TENTATIVE REPORT ON THE REEXAMINATION OF THE
GRAS STATUS OF SULFITING AGENTS

October 1984

Prepared for

CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20204

under

TASK ORDER #5
Contract No. FDA 223-33-2020

LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
9650 Rockville Pike
Bethesda, Maryland 20814
NOTICE

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

This tentative report was developed for the Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA) in accordance with the provisions of Task Order #5 of Contract No. 223-83-2020. FASEB recognizes that the safety of Generally Recognized as Safe (GRAS) substances is of national significance and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in this reexamination of the GRAS status of sulfiting agents.

During the course of the evaluation of the GRAS substances by the Select Committee on GRAS Substances (SCOGS) (1972-1982), FASEB and FDA agreed that when additional information and data became available subsequent to completion of an evaluation report, the FDA could request review and evaluation of the supplemental information and data. Based upon the evaluation of all available data, the Select Committee would prepare a supplemental report. While the Select Committee on GRAS Substances was terminated in 1982, similar mechanisms for review and evaluation have been maintained in FASEB by the LSRO.

The tentative report was prepared by an ad hoc Panel on Reexamination of the GRAS Status of Sulfiting Agents. Members of the Panel and others who assisted in preparation of the report are identified in Section VII. The scientists selected as members of the Panel were chosen for their qualifications, experience, and judgment with due consideration for balance and breadth in the appropriate professional disciplines. Certain members of the ad hoc Panel were formerly members of the Select Committee on GRAS Substances who participated in the review of GRAS substances which resulted in publication of the report, "Evaluation of the Health Aspects of Sulfiting Agents as Food Ingredients" (SCOGS, 1976).

The ad hoc Panel's evaluation of available information and data is being made independently of FDA or any other group, governmental or nongovernmental. The ad hoc Panel, as consultants to LSRO, and the LSRO accept responsibility for the contents of this tentative report.

The tentative report is being made available to the public for review in the Dockets Management Branch (HFA-305), FDA, Room 4-62, 5600 Fishers Lane, Rockville, Maryland 20857, and at
the LSRO at the address appearing on the title page. As announced by FASEB, and by FDA in the Federal Register, an opportunity (open meeting) is being provided for any interested person to appear before the ad hoc Panel on Reexamination of the GRAS Status of Sulfiting Agents to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the open meeting will be considered by the ad hoc Panel in reaching its final conclusions.

The final report will be approved by the ad hoc Review Panel and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists 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 reports will be approved and transmitted to FDA by the Executive Director of FASEB.

While this is a tentative report prepared by a Panel convened by an Office of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of FASEB, its constituent societies, or the individual members of each constituent society.

October 15, 1989
(date)

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
FASEB
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I. INTRODUCTION

This reexamination of the health aspects of using sulfiting agents as food ingredients is based partly on the information contained in a scientific literature review (monograph) furnished by the Food and Drug Administration (FDA) (The Franklin Institute Research Laboratories, 1972), which summarized the world's scientific literature from 1920 through 1972\(^*\), and the assessment of this information by the Select Committee on GRAS Substances (SCOGS) in 1976\(^*\). In its 1976 report on the evaluation of the health aspects of sulfiting agents (potassium bisulfite, potassium metabisulfite, sodium bisulfite, sodium metabisulfite, sodium sulfite, and sulfur dioxide) used as food ingredients, the Select Committee (SCOGS, 1976) concluded that:

There is no evidence in the available information on potassium bisulfite, potassium metabisulfite, sodium bisulfite, sodium metabisulfite, sodium sulfite, and sulfur dioxide that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.

Based in part upon the report of the Select Committee, the FDA, in the Federal Register of July 9, 1982, proposed to affirm the use of four sulfiting agents (potassium metabisulfite, sodium bisulfite, sodium metabisulfite, and sulfur dioxide) as GRAS [47 FR 29956]. At the same time, FDA proposed not to affirm the use of potassium bisulfite and sodium sulfite as GRAS. Subsequent to the completion of the Select Committee's report and the publication of the FDA proposal, additional information and data on uses of sulfiting agents in foods as well as reports of health effects possibly associated with sulfiting agents have become available.

