animals, it cannot be decided whether there is a risk in its long-term consumption (Coon, 1975).

However, notwithstanding these difficulties, a long history of widespread consumption of a substance, with no indication of harm, should be accepted at least as partial evidence for its safety, and should be considered in the evaluation of its safety. A quantitative value of the weight of such evidence is difficult to assign, but such a value would tend to increase with the duration of the historical use, the amount and frequency of its use by those consuming it, the number of consumers, and the quality of retrospective epidemiologic information.

Most of the natural products consumed by man have never been tested for their safety or margins of safety by modern methods of toxicological
evaluation. Natural food products originating from plants and animals and used by man throughout history, have been accepted as safe because long experience has revealed what can be eaten in reasonable amounts without harmful effects. For such products, and for their innumerable natural chemical components, history of use without known harmful effects is the primary basis for their acceptance as safe.

On the other hand, if a specific chemical component of a natural food product is isolated for use as a food additive, the apparently safe use of the natural product is not considered acceptable evidence for safe use of the component as a food additive. Safrole, for example, a natural component of several spices such as cinnamon, nutmeg and mace, was used as a flavoring agent for root beer, but this use was discontinued when studies showed that it could produce malignant liver tumors in rats (Long et al., 1963). In the case of natural products containing safrole, the history of use without evidence of harm has been the sole criterion in judging their acceptability for use in foods, whereas in the case of safrole itself, the history of its consumption in natural products provides no weight in favor of its use as a food additive. The case of safrole illustrates a general principle that care must be taken in the use of any specific component of natural food products as an additive in order to avoid increasing the amount of toxic substances that are already naturally present in food.

Many flavoring substances originally derived from plants are now synthesized chemically, and are chemically identical to the naturally occurring substances. There is no scientific basis for the common belief that these synthetic substances are more hazardous than those occurring in nature (Bernarde, 1971).

In perspective, man has added very few new synthetic chemicals to his food supply in proportion to the different chemical substances that occur naturally (Coon, 1973). Considering the variety of unidentified chemical substances in natural foods, it might be asked whether man has produced dietary components that, in the amounts used, cannot be handled by the defense mechanisms of the body. There is no reason to believe that the detoxication mechanisms of the body are less efficient in handling unnatural synthetic chemicals than in handling the natural chemical components of foods.

A long history of human use does not necessarily mean long-term consumption by particular individuals. A minimum of one human life-time, e.g., seventy years, would be necessary to eliminate possible effects such as arteriosclerosis and prostatic carcinoma. It is unlikely that disease susceptibility would remain stable over such a time period, and the possible role of dietary substances would remain moot unless their effects were rather striking and rapid. Nor should a record of consumption of certain substances by one group necessarily mean that the same level of intake could be adopted as safe for another group within the population.
In conclusion, food additives require both history of use and experimental testing before a definitive assessment of their safety can be made. However, because of the many uncertainties in extrapolating animal data to man, the results of toxicologic testing may be just as difficult to weigh as a long history of use in the definitive evaluation of the safety of food additives. A long history of use of a food additive without evident harm resulting may be useful in setting priorities for detailed toxicological examination; but alone it has limited value in providing an adequate assurance of the safety of lifetime consumption.

References


B. PREDICTABILITY OF TOXICITY IN SERIES OF ANALOGOUS COMPOUNDS

In making its decisions concerning the safety of flavor compounds, the FEMA Expert Panel placed considerable emphasis on analogies with chemically related substances of known toxicity or metabolism. This has been interpreted to mean that an organic chemical may be presumed to be toxicologically inconsequential at a level of 10 ppm or less in the human diet (Hall and Oser, 1968). The Food Protection Committee (1969), addressing the same issue, concluded that an organic chemical could be presumed to be toxicologically insignificant at a level of 1 ppm or less in the diet, if it met all of the following criteria: it is of simple and known structure and purity; its structure suggests that it will be readily handled through known metabolic pathways; and, it is a member of a closely related group of substances that, without known exception, are or can be presumed to be low in toxicity. "Closely related" in this context is understood to mean:

(a) near members of a homologous series;
(b) geometric or positional isomers that would not be expected to present serious differences in chemical reactivity or steric effects;
(c) substances of identical basic structure or differing only by (a) or (b) above, and possessing additional functional groups readily accommodated by known metabolic mechanisms; or,
(d) compounds readily metabolized into substances meeting the other criteria here listed (Food Protection Committee, 1969).

Thus it is implicit in this concept that an untested compound could be regarded as safe. In this respect, it is appropriate to examine the above assumptions as they apply to the evaluation of the safety of flavoring substances on the basis of information contained in the monographs being prepared on classes of flavor compounds.

The simplest types of homologous series of compounds are the alkyls containing methylene chains of gradually increasing length. In such homologous series various physicochemical constants, such as water solubility, lipid-water partition coefficient, vapor pressure, melting point, and critical concentration for micelle formation are expressions of the same basic properties of a compound, namely the presence of certain, and particularly of large, hydrophobic groups. Stereochemical fit on enzyme surfaces becomes a matter of importance in interactions due to electrostatic attractions, van der Waal's forces or hydrogen bonds.
When the effect on receptors is largely physical (Ferguson, 1939), several kinds of biological activity rise by small increments while the effective toxic concentration rapidly falls as a homologous series is ascended. Table 1 relates the biological action of homologous compounds to the thermodynamic activity, $a$, which is defined by the equation:

$$\bar{F} - F_0 = RT \ln a$$

where $\bar{F}$ is the partial molal free energy of the compound referred to a standard state, $F_0$. As each series is ascended, thermodynamic activity rises by small increments while the concentration at which toxicity occurs falls rapidly.

Moreover, where the biological activity is primarily dependent on the physical properties, it is usually observed that as the series is ascended there is a peak beyond which activity declines rapidly (Sexton, 1963). This trend is shown in Figure 1, Curve A. Curve B represents cases where the maximum potency is shown on the first member of the series. Curve C is encountered when the first member of the series has special properties, for example, in the toxicity of aliphatic thiocyanates to germinating seeds. High potency in the methyl compound is probably due to its chemical reactivity which decreases as the series is ascended. Meanwhile purely physical factors become dominant so that biological potency rises again. The descending leg of the curve sometimes goes down abruptly, often due to a drop in water solubility, so that the concentration of the compound at its site of action decreases markedly. As a general rule, water solubility in homologous series decreases in geometric progression as the number of methylene groups increases arithmetically (Fühner, 1924). However, alteration in the physical properties of homologous compounds with an odd and even number of carbons in their chains is well known. Figure 2 shows the effect on the melting point and water solubility of fatty acids and simple dicarboxylic acids. Note the alternation in the values of the parameters with values of $n$.

As a general rule, when the biological effect of a flavor is largely due to chemical reactivity, the toxic effects of lower homologs are reduced as the series is ascended. However, at some point in the series, which can only be determined experimentally, toxicity due to physical properties is predominant and the toxic dose decreases. Metabolism may produce toxic products. The alkyl thiocyanates are broken down by tissue reactions to yield cyanide (Von Oettingen, 1936). However, this property decreases with the number of carbon atoms in the thiocyanate so that at C12 there is little evidence of cyanide toxicity (Table 2).

The straight-chain, aliphatic alcohols, aldehydes, acids and their esters and acetals exhibit a distinction in the metabolism of odd and even-numbered carbon chains. The odd chains are metabolized to a three-carbon fragment while the even numbered chains yield a two-carbon fragment. This factor becomes important in bifunctional chains. Thus even
<table>
<thead>
<tr>
<th>Substance</th>
<th>Inhibition of development of sea-urchin eggs</th>
<th>Bactericidal concn. \textit{E. typhosa}</th>
<th>Fumigant toxicity, red spider</th>
<th>Tadpole narcosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mol/l</td>
<td>(a)</td>
<td>mol/l</td>
<td>(a)</td>
</tr>
<tr>
<td>Methyl alcohol</td>
<td>0.719</td>
<td>0.019</td>
<td>10.8</td>
<td>0.33</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>0.408</td>
<td>0.026</td>
<td>4.86</td>
<td>0.32</td>
</tr>
<tr>
<td>Propyl alcohol</td>
<td>0.136</td>
<td>0.034</td>
<td>1.50</td>
<td>0.34</td>
</tr>
<tr>
<td>Butyl alcohol</td>
<td>0.0454</td>
<td>0.043</td>
<td>0.45</td>
<td>0.37</td>
</tr>
<tr>
<td>Amyl alcohol</td>
<td>0.0204</td>
<td>0.070</td>
<td>0.13</td>
<td>0.52</td>
</tr>
<tr>
<td>Hexyl alcohol</td>
<td>-</td>
<td>-</td>
<td>0.039</td>
<td>0.63</td>
</tr>
<tr>
<td>Heptyl alcohol</td>
<td>0.00172</td>
<td>0.112</td>
<td>0.012</td>
<td>0.74</td>
</tr>
<tr>
<td>Octyl alcohol</td>
<td>0.0051</td>
<td>0.113</td>
<td>0.0034</td>
<td>0.88</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>Tadpole narcosis</th>
<th>Haemolysis</th>
<th>Substances</th>
<th>Narcosis of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mol/l</td>
<td>(a)</td>
<td>mol/l</td>
<td>(a)</td>
</tr>
<tr>
<td>Methyl acetate</td>
<td>0.08</td>
<td>0.026</td>
<td>1.15</td>
<td>0.43</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.03</td>
<td>0.035</td>
<td>0.47</td>
<td>0.55</td>
</tr>
<tr>
<td>Propyl acetate</td>
<td>0.01</td>
<td>0.045</td>
<td>0.16</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Pentane 0.0052 0.29
Hexane 0.0017 0.34
Heptane 0.00064 0.44
Octane 0.00032 0.82

Redrawn from Ferguson (1939).
FIGURE 1

TYPES OF VARIATION OF ACTIVITY AS AN HOMOLOGOUS SERIES IS ASCENDED

Redrawn from Fühner (1924); cited by Sexton (1963).

FIGURE 2

PHYSICOCHEMICAL PARAMETERS IN SOME HOMOLOGOUS SERIES OF COMPOUNDS

Redrawn from Karrer (1954).
<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimal Fatal Dose</th>
<th>Fatalities</th>
<th>Time Until Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc. per rat</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Methyl rhodanate</td>
<td>0.02</td>
<td>70</td>
<td>5 min. - 28 min.</td>
</tr>
<tr>
<td>Ethyl rhodanate</td>
<td>0.04</td>
<td>82</td>
<td>10 min. - 5½ hrs.</td>
</tr>
<tr>
<td>n-Butyl rhodanate</td>
<td>0.05</td>
<td>80</td>
<td>10 min. - 36 hrs.</td>
</tr>
<tr>
<td>Octyl rhodanate</td>
<td>0.30</td>
<td>80</td>
<td>3 hrs. - 8 hrs.</td>
</tr>
<tr>
<td>Decyl rhodanate</td>
<td>0.30</td>
<td>80</td>
<td>3 hrs. - 10 hrs.</td>
</tr>
<tr>
<td>Lauryl rhodanate</td>
<td>2-3</td>
<td>85</td>
<td>2 days - 5 days</td>
</tr>
<tr>
<td>Myristyl rhodanate</td>
<td>3-4</td>
<td>80</td>
<td>4 days - 7 days</td>
</tr>
<tr>
<td>n-Butyl-carbitol-rhodanate</td>
<td>0.15</td>
<td>80</td>
<td>35 min. - 3½ hrs.</td>
</tr>
</tbody>
</table>

Redrawn from Von Oettingen (1936).

---

**TABLE 3**

ALTERATION IN TOXICITY OF ALIPHATIC \( w \)-FLUORINE-COMPOUNDS*

<table>
<thead>
<tr>
<th>( n )</th>
<th>F(C)( n )COOH Odd</th>
<th>F(C)( n )COH Odd</th>
<th>F(C)( n )CO Even</th>
<th>F(C)( n )COH Even</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.6</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td></td>
<td>10</td>
<td>46.5</td>
</tr>
<tr>
<td>3</td>
<td>0.65</td>
<td>2</td>
<td>0.9</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>&gt;100</td>
<td>81</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.35</td>
<td>0.58</td>
<td>1.2</td>
<td>1.7</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>&gt;100</td>
<td>80</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>0.64</td>
<td>2</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>&gt;100</td>
<td>53</td>
<td>32</td>
<td>21.7</td>
</tr>
<tr>
<td>9</td>
<td>1.5</td>
<td>1.9</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>10</td>
<td>57</td>
<td>&gt; 40</td>
<td>&gt;100</td>
<td>15.5</td>
</tr>
<tr>
<td>11</td>
<td>1.25</td>
<td>1.5</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

*\( LD_{50} \) for mice in mg/kg intraperitoneally.
Redrawn from Ariens (1971).
FIGURE 3

BIOLICAL EFFECTS AND NUMBER OF CARBON ATOMS IN ALKYL AND METHYLENE CHAINS

Modified from Ariens (1971).
numbered ω-fluorine acids are converted to the highly toxic fluoracetic acid while the odd-numbered acids yield the much less toxic fluoropropionic acid (Ariens, 1971)(Table 3).

It is apparent that the relative positioning along a chain of two or more functional groups may have a profound effect on the chemical toxicity of a homolog. For example bis-(α-chloromethyl) sulfide and bis-(γ-chloropropyl) sulfide are relatively nontoxic compared to their homolog, the ethyl compound which is mustard gas. 1-Chloro-2-pentene, by virtue of its allylic chloride, is very reactive and toxic while 5-chloro-1-pentene is of much lower toxicity. Figure 3a shows further a relationship between the number of carbon atoms in alkyl chains or methylene chains for certain biological effects. If the principle of analogy were applied to an estrogenic compound (Figure 3b), selection of a 2-carbon chain from knowledge of either the 1- or 3- carbon compound would lead to increased toxicity (estrogenic activity).

Examples cited from a large number reported in the literature seemed to indicate clearly that prediction of a particular toxicological property of a near homolog can be reliable only in such series as the monofunctional fatty alcohols, aldehydes, and acids where considerable data on trends and exceptions exist.

In homologous series of polyfunctional compounds, selection of an untested structural isomer poses a risk that cannot be predicted reliably from its chemical and physical properties or from knowledge of the metabolism of a closely related compound.

References


C. THE NEED FOR LONG-TERM INGESTION STUDIES IN SAFETY EVALUATION OF FOOD FLAVORING AGENTS

Chronic toxicity studies are necessary because of insufficient knowledge of the relationships between the short-term effects of chemical-biological interactions and their long-term sequelae. Distinction should be made between chronic exposure and chronic effect (Casarett, 1975). Chronic exposure means only repeated frequent ingestion over a prolonged period of time. Chronic effect implies injury that persists for a prolonged period, either because the exposure is prolonged and the frequency of injury exceeds the rate of injury repair, or because of the irreversible and sometimes progressive nature of a short-term injury. Chronic effects can result both from chronic exposure or as sequelae to relatively short-term exposures. Chronic exposure is generally considered to be at least one-half of the life span of the organism.

Reversible or irreversible injury may result from three types of chronic exposure to chemicals: a) accumulation of the chemical to a critical concentration at sites of action sufficient to induce detectable injury; b) accumulation of the injury until physiological reserves can no longer compensate; or c) long latency period between an initial unrecognized biologic event and its manifestation as injury. In the first case, knowledge of the kinetics of chemical absorption, metabolism, and excretion obtained in short-term studies allows prediction of the probability of accumulation of the toxic chemical under prediction of the probability of accumulation of the toxic chemical under conditions of long-term use. Thus, the need for low-dose chronic exposure studies may be obviated. In the case of flavoring substances, this decision would be unequivocal only when the toxicokinetic studies indicate that the substance is not absorbed, or where other extensive tests clearly establish the concentration-effect relationships for interactions of the flavoring agent or its metabolites with certain critical biological macromolecules. Prediction of the possibility of chronic accumulation of injury, or of its latent development, depends upon a thorough understanding of kinetics of injury production and repair.

Reversible effects are deviations from normal structure or function induced by a chemical which return to normal limits after cessation of exposure to the chemical. With irreversible effects, the deviation persists or may progress. The time required for return to normality after exposure should be a reasonably small fraction of the remaining lifetime of a young animal for the effect to be considered "reversible." Reversibility may be qualified by the normal lifetime of a specific cell or macromolecule that serves as the endpoint for the effect. Adaptation by numerous mechanisms restoring the deviation to normality in spite of continued exposure to the chemical, is another possible mode of 'apparent' reversibility.
There are certain effects of toxic chemicals that are unmistakably irreversible. These include the production of terata or malignant tumors, production of mutations in offspring of exposed animals, and certain chronic neurologic changes or other chronic degenerative disorders, such as cirrhosis or emphysema. While there are gross manifestations of certain specific chemical-cell interactions, there most probably are early reversible effects either at the level of the first affected cellular process or at intervening points. Prediction of adverse effects in a shorter period of time may be possible if the critical dose and the rate-limiting factors that determine reversibility are understood. Until this type of mechanistic information is available, evaluation of toxicity will generally stress the importance of irreversible effects, and there will likely be more concern about the possibility of their production than emphasis on the detection of reversible effects.

The net rate of reversibility of an effect will be dependent upon the rate of cellular injury and the rate at which this injury is repaired (Casarett, 1975). The rate of injury will depend upon the concentration and duration or frequency with which a test chemical contacts responsive tissue constituents. It is, then, dose and dose-rate dependent. The rate of repair is determined intrinsically and involves several cell processes. It may vary between different tissues and will likely exhibit species and strain differences. From a practical standpoint, it will generally be impossible to measure the specific processes involved in injury and repair in the standard toxicity evaluation study. However, it is important to make measurements of reversibility of effects as they are perceived in early acute and subacute studies. Thus, the time required for a process to return to normal after single doses, which produce varying degrees of injury, will provide a guideline for selecting doses to be used in subsequent subacute or chronic ingestion studies.

The predictive value of such tests will depend upon the persistence of the chemical in the test organism. If the chemical produces an effect and then is rapidly detoxified or excreted, it may be possible to predict with reasonable accuracy the doses or exposure schedules which would not produce cumulative effects. The manner of exposure and possible effects other than the one being measured would be important in reaching such conclusions. For example, rapid reversibility after a single dose might not be indicative of the rate of reversal under a repeated dosing schedule if the first dose, in addition to the measured effect, also altered either the repair process or processes responsible for detoxification of the chemical (Murphy, 1967). Repeated exposure studies are necessary to evaluate these possibilities. Therefore, the design of short-term feeding studies should include groups of animals that can be removed from exposure either at the end of the experiment, or preferably, at selected intervals. The rate of reversal of effects in these animals can be measured at intervals or at some critical time after exposure ceases.
If the chemical persists in the organism, it may accumulate, and measurements and interpretations of rates of reversal of effects are then more complicated. It is useful to have kinetic data on absorption and disposition to correspond with data on rates and reversal of effects. Further discussion of these and related principles is provided by Hayes (1972) in relation to his proposed determination of "chronicity factors" \[ \frac{\text{1-dose } LD_{50}}{\text{90-dose } LD_{50}} \text{ (mg/kg/day) in diet} \] useful in predicting candidate chemicals requiring long-term studies. Application of this concept to evaluation of the toxicity of flavoring substances would have to be coupled with consideration of the potential for other effects such as tumorigenesis, that are not detectable in subacute studies.

In summary, it is important to conduct well-designed long-term feeding studies on chemicals which humans are likely to ingest routinely in their diet, in order to detect possible adverse effects which at present cannot be accurately predicted by conventional 90-day studies. Although carcinogenesis is the most obvious effect of concern, other progressive degenerative diseases, which have a long latent period or where very slow accumulation of the chemical or injury occurs, may require prolonged exposures for their manifestation. A thorough knowledge of disposition kinetics, concentration-effect relationships, and the kinetics of injury production and repair may obviate the need for long-term feeding studies. However, our current test methods and understanding of disease processes are generally not adequate to permit such shortcuts in the safety evaluation of chemicals to which a large spectrum of the population may be exposed daily. Certain types of recently developed in vitro screening procedures combined with adequate 90-day tests in intact animals and data on the biological disposition of chemicals in question are useful for setting priorities for long-term feeding studies. However, until sufficient research has been conducted to unequivocally demonstrate the predictive value of short-term test methods, prudence requires that high volume or frequent use food additives be evaluated under long-term exposure conditions.

References


D. BIOTRANSFORMATIONS AND ACTIONS OF FLAVORING AGENTS IN THE GASTROINTESTINAL TRACT

The enzymes of the microbial flora of the gastrointestinal tract catalyze numerous reactions which can alter the molecular structures of a wide variety of classes of chemicals, including many food flavoring agents. These microbial transformations may alter the physico-chemical properties of compounds to influence their rates of absorption and lead to metabolites which are either more or less reactive with biological macromolecules. Microbial inactivation, if it represents a significant protective mechanism for the organism under normal circumstances, may also provide the basis for unanticipated adverse effects of chemicals when these biotransformations are interfered with by other chemicals, diseases, or dietary conditions that alter the quantity and quality of intestinal flora (Williams, 1972).

1. Biotransformation of Ingested Substances

A variety of compounds in the diet escape digestion and absorption and hence become susceptible to bacterial alteration in the intestine. Flavors as well as coloring materials, food additives, drugs, alkaloids, terpenes, aromatic acids, undigested foodstuffs and contaminants such as pesticides are thus converted to products of varying biological activities (Drasar and Hill, 1974; Scheline, 1968; Williams et al., 1971). The metabolites may be either more or less reactive with biological macromolecules.