Accordingly, the FDA requested that the Federation of American Societies for Experimental Biology (FASEB), through its Life Sciences Research Office (LSRO), convene an ad hoc Panel on Reexamination of the GRAS Status of Sulfiting Agents to consider

\(^*\) These documents (PB-221 217 and PB-265 508, respectively) are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161.
the recent scientific publications and new information submitted to FDA in response to its 1982 proposal on the affirmation of the GRAS status of sulfiting agents. The ad hoc Panel was asked to address the following question:

"In view of the currently available information relevant to the use and safety of sulfiting agents, which of the five types of conclusions* for the appraisal of GRAS substances that were developed by the Select Committee now applies to the use of sulfiting agents? The conclusion which is reached should be supported by a discussion of the rationale behind that conclusion."

To insure completeness and currency as of the date of this tentative report, the information and data received from FDA has been supplemented by searches of scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; inquiry of leading investigators in the field and the combined knowledge and experience of members of the ad hoc Review Panel and the LSRO staff. In addition, an announcement was made in the Federal Register of July 9, 1984 [49 FR 27994] that opportunity is available for all interested parties to submit information, data, and reports on the health effects of sulfiting agents. An opportunity to appear before the ad hoc Panel at an open meeting to make oral presentation of data, information, and views on this tentative report on the health aspects of using sulfiting agents as food ingredients was published in the Federal Register of September 15, 1984 [49 FR 37670].

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Code of Federal Regulations [21 CFR 170.30], as revised April 11, 1984, that general recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. This section of the Code also indicates that expert judgment may be based on published studies which may be corroborated by unpublished studies and other data and information or, in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. FDA recognizes

* See Appendix
further [21 CFR 170.3] that it is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance.

LSRO's ad hoc Panel on Reexamination of the GRAS Status of Sulfiting Agents is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the ad hoc Panel, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. These conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO ad hoc Panel has reviewed the available information on sulfiting agents and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act, as amended.
II. BACKGROUND INFORMATION

"Sulfiting agents" is a term used to designate sulfur dioxide and any one of the five sulfite salts that may be used in some foods. Since the sulfite salts release sulfur dioxide, the effective agent under conditions of food processing, levels of sulfiting agents are usually expressed as sulfur dioxide equivalents (SDE). Sulfiting agents effectively serve a number of purposes in food processing including the inhibition of nonenzymatic browning; the inhibition and control of microorganisms; the inhibition of various enzymatic reactions such as enzymatic browning; as antioxidants and reducing agents, including dough conditioners; as bleaching agents; as processing aids; and, secondarily as pH control and stabilizing agents (Taylor and Bush, 1983).

The Code of Federal Regulations (CFR) permits use of potassium bisulfite (KHSO₃), potassium metabisulfite (K₂S₂O₅), sodium bisulfite (NaHSO₃), sodium metabisulfite (Na₂S₂O₅), sodium sulfite (Na₂SO₃), and sulfur dioxide (SO₂) as GRAS ingredients provided that they are not used in meats or foods recognized as a source of thiamin (vitamin B₁). Specific GRAS listings of sulfiting agents for use as chemical preservatives are to be found in the following sections of the CFR: potassium bisulfite [21 CFR 182.3616], potassium metabisulfite [21 CFR 182.3637], sodium bisulfite [21 CFR 182.3730], sodium metabisulfite [21 CFR 182.3766], sodium sulfite [21 CFR 182.3798], and sulfur dioxide [21 CFR 182.3862]. Other sections of the CFR pertaining to the use of sulfiting agents are: caramel [21 CFR 73.85(2)], dextrose monohydrate [21 CFR 168.111], glucose syrup [21 CFR 168.120], food starch-modified [21 CFR 177.892], boiler water additives [21 CFR 173.310(c)], cellophane [21 CFR 177.1200(c)], and water-soluble hydroxyethylcellulose film [21 CFR 177.1400].

The Food Chemicals Codex (NRC, 1981) provides specifications for food grades of potassium metabisulfite, sodium bisulfite, sodium metabisulfite, sodium sulfite, and sulfur dioxide. No specifications for food grade potassium bisulfite have been established.