The common microorganisms of the gut are Enterococci, Lactobacilli, Veillonella, yeasts, Clostridia, Bacteroides, Bifidobacteria and Enterobacteria; the last three types predominate. In addition, Pseudomonas, Neisseria, Propionobacteria and Corynebacteria have been cultured from intestinal specimens but frequently may not constitute large populations in vivo. In man the small intestine contains only a few bacterial species, predominately the Lactobacilli and Bifidobacteria.

It must be recognized that the population of bacteria in the gut is variable. Physiological mechanisms, bacterial interactions and environmental influences such as diet and intake of antibiotics all interrelate in determining the nature and distribution of bacteria in the gut. Aging and changes in bowel habits also exert effects on the flora (Draser and Hill, 1974).

The microbial alterations of chemicals that occur in the intestine are principally fermentative. Thus the processes of reduction, hydrolysis, deamination, and decarboxylation predominate with dealkylations, deacylation and dehydroxylation, aromatization, dehalogenation, and ring fission representing minor changes. The principal result of these bacterial actions
is the production of fatty acids, alcohols, ammonia, amines and reduced
sulfur compounds (Williams, 1972).

a. Microbial actions on functional groups

Glucuronides. Most of the glucuronides entering the intestine have been
formed in the liver and excreted via the bile. Glucuronidase is found in
most of the gut bacteria. Many glucuronides are hydrolyzed to release the
aglycone which is reabsorbed in the portal circulation. On returning to the
liver the compound is reconjugated to the glucuronide and reexcreted in the
bile. This enterohepatic circulation results in a much longer retention of
the compound in the body and allows time for bacterial production of other
possibly more toxic metabolites.

Glycosides. Most of the plant glycosides are glucosides, where the attach-
ment of the sugar moiety may either be α- or β-. Adult mammals lack
β-glucosidases, but the enzymes are produced by most intestinal micro-
organisms. The diet provides a wide range of glucosides some of which
produce harmful effects. Three major cyanogenic glucosides are amygdalin,
dhurrin and linamarin. These products are harmless when injected paren-
terally due to the absence of a mammalian β-glucosidase. However, in the
gut, all release cyanide as well as their aglycone, benzaldehyde, p-hydroxy-
benzaldehyde and acetone respectively, and hence are highly toxic. Cycasin,
the glucoside of raw cycad nuts, is hydrolyzed to its aglycone, methyl
azoxymethanol, which produced tumors of the liver in rodents (Drasar and
Hill, 1974).

Disaccharides. The major disaccharides of the diet, sucrose, lactose and
maltose, are sometimes added to foods for flavoring purposes. These
sugars are hydrolyzed by enzymes located in the enterocytic brush border
and thus do not reach regions colonized by microbes. In certain disease
states, however, the disaccharides are not hydrolyzed by mucosal enzymes.
Under these conditions microbial enzymes are important in production of
fermentative diarrhea. The most common condition is lactase deficiency
which makes most of the adult population of the world lactose intolerant.
Lactose fermentation yields fatty acids, ethanol, carbon dioxide and hydro-
gen giving rise to abdominal discomfort, flatulence and diarrhea. Defici-
cencies of sucrase, isomaltase and maltase are much rarer conditions.
Other oligosaccharides in the diet are present in rather small amounts.

Primary Amino Groups. Gut microorganisms release ammonia from amino
acids and amines by four direct pathways -- oxidation with formation of an
aldehyde and ammonia, reduction with formation of a fatty acid, hydrolysis
with substitution of an hydroxyl group, or removal of ammonia to produce
an unsaturated product. Reductive deamination is the predominant reaction.
Hydrolysis and loss of ammonia is usually followed by reduction so that a
fatty acid is the common product of these pathways.

Secondary Amino Groups. Secondary amines may be dealkylated. This
is often accompanied by ring opening of cyclic secondary amines to form
primary amines. Gut bacteria can induce opening of the pyrrolidine ring of proline and of purine and pyrimidines, producing ammonia, carbon dioxide, and ethanol. Enterococci, Clostridia, Bacteroides and Bifidobacteria can nitrosate secondary amines using nitrite as the nitrosating agent. The amount of nitrosation varies widely, being related to the alkalinity of the amine. Diphenylamide is 68 percent converted while dimethylamine is less than 0.01 percent converted.

Tertiary and Quarternary Amines. In these classes of compounds the principal action of bacteria is N-dealkylation. For example, choline is converted to trimethylamine, dimethylamine, methylamine and ammonia.

Diazo Compounds. A wide range of the azo dyes used as coloring agents are reduced by gut bacteria (Drasar and Hill, 1974).

Nitro Groups, Nitrate and Nitrite. Reduction of nitrate to nitrite can be carried out by Enterococci under either anaerobic or aerobic conditions. In high nitrate concentrations, nitrite is the principal product but at low nitrate concentrations, ammonia is formed. Many organisms produce a nitrite reductase; the end product is free nitrogen or ammonia. Aryl nitro-groups are reduced with some difficulty to aryl amines. Bacteria deaminate tyrosine reductively to phloretic acid which is then decarboxylated to 4-ethyl phenol or oxidized to phenyl acetic acid, p-cresol and phenol. Dihydroxyphenylalanine produces similar products including an 0-methylation product, 4-methylguaiaicol.

Sulfate Esters. Sulfate esterification is a common pathway of detoxication of foreign compounds in the liver. Since most of these are excreted in the urine and not in the bile, little bacterial metabolism occurs. However, hydrolysis by intestinal bacteria has been reported. Taurine is converted to free sulfate. Cyclamate (cyclohexyl amine-N-sulfonate) is converted to cyclohexylamine most of which is excreted in the urine (Drasar et al., 1972). It is generally supposed that the sulfatase activities of bacteria which normally inhabit the intestine are very low.

Ester and Amido Groups. Both simple and complex esters may be hydrolyzed by bacterial lipases. Furthermore, pancreatic lipases are capable of hydrolyzing esters in the small intestine. Amide hydrolysis produced by bacteria includes action on the bile acid conjugates, the sulfonamides, and chloramphenicol. Hippuric acid, the detoxication product of benzoic acid, is hydrolyzed by the gut bacteria. Two types of activity are observed with the penicillins. The so-called penicillinases open the β-lactam ring while a second type of hydrolysis involves removal of the side chain.

Heterocyclic Rings. Ring fission by the intestinal microflora is a common reaction with heterocyclic oxygen compounds. Rutin is converted to m-hydroxyphenyl propionic acid. Coumarin is converted to melilotic acid.

Unsaturated Rings. Reduction of double bonds by intestinal organisms is extensive and accompanies other reactions in which ammonia or water are eliminated. For example, ricinoleic acid is hydrogenated to hydroxystearic acid and caffeic acid is partially reduced to 3,4-dihydroxyphenyl propionic acid.
Aromatic Rings. Microbial conversion of nonaromatic cyclic compounds to aromatic substances has been recognized for more than a century. Quinic acid (1, 3, 4, 5-tetrahydroxycyclohexane carboxylic acid) may be converted to benzoic acid and catechol.

b. Mucosal cell actions on functional groups

In addition to chemical biotransformations catalyzed by enzymes of intestinal microflora, the cells of the gastrointestinal mucosa also produce many of the same biotransformations (Hartia, 1973). Although both types of cells can catalyze all of the major types of reactions that are involved in foreign compound metabolism (oxidation, reduction, hydrolysis and conjugation) microbial reactions are principally reductive, while oxidative reactions predominate in mucosal cells.

Recent research on biotransformations in intestinal mucosa has focused primarily on the mixed-function oxidase system (MFOS). Although the spectrum of foreign-chemical biotransformations catalyzed by MFOS in the intestinal mucosa appears more restricted and the specific activities generally much lower than in the liver (Lake et al., 1973; Wattenberg, 1972), these biotransformations in intestinal mucosa are of considerable importance in modulating local toxic actions of low concentrations of ingested substances. The MFOS activity of the gastrointestinal mucosa can be increased by certain of the so-called "inducers" of drug metabolism (Lake et al., 1973), and it is of particular importance that the activity of gastrointestinal mucosal MFOS can be markedly influenced by constituents of natural diets (Wattenberg, 1972).

2. Toxicological Implications of Biotransformations

The biotransformation of ingested substances by either gut microflora or mucosal cells can play a role in regulating the quality and quantity of these chemicals or their metabolites that are absorbed. However, in the case of food flavors, the influence of biotransformations on chemical injury within the gastrointestinal tract itself, takes on special significance because of the low total dose but possibly high local concentration under which flavors would generally be encountered. It is possible that the amount of flavor absorbed might be insufficient to cause injury remote from the site of absorption, but the local concentrations on the mucosal cells may be sufficient to cause injury at first site of contact. The concept that the specific cellular site of injury of certain foreign chemicals may be determined by the site of biotransformation to reactive metabolites that bind covalently to cell membranes, critical soluble proteins or nucleic acids, has received considerable support from recent studies in other tissues (Reid et al., 1973a, 1973b).
The 'local' injury that might result could include reversible inhibition of enzymes involved in the metabolism of nutrient or non-nutrient chemicals, irreversible cell membrane damage leading to death of cells, or alkylation of nucleic acids with resultant induction of mutations followed by the possibility of a delayed manifestation of gastrointestinal cancer. Intestinal cancer has a geographic distribution that suggests an environmental influence. It has been suggested that dietary factors contribute to this disease. Furthermore, cellular biotransformations and resultant injury that occur within the cells of the microbial flora could have adverse effects such as altered quality of the bacterial flora or altered capacities of the existing flora to metabolize both nutrients and non-nutrients.

3. Other Chemical Interactions

Ethylene diamine tetra-acetic acid (EDTA) is a chelating agent that enhances the absorption of some foreign compounds from the gastrointestinal tract (Feldman and Gibaldi, 1969; Schanker and Johnson, 1961). It has been suggested that the action of EDTA on absorption from the gastrointestinal tract is "potentially dangerous to the organism, since it might promote the absorption of ingested allergens and also that of toxic substances normally produced within the intestinal contents" (Schanker, 1971). Some food flavoring compounds, or their biotransformation products have properties of chelating agents and thus might alter the absorption of foreign compounds or essential trace minerals.

Direct chemical interactions of food additives with food constituents occur during processing or storage of food and result in either the formation of toxic products or destruction of nutrients (Golberg, 1967). A classic example of this type of interaction is the formation of the convulsant compound methionine sulfoximine resulting from the treatment of flour with nitrogen trichloride.

The possibility of formation of carcinogenic nitrosamines and nitrosamides in the gut from a variety of naturally occurring substances or from direct or indirect additives, has been the source of considerable concern. The chemistry of the formation of N-nitroso compounds has recently been reviewed by Mirvish (1975). Although food flavoring compounds have not been specifically implicated as reactants in the formation of nitrosamines, piperidine is a nitrosable compound in acid pH and piperidine derivatives are used as food flavors. Mirvish (1975) has suggested that it should be possible to predict nitrosamine formation from a consideration of chemico-physical properties of a compound. Such considerations should be applied to the evaluation of existing or potential food flavors.

In summary, microbial and mucosal cells can play a role in altering the residuum of compounds from digestion and absorption. Microorganisms
have a large range of metabolic capabilities far in excess of many mammalian cells, but both systems probably produce changes in flavoring compounds. Some metabolic alterations of flavors may produce compounds of increased biological activity or toxicity. This may lead to liberation of such active compounds as aglycones, toxic arylamines, epoxides or phenols. Intestinal biotransformations of flavors may result in local cellular injury. Altered absorption and systemic actions are also possible. Other chemical interactions between flavor chemicals and nutrient or nonnutrient chemicals present in food may occur during food processing or within the gastrointestinal tract. The potential for forming toxic products or altering biological availability of essential nutrients in the gastrointestinal tract should also be considered in evaluation of safety of food flavors. However, because food flavorings are present in foods in minute quantities, there is a reduced likelihood that detectable quantities of biotransformation products will be present.

References


E. THE SIGNIFICANCE OF UNTOWARD REACTIONS TO FOOD FLAVORING SUBSTANCES

Untoward reactions to normally innocuous food ingredients can involve toxicity, idiosyncracy, and hypersensitivity. These reactions are essentially unpredictable. Many individuals believe and state categorically that they have experienced allergic reactions to natural or processed foods, but in very few instances have specific antibodies been identified or appropriate challenge tests conducted. As currently understood, true or immunologic allergy is unlikely to be caused directly by low molecular weight, nonproteinaceous compounds ingested at the level of most food flavoring agents.

Certain signs and symptoms which are classical examples of the allergic response may occur without a demonstrable immune response. This helps to explain why few proven cases of hypersensitivity reactions to food flavoring substances exist despite the numerous anecdotal reports of allergic-type signs and symptoms. Nonimmunologic mechanisms which may be associated with adverse reactions to foods and food chemicals include enzymatic deficiencies as in disaccharide and gluten intolerance; chemical irritation of the gastrointestinal tract by such foods as cabbage, onions, and spices; toxic reactions from tainted foods including improperly preserved fish and shellfish, which may develop a high histamine content; untoward reactions to food contaminated by bacteria and their products; and toxic effects of naturally occurring or added chemicals in foods.

Toxic effects occur when the amount of toxic material exceeds the tolerance and these reactions are independent of the immune response. Reactions of certain individuals are often termed idiosyncratic in cases where the exact nature is unknown but genetically determined factors are suspected. Untoward idiosyncratic reactions may be mediated by either immune or nonimmune mechanisms and may be precipitated by minute amounts of the eliciting substance. The clinical signs and symptoms of toxic reactions are generally distinguishable from those of hypersensitivity and idiosyncrasy; however, differentiation between idiosyncrasy and hypersensitivity solely on the basis of clinical signs and symptoms is not feasible.

Most authorities agree that hypersensitivity reactions to normally innocuous substances are mediated by the immune system. With respect to food additives such as flavoring agents, an interaction with humoral antibodies or specifically sensitized lymphocytes is assumed to be a necessary part of the phenomenon. When the effects of the immune response are beneficial to the host, they are called "immunity"; when detrimental, they are variously known as "hypersensitivity," "allergy," or "sensitivity." Hypersensitivity may be defined in terms of four types of reactions (Lakin, 1972):
anaphylactic reactions (Type I); cytotoxic reactions (Type II); toxic-complex reactions (Type III); and, cellular hypersensitivity (Type IV).

Many authorities prefer to use the traditional immediate and delayed classification of hypersensitivity (Austen, 1970; Gordon and Ford, 1971; White and Timbury, 1973a). Immediate hypersensitivity depends upon circulating antibodies and includes all the allergic responses that begin within minutes or a few hours of any antigen-antibody interaction. Among these are anaphylaxis, the Arthus reaction, and responses such as the hemolytic blood transfusion reaction caused by antibodies acting against cell or tissue antigens.

Delayed or cell-mediated hypersensitivity typically manifests itself in inflammatory reactions which develop in 12 to 48 hours and feature local accumulations of mixed cells, particularly lymphocytes and macrophages. Examples are the positive tuberculin skin tests, the well-known rejection of homografts, and the contact or eczematous dermatitides. Delayed hypersensitivity reactions do not result from antigenic interactions with circulating antibodies; instead, the reaction to the local application of antigens is mediated by nucleated cells of the peripheral blood. These cells, thymus-controlled small lymphocytes (T cells), are able to react with specific antigens and, in some way, influence macrophages to participate in the reaction (Austen, 1970; White and Timbury, 1973b).

Accurate estimates of the prevalence of food allergies are difficult to obtain. Barkin and McGovern (1966) did not report food allergy as a separate category in their review of allergy statistics. Patterson (1972) states that food allergy is uncommon and the incidence of food allergy decreases with age. On the other hand, Feingold (1973) and Rowe and Rowe (1972) suggest that food allergies are more common than most people appreciate. Food allergy per se is not a separate category listed in estimates of allergy prevalence completed by the National Center for Health Statistics.

While no pertinent incidence data are given, Feingold (1973) and Rowe and Rowe (1972) indicate that milk, eggs, cereals, and chocolate are the most common allergenic foods. In the cereal group, wheat and corn are the most frequent offenders. Randolph and Yeager (1949) have reported that corn was the most frequent cause of sensitivity reactions in 200 consecutive cases of food allergy. There is little definitive information available on prevalence of allergy or hypersensitivity to food flavoring substances.

Unfortunately, there are no standard animal tests for predicting chemical allergenicity in humans. In view of the assumed low level of prevalence of allergy to food flavoring agents, allergenicity testing of flavoring agents would be impractical, but surveillance and reporting of such effects would be desirable.
References


F. THE IMPORTANCE OF TASTE AND OLFACTION IN SAFETY EVALUATION OF FOOD FLAVORING AGENTS

The sensory receptors, particularly those for taste and olfaction, are the gatekeepers that determine what food will be introduced into the digestive tract. Body response to a food occurs via the various sensory receptors which transmit information to the central nervous system, and the perception of a food flavor is relatively rapid. Thus taste permits a prompt decision as to whether or not food will enter the digestive tract.

The preoccupation with toxicity should not detract from consideration of the special functions of flavors in food. Physiological effects on the digestive system by food flavors can be different from those of preservatives, stabilizers, and many nutrients. Some foods, sugar for example, may be added for flavor, although generally flavors are present in concentrations that do not contribute significantly to the caloric or nutrient value of the food.

While caloric value of a unit weight of a food, such as starch, may double when the amount is doubled or remain constant in a mixture of ingredients, a flavor is a flavor only within a narrow range of concentrations and only in well-defined mixtures. An analogy can be made to primary colors and the new colors that result in mixtures; even further, flavors and their contribution to a prepared food can be considered as analogous to colors and their effects in a painting.

The potential toxicity of a food ingredient is important enough to justify testing and evaluation. For less deleterious effects, the evaluation should be relevant to the intended use of the substances. For example, chemicals used to provide sour and salty flavors are commonly present at substantially higher concentrations than those that provide bitter taste or the various odors; in addition, many flavors are detected and judged in concentrations below the sensitivity of available analytical measuring instruments. The intake of flavors is self limiting and most are effective in minute quantities. Massive concentrations that significantly exceed a pleasant effect simply do not gain entrance to the digestive tract. For these reasons toxicity testing at high dosage levels is inappropriate.

It is still not possible to predict the sensory quality of a flavor on the basis of chemical structure. This is illustrated by the diversity of chemical structures that provide man with a sweet sensation, for example: simple carbohydrates, saccharin, cyclamates, dihydrochalcones, lead acetate, dipeptides, and even proteins. In addition, there are simple carbohydrates that are not very sweet and many dipeptides that are bitter.
With odors a similar absence of predictability based upon structure exists. For example, the mirror image forms of the molecule carvone have different odors--spearmint and caraway. Minor changes in structure within an homologous series can result in an odor totally unrelated to that of the parent compound. This situation contrasts with other physiological systems because structurally related drugs or hormones would be expected to provide similar physiological effects.

Within an individual as well as in related species, emphasis should be placed on the fact that molecular structure cannot be used reliably to predict flavor. However, there are some continuities: sour taste is related to free hydrogen ion concentration; simple carbohydrates are generally sweet; and various salts such as lithium and sodium chloride taste "salty." Within some chemical series, the toxicity of one member of a homologous series may possibly be estimated from the toxicity profile of the remainder of the series; however, no such predictions can be made about the sensory qualities of structurally related compounds.

An important function of flavor is to encourage eating. This need is not apparent to many Americans. However, where nourishment is critical such as in the infant, the hospital patient, or the individual in an unusual environment, food flavor may be significant in determining whether the food is consumed or rejected. Sensory stimuli can influence salivary secretion, mastication, and the pattern of deglutition. Digestive tract activity, including gastric contractions and intestinal motility, can respond to oral stimulants. Recent studies have confirmed and extended Pavlov's early observations that sensory stimuli can influence digestive secretions (Kare, 1971). Apparently, taste stimuli can modify the volume and nature of digestive secretions. There is also some evidence that oral stimuli can affect the release of metabolic hormones (Hommel et al., 1972).

These physiological functions of flavors in food should be given consideration in any testing procedures. These "invisible" functions challenge the dismissal of flavors as contributing merely cosmetic and hedonic qualities to the diet. If food is not consumed, monitoring the nutritional quality or safety of food is meaningless.

Flavor stimuli can be mixtures as complex as coffee or as simple as glucose. One part in many millions can serve as an adequate stimulus, particularly for odors. However, in the case of a poor taste stimulus, such as sucrose, considerable mass is necessary. The stimulus molecule must come in contact with a receptor. The best evidence indicates that the stimulus binds to the receptor in a weak reversible interaction. However, it is now known that the large molecule, monellin (molecular weight 10,700), can evoke a sweet taste response, suggesting that penetration of the receptor cell by the stimulus is unnecessary. The interaction is then converted to a
nerve impulse at the receptor, and transmitted to the brain to evoke the sensation. There are many points at which the sensation of a flavor could be influenced. For example, the receptor could be modified to either alter the nature of the perceived stimulus or to increase or decrease the sensitivity. As new modifiers, potentiators, and flavor enhancers become available, it may be necessary to know their mode of action before deciding if they should be evaluated as flavors.

There is enormous variation among individuals in their senses of taste and smell. These differences are chemical-specific and concentration-dependent. The inherent differences in what can be perceived by an individual are further complicated by learned responses to flavor stimuli. This diversity in response to tastes and smells argues against the suggestion that the number of flavors be limited to reduce the testing problem and it is independent of the desire or possible need by man for variety in food.

Human genetic differences in perception are well documented in the case of bitter taste; other individual variations extend across the spectrum of taste and olfaction. These differences can be further magnified by circulating hormones, helium, age, and the impact of other sensory information (Kare, 1971).

Unique sensitivity of a deleterious nature to a food additive by even a small group of people is of serious concern. However, unique response to flavor is common, and unusually high or low thresholds for detecting flavors used in food is to be expected. In the latter instance, the major effect on the individual might be a transitory unpleasantness. A commercial level of added flavor might be imperceptible to some individuals and at the same time offensively concentrated to others.