Limits are not currently set for levels of use of sulfiting agents in most foods. The residual amount of SDE's in foods as consumed is less than the amount added during processing because portions of sulfite salts and sulfur dioxide combine with other food components, are liberated as sulfur dioxide, or oxidized to sulfate. Under acidic conditions, the percentage of sulfur dioxide liberated from sulfurous acid ranges from 0.5% at pH 4 to 37% at pH 2 (Green, 1976). The residual amount of sulfite in foods is limited by its organoleptic properties; the taste becomes noticeable at residual levels above 500 ppm (Furia, 1972).
III. CONSUMER EXPOSURE DATA

Three basic pieces of information are needed for estimating intake of food ingredients: a) the amount of the ingredient present in the various components of a diet at the time of consumption, b) the kinds of foods in the daily diet and the frequency with which each is consumed, and c) average portion size for the various foods consumed. From this information it is possible to compute the weight of the ingredient consumed from each food in the diet by multiplying the weight percent of the ingredient in the food by the portion size and the frequency with which it is consumed.

Information on the kinds of foods consumed, the frequency of consumption, and the usual portion size can usually be obtained with reasonable accuracy. However, obtaining reasonably accurate information on the amount of an added ingredient present in the food at the time of consumption is much more difficult. This is especially true with substances like the sulfiting agents which can be lost from foods during processing, storage, distribution, and home preparation. True loss can occur through volatilization of sulfur dioxide at pH below 4, and leaching; loss of sulfite, per se, i.e., "free sulfite" can occur through reversible reactions with other food constituents, particularly those containing aldehyde or ketone groups. Total sulfite, which consists of free sulfite and reversibly reacted sulfite, is generally defined as that measured, for example, by the Monier-Williams analytical method (AOAC, 1980).

The Monier-Williams method measures those sulfite species present, including reacted sulfite, that are converted to sulfur dioxide under strongly acidic conditions. These include reaction products with aldehydes and ketones. However, all reaction products are not converted to sulfur dioxide under these analytical conditions. Some sulfite is oxidized to sulfate in certain foods during processing (Thewlis and Wade, 1974). Furthermore, in some cases sulfite reacts with food components to produce organic sulfur compounds that are stable under the conditions of the Monier-Williams analytical method. Several examples can be cited. In laboratory dehydration of potatoes, cabbage, and carrots using $^{35}S$-sulfur dioxide, about 55-65% of residual $^{35}S$ was present in stable organic compounds and not released as SO$_2$ in a modified Monier-Williams procedure (Gilbert and McWeeny, 1976). After 6 months storage of strawberries in $^{35}S$-sulfite solution, 62% of the total $^{35}S$ present in the berries was recovered as sulfur dioxide by the Monier-Williams method, but 32% was present as irreversible reaction products. Only about 3% of residual $^{35}S$ in jam prepared from the strawberries was recoverable as sulfur dioxide (McWeeny et al., 1980). Some of the stable reaction products present in dehydrated cabbage have been identified as 5-deoxy-4-sulfo osuloses, derived from the reaction of sulfite with reducing sugars and ascorbic acid (Wedzicha and McWeeny, 1975).
In most animal feeding studies, sulfite salts are fed as dry mixtures with other components of the diet or as a component of the drinking water. Much less interaction with dietary components to produce irreversibly bound sulfur compounds would be expected in such animal diets than would likely occur in processed foods containing added sulfites, particularly those subjected to high temperatures in the processing operation and/or long storage times. To the extent that animal studies reflect the effect of free sulfite, or that which is reversibly bound and released in vivo, the sulfite content of foods as measured by the Monier-Williams or similar methods is a relevant quantity. However, animal studies with diets as conventionally prepared are less likely to reflect the physiological and toxicological properties of the stable organic sulfur compounds that may be present in many sulfited foods.

After considering all available information, the following data sources were used in estimating food intake of sulfites in the U.S.: a) the 1977 Survey of Industry on the Use of Food Additives by the National Research Council (NRC, 1979); b) responses of the food industry to FDA's proposed order on the GRAS status of sulfites in 1982 (FDA, 1982); c) the 1977-1978 National Food Consumption Survey (NFCS) of the U.S. Department of Agriculture (USDA, 1983a,b; Pao et al., 1982); d) Agricultural Statistics (USDA, 1983c) and other USDA statistical publications (USDA, 1984a,b); and e) U.S. Imports for Consumption and General Imports (USDC, 1984). Using information obtained from these sources supplemented by contacts with manufacturers and users in certain instances, Table 1 was prepared to assemble the following information: added and residual levels of sulfiting agents in processed foods, estimated level of sulfites in the food as prepared for consumption, human intake of specific foods and/or food categories, and estimated per capita daily intake of sulfites. All sulfite levels given are total sulfites expressed as sulfur dioxide equivalents (SDE), i.e., the proportion of the weight of the various sulfiting agents that can dissociate into sulfur dioxide. The added and residual SDE levels in Table 1 are the levels reported in the NRC 1977 survey and those reported in comments to FDA after July 1982 and/or to LSRO after July, 1984. Levels for a given food reported in the 1977 NRC Survey are generally weighted mean values obtained by weighting the added or residual level of each company reporting the use of sulfite in the food by its fraction of the total poundage of sulfites for all uses reported by these companies. Information on the analytical methods used and the standard deviations of the values listed in Table 1 were not reported for most food items. For some products, levels were stated to be calculated values based on reported sulfite concentrations in the ingredients, e.g., the sucrose, high fructose corn syrup, and caramel ingredients in soft drinks.