Perception of flavor has been demonstrated in the fetus. The human newborn will respond to sugar solution at concentrations in the range perceived by the adult (Desor et al., 1973). The preference for high concentrations of sugar and salt solutions by the adolescent differs from those of the adult (Desor et al., 1975). Contrary to popular opinion, the major loss of sensory cells in the aged may have little or no effect on flavor preference although detection threshold may be altered (Kare, 1975). It is apparent that flavor in food is of consequence in all age groups although a small percentage of the American population, estimated to be one to two percent, suffers from chemically evident alterations in taste or smell.

Most criteria used to establish safety of flavoring substances require screening in animals. These criteria are based upon procedures often used for food additives or drugs. However, special problems arise concerning flavor in animal studies. Each species has evolved senses to supply its own needs (Kare, 1970). Man cannot, using his own senses, reliably predict what will appeal to or offend the senses of another species. For example, many
of the noncarbohydrate sweeteners that humans describe as sweet, such as saccharin, have no appeal to or are actually rejected by many animal species. Even in extreme dilution, dimethyl anthranilate, a constituent in flavor mixtures is uniquely offensive to birds. On the other hand, the coprophagous behavior of dogs and rats, or the boar's salivary pheromone that evokes mating behavior in the sow, suggest that there are sensory qualities eluding man's receptive mechanisms. Clearly, we cannot be anthropomorphic in dealing with the sensory world of animals, and unlike tests for toxicity, the use of animals in flavor testing requires special precautions.

In animal testing, the use of massive quantities of a flavor might provide misleading results related to unusual sensory impact. The test animal may be offended by the material, even at levels used by man. Enormous concentrations used to magnify an effect may render the material so offensive that it constitutes a serious stress. If the oral receptors are bypassed, as when the material is administered by stomach tube to overcome unpalatability, the material is no longer being tested as a flavor.

The sensory effect of a flavor in an animal test should provide behavioral responses similar to those anticipated in man. The introduction of undefined oral stimulation to a test animal could result in altered digestion which might produce deleterious effects related to its abnormal sensory effect and not the chemical. It has been suggested that specially sensitive species might be employed for screening tests. For example, trout are a preferred species in testing for aflatoxins. However, any uniquely low threshold for the olfactory and gustatory stimuli should be ruled out before adopting a species for food flavor toxicity studies.

Newborn or young animals are commonly used because of their high sensitivity and nutritional needs. However, this may be a period of rapid change in sensory perception and learned responses based on taste or odor cues by the animal. The time of appearance and numbers of sensory receptors after birth differ among species. The sensory behavior of adult animals cannot be assumed to be identical to that of young animals. The special physiology and behavior of any test species must be taken into consideration before its use as a test animal for flavors.

Some suggested criteria for use of animal species in screening flavors include:

- The flavor should be tested in a species whose behavioral and metabolic responses to the material are as similar as possible to those of man;
- the concentration should be in the range that is appealing to the animal; at the least, it should not be unreasonably offensive to the test animal;
• where a flavor is tested in a mixture, the other materials should have been established not to have an overwhelming sensory effect, or not ones to which the animal is uniquely sensitive;

• normal oral administration or self-selection is suggested;

• unique species, age groups, or other conditions would be acceptable only where they have been established as useful with the specific flavor under consideration.

It is recognized that these considerations constitute a possible dilemma in selection of specific tests or test species to be included in the toxicological evaluation of flavoring substances.

References


G. IMPLICATIONS OF NEW TECHNIQUES FOR EVALUATING THE
HEALTH ASPECTS OF FOOD FLAVORS

Reconsideration of minimum acceptable standards for concluding that
a negligible human health hazard exists for a food flavoring agent is confound-
ed by the fact that numerous compounds have been assigned such status based
on testing conducted over a decade ago when the extent of toxicological knowl-
edge was meager. Societal concerns for the safety of foods have recently
focused attention on levels of acceptable risk and a need for more critical
evaluation of possible toxicity of food additives. The application of newer
scientific developments and techniques will provide a better basis for the
toxicological evaluation of food flavoring substances.

In less than two decades, toxicity evaluation of chemicals has evolved
from the empiric practice of administering test substances at various dose
levels and recording symptoms and death, to a recognized scientific disci-
pline. Toxicologists utilize the tools of pharmacology, pathology, biometrics,
and other disciplines to make reasonably predictive estimates of human health
hazard based on controlled studies in animals under analogous conditions of
use. Suitable animal models are available for testing particular forms of
toxicity, and multispecies comparisons, often including subhuman primates,
are frequently conducted.

Analytical techniques such as atomic absorption spectroscopy,
neutron-activation analysis, gas chromatography and mass spectroscopy,
have been introduced recently and provide a means for specific detection of
inorganic and organic molecules at sensitivities as low as the picogram and
part per trillion range. Thus the limits of "zero" must be reconsidered as
toxicologically significant impurities and contaminants may be detected in food
flavoring substances. In addition, the presence of impurities in extremely
low concentrations or expected human exposure at very low levels can no
longer be viewed as \textit{prima facie} evidence of being toxicologically insigni-
nificant.

The biotransformation of chemicals foreign to the body has taken on
new toxicological significance. This process may involve activation of
compounds to highly reactive and potentially toxic intermediates which are
thought to mediate carcinogenicity, mutagenicity, parenchymal cell destruc-
tion, hypersensitivity and perhaps teratogenicity. Concern for these tox-
icologically significant events is heightened by growing evidence that con-
comitant exposure to other chemicals can enhance the formation of these
potentially toxic substances and that because of genetic differences, some
individuals may be particularly susceptible.
The recent development of relatively simple tests for mutagenicity in \textit{in vitro} systems has provided a possible means of rapidly screening compounds for carcinogenic potential. While mutagenic compounds need not be tumorigenic, a strong relationship between these forms of toxicity is being established. In the past, carcinogenicity evaluation required chronic and often life-time study of test compounds because of the long latency between exposure and manifestation. It is possible to envision \textit{in vitro} mutagenicity testing as a minimum criterion and to require carcinogenicity testing in cases where positive mutagenic results are obtained.

The confidence in estimates of human health hazards from toxicity evaluation in animals has been enhanced by observing correlations between the results of animal testing and human epidemiologic surveys. For example, confidence in the value of teratogenicity testing has been at least partially restored by the finding that rhesus monkeys and rabbits are sensitive to the teratogenic effects of thalidomide and that these two species could have predicted that this drug would be a human teratogen. Unfortunately, the commonly used species - rat - is relatively insensitive.

The promulgation of new criteria creates at least two significant problems related to the development of new toxicological techniques:
1) Regulatory decisions based on earlier methods of toxicological evaluation may appear inappropriate in light of more recently adopted test techniques; and
2) Recommended minimum criteria, reflecting the current state-of-the-art, may appear excessively stringent by comparison with previous standards.

The first problem may be addressed in one of the following ways and the most rational choice appears to be the third option:

- Continue to accept "Grandfather" compounds that are currently recognized as safe;

- Require reevaluation of any compound when available toxicity information does not meet the new criteria; or

- Determine priorities for reevaluation of existing compounds on the basis of known animal toxicity, chemical structure, level of intake, or other similar factors.

With regard to the second problem, the only reasonable option is to delineate the justification for more stringent requirements, recognizing that what was adequate safety assurance years ago may be inadequate by current standards.
H. PUBLIC POLICY ISSUES AS RELATED TO EVALUATION OF THE HEALTH ASPECTS OF FLAVORING COMPOUNDS

Theoretically, assessment of safety of a compound is a scientific question requiring a review to determine whether available data are adequate for a decision. The methodology available for accurate evaluation of toxic responses continues to improve almost daily. Yet, decisions on criteria of safety must be made at a given moment on the basis of currently available methodologies. Thus, the evaluation is not only a scientific one but in the broader sense becomes a matter of judgment in which the individual's scientific knowledge and experience interact with his views on the role and nature of public policy.

The purpose of this discussion is to recognize that public policy issues exist, not because they belong in the scientific decision-making process, but rather to note that scientists asked to make judgmental decisions are aware of these issues. There are three major areas of concern:

1. Fear of environmentally-derived diseases;
2. The reduction of opportunities for individual discretion in utilization of certain foodstuffs; and
3. The significance of flavors in the development of new food products.

Cancer is an outstanding example of an environmental factor that individuals literally fear and desire to avoid. Other concerns include allergies, teratogenic influences, and numerous chronic diseases suspected as related to environmental causes. The public, taking the view that prevention is the best approach, is becoming increasingly suspicious of unknown or poorly understood elements of the environment that might be related to these undesirable conditions. The prevailing cultural thinking would reason that benefits should have no risk; one should be able to eat anything without having to worry about the consequences, including obesity. However, it is becoming increasingly clear that as more people are at risk there is an increasing public challenge of the so-called benefits of a number of foods and food additives.

Particular public attention has been directed to food additives, including flavoring materials. However, there is the mistaken notion that the only "chemicals" in foods are those identified on the label. These are the additives, as well as colors and flavoring materials. As we learn more about substances naturally present in foods and the chemical reactions that take place among natural components by such processes as broiling or roasting, the distinction between known and unknown substances becomes blurred. Nevertheless, given a choice, most consumers will opt to purchase foods that have a shorter rather than longer list of added ingredients on the label.
The second area of concern is related to the number of discretionary food choices available to the public. Depending on the definition of a processed food, it is estimated that over 50 percent of the foods eaten in the United States are processed. These are foods for which the individual has a restricted choice of ingredients. A good example is sodium chloride. The amount of salt that can be added at the table or in the kitchen is discretionary but the amount already available in the products as purchased is unknown. Estimates of discretionary consumption of salt range from only 10 to 30 percent of the total daily intake. Similarly, discretionary consumption of sugars and fats are a lower proportion of the total consumption than in previous decades.

As a result of these developments, the consumer has, for sake of convenience and in order to increase sensory variety, abrogated in part the prerogative of discretionary selection of foods according to individual taste. With the loss of discretion comes a reduction in control, and it becomes more difficult for the individual to meet what he considers to be his own nutritional needs.

If people decide they need or want more flexibility in the composition of their food, they must either select a set of conditions where there will be more discretion or require more stringent rules defining nondiscretionary dietary items. Added flavors are among these nondiscretionary items. It seems inevitable that public demand for more assurance of the safety of these nondiscretionary substances will continue.

The third area of concern is that flavors are a key factor in the development of new foods. Food manufacturers employ the flexibility that is provided by a broad array of flavoring substances to make new products attractive. The consuming public recognizes that the proliferation of flavoring materials requires control, including some control over the multiplication of new products. Therefore, the public decision either to encourage a broader variety of new products or to reduce the number of new products, will eventually be a determining factor in the number of flavoring substances in the diet.

In the final sense it matters little whether any of these issues are correctly stated; it is the public conception of the issues that is important. Each individual scientist will likely differ on these public questions; however, these issues will be ever present as each scientist attempts to focus upon what should be only scientific questions. The end product, nevertheless, must reflect, to the best of each scientist's ability, a scientific judgment.

The foregoing issues of policy are matters that invite and should receive serious public attention. The scientist who is asked to give an
opinion is exposed to these same concerns and as citizens are subject to political issues or pressures. At best, the most fitting environment should be conducive to the development of decisions on safety criteria based on existing scientific knowledge. It is possible to approach these conditions by confining the selection of participating scientists to those who are competent in the areas under consideration and those who, as a matter of record, are not advocates for a certain position. Thus the evaluation of the available data base is a scientific judgment tinctured with the recognized concerns of the participating scientists for public issues.

Sequentially, after the opinions of the scientists have been obtained, the risk-benefits of flavor food additives can be assessed. At this point the advocates for and against issues of public policy should inject those considerations that will eventually lead to appropriate final decisions for the regulatory agency. In this step-wise progression each segment of the public has contributed its part in reaching a judicial decision.

The validity of any scientific judgment is not quickly or easily determined; the best measure is the test of time and history. If decisions or opinions are affirmed following evaluation by other scientists, the validity of the original conclusion is reinforced. But the ultimate test of any scientific judgment or decision is its reaffirmation by subsequent experimental investigation. No other procedures can adequately attest to the validity of the original decision and manner by which it was reached.
II. GUIDELINES FOR TOXICOLOGICAL TESTING OF FOOD SUBSTANCES DEVELOPED BY OTHER SCIENTIFIC PANELS

The need to evaluate the potential toxicologic effects of food additives, drugs, pesticides, and other substances has been a primary focus of many organizations, committees, and commissions. In most cases, the reports of these groups have dealt with methodology related to specific types of test protocols, such as mutagenicity, or the broad range of evaluative tests needed for individual substances. Few reports have specifically reviewed criteria to evaluate flavoring substances beyond noting that such substances were included in a broad definition of food additives.

However, a number of these reports contain pertinent information on test procedures, concepts of evaluation, and proposed criteria. For these reasons, the SCOFEC reviewed these for background information.

A. PROCEDURES FOR INVESTIGATING INTENTIONAL AND UNINTENTIONAL FOOD ADDITIVES

In 1966, the World Health Organization (WHO) (1967) organized a Panel to evaluate procedures for investigating intentional and unintentional food additives. This Panel reviewed criteria to establish acceptable daily intakes and suggested additional toxicological procedures to evaluate the health hazards of intentional and unintentional food additives.

This Panel agreed with previous WHO groups and concluded that all intentional food additives should be subjected to individual toxicological testing. However, they suggested four reasons for modifying this general procedure:

- Many additives are already in foods or elsewhere in the environment. The natural occurrence of a substance should be taken into account in the evaluation of its safety.

- Certain additives may be altered to substances which are already present in food in greater amounts. If biochemical studies show the additive makes only a small contribution to existing metabolic pools of that substance then there would be no need for detailed toxicology studies.
If a series of chemical analogs could be shown to give rise to the main metabolic product or other compounds that are already present in greater quantities or that can be readily and safely metabolized, then it may be sufficient to carry out toxicology studies on only one suitable representative of this chemical series.

Toxicologic information on safety evaluation of food additives is often inadequate. Substances that are urgently needed in food processing or preserving or are present in relatively minute amounts might be given temporary clearance or have temporary acceptable daily intakes established.

The WHO Panel concluded that the 100-fold safety margin was a useful general guide but that it should not be applied too rigidly. They established techniques for developing temporary acceptable daily limits and acceptable daily intakes of various food additives and pesticides. The WHO Panel did not recommend any specific test for the assessment of mutagenicity or teratogenicity beyond that which might occur in multigeneration studies nor did they recommend specific testing for teratogenicity except that which occurs in multigeneration studies.

B. GUIDELINES FOR ESTIMATING TOXICOLOGICALLY INSIGNIFICANT LEVELS OF CHEMICALS IN FOOD

The Food Protection Committee of the National Research Council (NRC) in 1958 concluded that for every chemical there was some finite level ("safe level") at or below which the substance could be present in food without prejudicing safety (Food Protection Committee, 1969). The "safe level" of any substance would be established through extensive toxicological study and expert evaluation and could be determined by applying a safety factor to the highest dietary intake that did not produce harmful effects in experimental animals exposed to the substance in long-term feeding trials. The Committee indicated that a safe level of 1/100 of the experimentally determined "no-adverse-effect level" would be an acceptable one. A number of organizations concerned with food safety have adopted this procedure and the term "safe level" usually refers to a level set arbitrarily.

The Food Protection Committee (1969) suggested that the possibility of hazards from use or misuse would be very remote if the human dietary use level was well below the acceptable safe level. Such low use levels were sufficiently presumptive of safety so that they were termed "toxicologically insignificant."
A special task force of the Food Protection Committee (1969) was formed to review the concept and estimation of toxicologically insignificant amounts in a wide range of natural and synthetic substances of known and possible toxicity. Major emphasis was placed upon evaluation of pesticides, antipersonnel agents, certain drugs active at low dosages and naturally occurring toxins. The task force concluded that compounds in commercial use that may have deleterious effects at low levels fall into four general groups: a) impurities or contaminants of natural origin; b) essential nutrients or hormones; c) heavy metals and their compounds; and d) certain organic compounds employed for their biological activity.

The task force stated,

"Chemicals that exert significant biological effects and that are useful or unavoidable in food or any other part of the environment will continue to be subjected to laboratory investigations to establish safe levels, and the experience gained thereby will constantly provide the basis for confirming or modifying earlier conclusions."

While the major thrust of the task force report did not include flavoring agents, the statement suggests that they were aware of flavorings as a category of substances used at extremely low levels in foods. For example, the task force reached several conclusions on estimation of toxicologically insignificant levels of substances which are pertinent to the evaluation of the safety of flavoring substances used in foods. The first of these concerns chemicals in commercial production. If a chemical a) had been in commercial production for five years or more without evidence of toxicological hazard associated with its production or use, b) is not a heavy metal or a compound of a heavy metal, c) was not intended for use because of its biological activity, then it would be consistent with sound toxicological judgment to conclude that a level of 0.1 ppm of the chemical in the diet of man would be toxicologically insignificant.

With respect to other substances, the task force concluded that certain organic chemicals meeting special structural restrictions about which complete toxicological data were unavailable might also be present in toxicologically insignificant levels. Many substances are functionally effective in food at dietary concentrations above 0.1 ppm. For those substances where metabolism or toxicity were already known, the task force considered it justifiable to employ accumulated scientific knowledge and to recognize the need for structural analogy to other chemicals. Reasoning by analogy could be used to arrive at conclusions of toxicological insignificance. The task force defined presumable toxicological insignificance at a level of 1.0 ppm or less in the human diet if the substance met the following criteria; a) the substance in question is of known structure and purity; b) it is structurally simple,
e.g., straight chain or simply-branched aliphatic alcohols and acids or esters; linear polymers of ethylene or ethylene oxides; cellulose ethers; and, mononuclear aromatic compounds containing only carbon, hydrogen, and oxygen possessing one or more functional groups including hydroxyl aldehyde or ketone groups; 3) the substance is readily metabolized through known pathways; and 4) the substance is a member of a closely related group of substances that without known exception are or can be presumed to be low in toxicity. The closely related substances were considered to be near members of a homologous series, geometric or positional isomers, substances of identical basic structure or differing only by minor side chains, and substances readily degraded into compounds meeting the other criteria listed.

C. EVALUATION AND TESTING OF DRUGS FOR MUTAGENICITY: PRINCIPLES AND PROBLEMS

In 1971, the World Health Organization convened a special scientific group that suggested that all drugs should be evaluated for mutagenicity potential. No specific test or panel of tests was recommended but testing procedures and methods were identified for possible inclusion in any protocol for evaluating the mutagenic effects of drugs.

D. REPORT ON REPRODUCTION STUDIES IN THE SAFETY EVALUATION OF FOOD ADDITIVES AND PESTICIDE RESIDUES

A Food and Drug Administration, Advisory Committee on Protocols for Safety Evaluations (1970) was organized in 1967. One Panel of the Committee reviewed the role of reproduction studies in the safety evaluation of pesticides and food additives and reviewed the adequacy of methods being utilized at that time. The Panel addressed itself to specific hazards associated with the reproductive process, including gametogenesis, teratogenicity, hazards to the pregnant mother, hazards to the post-partem infant, and the effects on lactation. The Panel also considered suitability of various types of testing for mutagenicity and teratogenicity, interpretation of the results of reproductive studies, and the significance of animal tests with respect to possible hazard to man. The report does not indicate if the Committee considered different approaches for various types of food additives such as flavorings.

The Panel concluded that information regarding potential effects on the reproductive process is essential in any adequate safety evaluation program. However, they cautioned that judgment must be exercised in determining when reproduction data are needed. They concluded that the requirement for
reproductive data may vary in each case depending upon what other information is available at that time. They recognized that data on reproduction in nonrodent species, particularly in subhuman primates, would be desirable, but suggested that extrapolation of data from rodents to humans was generally as relevant as extrapolation from nonhuman primate species. The Panel also recognized that having comparative metabolic data from various species, including man, was desirable. The species of choice for reproduction tests should be one in which the metabolism of the compound is as similar to that in man as possible.

They concluded that a modified multi-generation test appeared to be the most satisfactory approach to the acquisition of data on the overall reproductive test. Both teratology tests involving a single dose given during embryogenesis and mutagenicity tests in mammals during reproductive studies were proposed as requirements. Tests in microorganisms, cell cultures, insects, and avian eggs were considered useful screening procedures but actual tests in mammals were preferred.

The Panel recommended to the FDA that they require the following information about food additives and pesticides:

- a multi-generation reproduction study, including an "effect" dose level but not necessarily a reproductive effect dose level;

- data on teratogenic potential;

- data on mutagenic potential.

They suggested that the mutagenicity and teratogenicity data might come from specific protocols designed to detect such effects, or less preferably, from the modification of the multi-generation study in which reproductive effects were being evaluated. Finally, they noted that if a substance under test belonged to a class of known teratogens or mutagens, then specific studies should be carried out.

The Panel concluded that these studies were necessary to supplement information on anticipated amounts and patterns of consumption, chemical and physical properties of the substance, specifications for identity of the commercial product, the intake of the substance, and consideration of products to which it was converted in foods. This latter requirement would include all toxicologic, metabolic, and nutritional effects. These were the data considered necessary for safety evaluation as prescribed by the Food Protection Committee (1959).

The Panel noted that the general goal of safety evaluation studies is to determine the maximum dose at which no detectable deleterious effect
can be discerned. However, they cautioned that this "maximum no-effect level" is obviously related to the nature of the phenomenon under study and the accuracy and sensitivity of methods used to detect and measure the effect. The Panel did not discuss possible effects of substances consumed in the diet, such as flavors and extracts, that would have gustatory or olfactory effects at levels below the no-toxicological effect level.

E. REPORT ON CANCER TESTING IN THE SAFETY EVALUATION OF FOOD ADDITIVES AND PESTICIDES

In 1971 an FDA Panel on Carcinogenicity Testing in the Safety Evaluation of Food Additives and Pesticides of the Advisory Committee on Protocols for Safety Evaluation prepared a special report on evaluating food additives and other substances for carcinogenic potential. The report focused upon the then current status of testing the carcinogenic action of food additives and other chemicals which were known to come into contact with man primarily through dietary intake. The Committee considered the spontaneous occurrence of benign and malignant tumors in several animal species, the significance of viral oncogenesis in testing possible chemical carcinogens, and the various aspects of updating test procedures. The Committee made recommendations concerning the protocol for studies but concluded that there was no rapid test which could be recommended for routine screening of potentially carcinogenic chemicals (Food and Drug Administration, Advisory Committee on Protocols for Safety Evaluations, 1971). Drawing largely upon the work of Mantel and Bryan (1961), they recommended that a feasible cancer testing program would include the following:

- testing done at doses and under experimental conditions likely to yield maximum tumor incidence. This would involve use of doses several orders of magnitude above actual use level;

- positive evidence of carcinogenicity supplied within confidence limits rather than as a single test of significance;

- a virtually safe dose estimated for compounds judged to be carcinogenic at test levels by extrapolating downwards, using some arbitrarily selected but conservative dose response curve. The Committee recognized the uncertainties involved in extrapolating in this manner and that these data might lead to conflict when applying the Delaney Clause. Unless the test level was at least \(10^6\) times the proposed use level, a finding of carcinogenicity at the testing level would lead to so low a safe use level recommendation as to be virtually unacceptable; and,
estimation of safe dosage levels for substances found to be noncarcinogenic in tests by similar methods might be so low as to virtually exclude substances for which there was no positive evidence of carcinogenicity.

These latter two points suggest that the Panel on Carcinogenesis recognized the difficulties attendant to downward extrapolation. For these and other reasons they suggested that FDA should require evaluation of all food additives and pesticides for carcinogenicity, but that FDA scientists should exercise judgment in the extent of testing. They suggested that carcinogenicity testing should be carried out in at least two species, but two rodent species would not be satisfactory. Animals should be randomly bred, and fed on a diet of known composition at several dosage levels. Carcinogenicity testing should include both negative controls and positive controls and should be done only in cases where metabolic pathways are known.

The Panel (Food and Drug Administration, Advisory Committee on Protocols for Safety Evaluations, 1971) recognized that an excessively large number of animals might be needed for testing and that it is impossible to provide absolute certainty that substances do not induce tumors. They also recognized the potentiation of possible dietary cocarcinogens and recommend that further tests be developed to assess the effects of such substances. They noted that although it is plausible, in principle, to estimate a safe level for a carcinogenic substance, the uncertainties involved in downward extrapolation from test levels resulted in permissible levels that would be zero for all practical purposes.

F. PRINCIPLES FOR EVALUATING CHEMICALS IN THE ENVIRONMENT

With contractual support of the Environmental Protection Agency, the National Academy of Sciences (1975) has recently completed a massive report on safety assessment of chemicals with respect to human health and ecological impact. The report summarizes the work of numerous panels and committees which addressed the ramifications of the effects of chemicals on man and on the environment. The participants reached a consensus on several general principles that are related to developing criteria for evaluating substances in foods such as flavoring substances. They recognized that prediction of all possible hazards for each environmental chemical is not possible and that it is more reasonable to minimize hazards within the limitations imposed by current knowledge and available resources. Periodic review would be required in cases where production methods or use patterns change.
The participants agreed that an inflexible scheme of evaluation tests should be avoided because new methodology is continually being developed and because any test technique does have some level of imprecision. Multiple tests were preferred to a single or dichotomous test sequence. The need for mature and sound judgment by experienced professionals was stressed.

The National Academy of Sciences report made several points that bear directly on evaluation of chemicals, such as flavoring substances. Resources should not be wasted on lengthy evaluation of chemicals that are unlikely to create problems for man or the environment. Similarly, facilities, manpower, and funds should not be used in testing substances that are known to be too hazardous to release. Rather, resources should be focused on thorough evaluation of chemicals termed "marginal cases." Secondly, the report noted that many decisions, including those related to thoroughness of testing, could be made without actual experimental work on the basis of analogies with other substances. Because structure-activity relationships are frequently well known for certain types or groups of substances, resources should be concentrated on evaluation of unknown groups or series of chemicals. The less well-known the chemistry of a single compound or a group of substances, the more difficult the evaluation process.

Finally, the panel on acute and subchronic toxicity proposed certain priorities as guidelines designed to minimize investment in unnecessary research. They suggested that substances that had a high priority for evaluation were those that: 1) did not have a safety margin of 1000 or more in a 90-day subchronic test; 2) are produced in high volume; 3) are related chemically to known carcinogens; 4) are chemically nonreactive and thus persistent; or 5) are substances that would involve extensive public exposure. Intermediate priority was suggested for substances that produce toxic effects at doses less than 3000 to 5000 mg per kg or whose use would lead to exposure levels of greater than 0.01 mg per kg per day. Acute and subchronic toxicity testing would be the basis for further toxicological evaluation.

Compounds that exhibited a low acute toxicity (doses greater than 3000-5000 mg per kg) and whose use would lead to exposure rates less than 0.01 mg per kg per day were considered low priority substances. On this basis, approximately half of the flavoring substances would fall into the latter category of low priority for testing because of their low level of use.

G. THE TESTING OF CHEMICALS FOR CARCINOGENICITY, MUTAGENICITY, AND TERATOGENICITY

Because of increasing concern about carcinogenicity, mutagenicity, and teratogenicity of food additives, drugs and industrial pollutants, a panel of expert scientists was convened by the Health and Welfare Department of
Canada (Health Protection Branch, 1975). The panel reviewed and evaluated potentially useful methodology and the interpretation of test results and general principles of evaluating these three parameters of toxicity. The report of this panel is a current summation of principles and methods applicable to evaluating environmental chemicals for carcinogenicity, mutagenicity, and teratogenicity.

While the report does not address the specific issue of intentional food additives such as flavoring agents, the guidelines on cancer testing in animals recommend that chronic toxicity studies on food additives should use at least three doses administered orally and at levels that are selected from experience or from results of short-term toxicity tests. This would imply testing at the "minimal effective dose level" rather than at the "as consumed level."

Evaluation of mutagenic potential should begin with efforts to predict mutagenic hazards from data on chemical structure and knowledge of metabolic activities of the substance. Dose levels would be related to the mutagenicity screening techniques selected for use in each particular case. The panel suggested that teratogenic evaluation should involve the same route of administration by which the suspect chemical normally entered the body and that administered dose levels would vary with route of administration and test species. Establishment of the LD$_{50}$ values from at least three or more equal, logarithmically subdivided dose levels was suggested for further consideration.

In summary, the Canadian review panel recommended in its report that testing for carcinogenicity, mutagenicity, and teratogenicity should be considered and probably included in evaluation of food additives and other chemicals. Specific methods are discussed and compared. Protocols for actual experimental procedures are developed. However, criteria for selection of candidate substances are discussed only indirectly.

Reports Cited


APPENDIX B

RECOMMENDATIONS ON TOXICOLOGICAL EVALUATION
OF FOOD FLAVORING SUBSTANCES BY OTHER ORGANIZATIONS

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I. CRITERIA FOR NATURAL AND ARTIFICIAL FLAVORING
   SUBSTANCES DEVELOPED BY THE COUNCIL OF EUROPE

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IV. BIBLIOGRAPHY
I. CRITERIA FOR NATURAL AND ARTIFICIAL FLAVORING SUBSTANCES DEVELOPED BY THE COUNCIL OF EUROPE

The Council of Europe, established in 1949 by ten European countries, currently has 17 member states that have agreed to discuss economic, social and technological issues of common concern. An ad hoc Working Party of the Subcommittee on the Health Control of Foodstuffs of their Public Health Committee has prepared an extensive review on natural and artificial flavorings used in food that includes: 1) lists of natural and artificial flavorings which may be added to foodstuffs without hazard to public health; 2) lists of substances with temporary acceptance pending further review; and 3) lists of flavoring substances that might be hazardous and therefore unacceptable. Available toxicologic information on all natural and artificial flavors used in Europe was reviewed in the preparation of these three lists (Council of Europe, 1974).

Most important to this current review by the SCOFEC, the Council of Europe proposed that as of July 1, 1970 (or some date to be established by mutual accord), no new flavoring substances should be added to foods unless the manufacturer supplied data on the possible health hazard of that flavoring substance. The tests required to establish the absence of health hazards were specified by the Council in their report.

The Council of Europe defined a flavoring substance as one that had primarily odor-producing properties and which possibly would affect gustatory sensitivity. They defined a natural flavoring as a substance obtained from plant and perhaps, animal sources through exclusively appropriate physical processes (biological processes and roasting were considered as physical processes). Artificial flavorings were defined as substances with flavoring properties obtained by chemical processes; artificial flavorings thus defined, would include substances which might be as yet undiscovered in natural products as well as substances known or unidentified which do occur in nature. Substances produced artificially although known to be present naturally would be classified as natural flavoring substances only in the case of their absolute chemical identity.

In establishing their list of natural and artificial flavorings which posed no hazard to health and the list of substances thought to be hazardous, the Council of Europe considered that flavorings could be regarded as acceptable if they did not contain toxic, non-flavoring constituents. The same general principles of toxicologic evaluation would be applied in setting limits for both natural or artificial flavorings.
They proposed the following important parameters as a basis of safety evaluation of flavoring substances:

- available information on quantitative and qualitative use patterns, particularly if the reported amounts indicate a negligible toxicologic risk;
- incorporation in staple items of the normal diet;
- preferential consumption by certain segments of the population;
- comparability of the chemical structure with that of compounds known toxicological and biochemical properties;
- information available from possible drug use of that substance;
- the presence of qualitative characteristics of the substance insuring self-limited use as a flavoring;
- the metabolic contribution of the flavoring substance to the total dietary load of that substance that might arise from other chemically similar food additives.

Natural flavoring substances were divided into acceptable, temporarily acceptable, and unacceptable either because relevant toxicologic information was lacking or the natural flavoring substance contained some component which was toxicologically unacceptable. The same type of classification was utilized in the review of artificial flavoring substances. Recognizing a paucity of technological and toxicological data on many of these substances, the Council relied upon long history of use, chemical structure and analogy to compounds of similar or related constitution, and knowledge of biochemical behavior of the substance in metabolic pathways. Decisions concerning such substances which might be added temporarily without hazard or substances not fully evaluated rested in part upon acute toxicity studies in selected species and 90-day studies in a known sensitive species. Data collected in the 90-day study were considered sufficient evidence for safety or hazard if and when these were available.

Recognizing that experts assessing toxicological properties of flavoring substances had, in many instances, to rely upon "common sense arguments" rather than on solid scientific data, the Council of Europe proposed that new natural and artificial flavoring substances should undergo a standard series of toxicologic evaluation tests. They proposed that at some
defined date, flavoring substances which have not been carefully evaluated
toxicologically should no longer be automatically acceptable on the basis
of reviewing existing data. Rather, they specified a series of guidelines
which they considered to be a minimal satisfactory basis for decision making.
The following paragraphs are a summary of the suggestions for credible toxi-
cological testing advanced by the Council of Europe:

- **Physical and chemical properties**

The chemical identity and purity of the substance should be
established for any artificial or natural flavoring which has
been chemically defined. The chemical formula should be
stated if it is known; if not, analytical characteristics must
be as precise as possible. Manufacturers would be required
to submit information concerning chemical identification
techniques as well as methods of testing purity and adequate
information on manner of use. Natural flavoring substances
chemically undefinable would be distinguished by their physi-
cal properties and by explicit botanical, mineralogical or
zoological description. Methods of use and methods to deter-
mine levels in foodstuffs must be specified.

- **General toxicity studies**

All flavoring substances would have to undergo experimental
toxicological evaluation in several species of warm blooded
animals. These would include acute toxicity, short-term
toxicity with emphasis upon biochemical and metabolic be-
behavior of the test substance. In principle, studies on long-
term toxicity would be required with emphasis on examination
of reproductive effects, particularly embryotoxic, teratogenic
and the possibility of carcinogenic effects.

- **Acute toxicity tests**

Acute toxicity tests would include oral administration of the
substance to not less than three species of animals, at least
one of which would be neither rodent nor bird. Acute toxicity
would also be studied in at least one mammalian species by
intraperitoneal administration where possible. \( L_{D_{50}} \) values
should be determined within acceptable confidence limits.
Acute toxicity studies would include administration of single
doses over at least a 7-day period; all animals dying from
a lethal dose would be autopsied and organs and tissues show-
ing macroscopic change would be examined histologically.
Short-term toxicity studies

These tests would include oral administration or administration by gavage in at least two species of warm-blooded animals. Administration of the substance would extend for a period equal to at least one-tenth of the average life span of the species which would be a minimum of 90 days in rats or one year in dogs. Substances would be incorporated into the diet in at least three or four different levels of concentration and each study would include a control group. Short-term toxicity studies on small species would include a minimum of 10 animals of each sex and with large species a minimum of 2 of each sex. The quantity of food consumed, behavior and appearance of the animals, and physicochemical cytological analysis of blood, and chemical analysis of the urine would be carried out during these studies. Animals would also be subjected to organ function tests, particularly hepatic and renal function tests. At the end of the short-term toxicity study all animals would be sacrificed and subjected to thorough microscopic pathological examination of all tissues and organs; pathological and histological study of all tissues and organs would also be carried out in animals in the control group.

Long-term toxicity studies

These studies would extend over four-fifths of the average life span of the animal, and if the species were the rat, the duration of the testing would be a minimum of two years. Long-term toxicity studies would be required in a second species if short-term studies made it appear likely that there could be differences between various species in response to the substances. The same set of pathological tests required in the short-term study would be required in long-term studies.

Reproduction studies

Tests would be required in at least one species and would be carried out for a minimum of two generations. Each group would consist of a minimum of 10 males and 20 females; in the case of rats, the experiments should begin at the weanling age with mating permitted at three months. Dosage levels to be used would be similar to those used in the long-term studies. Each of the two generations would be allowed to mate at least twice in order to produce two or more litters. If the number of animals in the first litter
was insufficient, then further litters would be produced. Pregnant animals from subsequent matings could then be sacrificed in order to study embryotoxicity. For each of the litters, the number of litters, the number of pups per litter and the weight of all pups at day 1, day 5, and at weaning would be recorded. Special attention would be paid to the number of resorption sites, the number of embryos and the development of embryos in females examined pathologically prior to delivery. The full macroscopic and histopathological examination would be carried out as well. If these findings indicated the presence of fetal abnormalities then separate teratological studies would be conducted. These would be so designed as to demonstrate possible deleterious effects on the developing embryo and would be supplemented by appropriate pathological and histological studies.

- **Biochemical studies**

Studies to determine absorption, distribution and excretion of the flavoring substances in some mammalian species would be necessary. Effects upon enzymes were considered of particular importance as well as metabolic behavior of the substance with respect to normal food constituents.

- **Human studies**

Upon completion of animal tests, observations in man would be valuable. These could be epidemiological studies in exposed human groups either by virtue of their occupation or by testing volunteers.

The Council of Europe acknowledged that these guidelines were potentially quite stringent and while they espoused the concept that all toxicologic evaluation would be carried out in accordance with the principles and concepts adopted for other classes of food additives, they recognized that the safety factor of a 100 would normally be adequate for flavoring substances.

In addition, they recognized that certain flavoring substances might be chemically or metabolically similar to substances already in use. In these cases, the toxicological examination might be limited to acute toxicity, short-term toxicity and biochemical studies. If these investigations confirm the close similarity of results between the flavoring substance under test and substances already in use, then the substance under test could be acceptable. If these tests were inconclusive or left any doubt concerning toxicologic similarities or biochemical effects, then more thorough testing would be required.
Furthermore, the Council provided that provisional acceptance could be obtained for substances that would be ingested at levels no higher than 0.1 ppm (0.1 mg per kg). Similarly, flavorings from edible plant parts might receive provisional acceptance if the amounts consumed did not exceed the quantity normally present in the natural product. Provisional acceptance for finite time periods as specified would allow marketing and use without completion of the entire testing procedure.

The Council of Europe report does not provide recommendations or guidelines on evaluation of carcinogenic potential of flavoring substances.
II. RECOMMENDATIONS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES CONCERNING FOOD FLAVORING SUBSTANCES

In the tenth report of the Joint FAO/WHO Expert Committee on Food Additives (1967), the Committee suggested that any food additive that is degraded completely in the food or in the digestive tract to common dietary substances or to body constituents might be evaluated satisfactorily on the basis of appropriate biochemical and metabolic studies. Usual toxicological investigations for such food additives in minute amounts were considered unnecessary.

In a subsequent report, the Joint FAO/WHO Expert Committee on Food Additives (1968) considered specifications for and toxicological evaluation of certain flavoring substances and nonnutritive sweeteners. Previous reviews had been limited because the number of flavoring substances was quite large, the majority of flavoring substances occur naturally, and their level of use is generally low. However, certain flavoring substances such as safrole, coumarin, capsaicin, and zingiberin had been shown to be toxic; thus, the Joint Committee concluded that the toxicological hazards of flavoring substances should be reviewed.

Unconditional and conditional acceptances and Acceptable Daily Intakes (ADI) for 35 flavoring substances were proposed; temporary or no ADI were established for eight other substances (Joint FAO/WHO Expert Committee on Food Additives, 1968). The decisions were based on biological data and toxicological studies as well as levels of use and occurrence in natural foodstuffs.

In establishing priorities for further evaluation of flavoring substances, the Joint Committee proposed several guidelines. They made no distinction between a naturally occurring or synthetic form of a substance; consideration would be given to all flavoring substances irrespective of whether they were natural or artificial. They also suggested that priority be given to evaluation of flavoring substances on the basis of the following criteria:

- appearance on restrictive list of various governments;
- estimated per capita consumption in excess of 3.65 mg per year;
- use at levels above 10 mg per kg of food, with the exception of substances lost by volatilization during heat processing;
• valid reasons for doubting safety of a particular substance; and,

• in the case of esters, the fact that acidic or alcoholic components had already been evaluated, either as such or as components of other esters.

More recently, the Joint FAO/WHO Expert Committee on Food Additives (1974) indicated that the procedures adopted by the Council of Europe represented a useful, practical approach to safety evaluation of flavoring substances. They called attention to the difficulties associated with traditional methods of safety evaluation used by the Joint Committee on Food Additives and pointed out that these lists might not be complete because of regional or ethnic uses of flavors. More importantly, the Joint Committee stated on page 13 that:

"In the opinion of the Committee, the guide to the testing and toxicological evaluation of flavouring substances provided by the Council of Europe goes too far beyond the enunciation of principles, entering into detailed protocols that are not only unnecessary but also tend to create a rigid set of testing requirements. The Committee cannot emphasize too strongly the need to maintain flexibility at all times in the approach to toxicity testing since each compound presents an individual and unique problem."
III. REPORT ON THE REVIEW OF FLAVORINGS IN FOOD

The Food Additives and Contaminants Committee, Ministry of Agriculture, Fisheries and Food has recently completed a report on the status of food flavorings used in Great Britain (1976). The Committee noted that the toxicological and other data available was disappointingly small, but acknowledged the recent efforts of the Council of Europe (1974) in preparing criteria for evaluation and in collating the lists of natural and artificial flavoring agents.

The Committee, in conjunction with the Toxicity Sub-committee of the Committee on Medical Aspects of Chemicals in Food and the Environment, reviewed the work of the Council of Europe and prepared an extensive list of "permitted food flavoring agents" which is contained within the report. The British permitted list of natural and artificial flavors are essentially similar but not identical to those prepared by the Council of Europe. The Committee adopted the position that use should be sanctioned by inclusion on a permitted list and that flavoring agents could not be used until they were published on the permitted list. This process implies review of available data prior to inclusion on official permitted use lists.

However, the Committee suggested modifications of the definition of flavorings which would exempt from regulation:

- natural food substances;

- naturally occurring constituents of a natural food substance present in food solely as a result of the natural food substance being used as an ingredient;

- substances present in food as a result of changes that take place during food preparation; and

- food additives already controlled by other regulations.

The report of the Committee and the appended lists of permitted and prohibited flavoring substances provides a rational basis for regulation of food flavoring agents in Great Britain under terms of their Food and Drugs Act of 1955. However, the Committee did not address the criteria that were used in establishing the lists in the current report or the criteria to be used in evaluating new flavoring agents.
IV. BIBLIOGRAPHY


APPENDIX C

LISTS OF FLAVORING SUBSTANCES
I. FOOD AND DRUG ADMINISTRATION

Flavorings, seasonings, and related substances approved for use in foods are listed in three parts of Section 121 in the Code of Federal Regulations, Title 21. These are:

A) Spices, seasonings, essential oils, oleoresins, and natural extractives that are generally recognized as safe for their intended use, within the meaning of Section 409 of the Act. Section 121.101 (e) and (g) in Code of Federal Regulations, Title 21, Food and drugs, parts 10 to 199 rev. Government Printing Office, Washington, D.C. (1975)


II. THE FLAVOR AND EXTRACT MANUFACTURERS' ASSOCIATION

Several lists of flavoring substances generally recognized as safe by the FEMA Expert Panel have been published in Food Technology from 1961 to 1976. Each list is alphabetical, but contains only those substances approved by the Expert Panel in the interim between reports.

The FEMA has recently (1976) prepared an Alphabetical Cross-Reference List of FEMA GRAS Substances and Synonyms. Further information on availability of the complete list of FEMA GRAS Substances may be obtained by writing:

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