Several characteristics of source (a), the 1977 NRC Survey, should be noted because they have a bearing on the
Table 1. Levels of Sulfiting Agents (Sulfur Dioxide Equivalents, SDE) in Foods and Estimated Per Capita Daily Intake

<table>
<thead>
<tr>
<th>Food Category and Subcategory</th>
<th>Level in Product as Processed ppm</th>
<th>Level in Product as Consumed ppm</th>
<th>Food Intake g/capita/d</th>
<th>SDE Intake mg/capita/d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NRC 1977 Survey1</td>
<td>Raptd. to FDA/LSRO2</td>
<td>Reported2</td>
<td>Estimated3</td>
</tr>
<tr>
<td>BAKED GOODS and BAKING MIXES6</td>
<td>Added</td>
<td>Residual</td>
<td>Added</td>
<td>Residual</td>
</tr>
<tr>
<td>Cookies</td>
<td>100</td>
<td>250</td>
<td>5</td>
<td>6.2</td>
</tr>
<tr>
<td>Cake w/dried carrots</td>
<td>300</td>
<td>0-20</td>
<td>10</td>
<td>0.1</td>
</tr>
<tr>
<td>Crackers</td>
<td>250</td>
<td>5</td>
<td></td>
<td>2.9</td>
</tr>
<tr>
<td>Dough conditioner</td>
<td>60</td>
<td>40</td>
<td>&lt;1.5</td>
<td></td>
</tr>
<tr>
<td>Dough conditioner</td>
<td>25</td>
<td>&lt;6</td>
<td></td>
<td></td>
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<tr>
<td>Ple dough</td>
<td>155-215</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ple dough (frozen)7</td>
<td>5.1, 204</td>
<td>11.6, 20.4</td>
<td>5</td>
<td>1.38</td>
</tr>
<tr>
<td>Pizza dough (frozen)7</td>
<td>11.6, 20.4</td>
<td>5</td>
<td>1.78</td>
<td>0.009</td>
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<tr>
<td>Pizza crust</td>
<td>2500</td>
<td>0.3</td>
<td>5</td>
<td>1.6</td>
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<td>Ple crust</td>
<td>0.3, 25</td>
<td>800</td>
<td>5</td>
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<td>Tortilla shells</td>
<td>25,200</td>
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<tr>
<td>Unspecified</td>
<td>27</td>
<td>&lt;125, &lt;245</td>
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<td>GRAIN PRODUCTS9</td>
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<tr>
<td>Corn starch</td>
<td>0, 0, &lt;65</td>
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<td>3.7</td>
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<td>Modified corn starch</td>
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<td>Hominy</td>
<td>15010</td>
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<td>Spinach pasta</td>
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<td>Unspecified</td>
<td>160</td>
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<tr>
<td>COFFEE/TEA</td>
<td></td>
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<tr>
<td>Instant tea</td>
<td>6011</td>
<td>50-1800</td>
<td>5-6</td>
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<td>Liquid conc. tea</td>
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See footnotes at end of table.
Table 1. Levels of Sulfiting Agents (Sulfur Dioxide Equivalents, SDE) in Foods and Estimated Per Capita Daily Intake (continued)

<table>
<thead>
<tr>
<th>Food Category and Subcategory</th>
<th>Level In Product as Processed</th>
<th>Level In Product as Consumed</th>
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<tbody>
<tr>
<td></td>
<td>NRC 1977 Survey(^1)</td>
<td>Rprt. to FDA/LSRO(^2)</td>
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<tr>
<td></td>
<td>ppm</td>
<td>ppm</td>
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<tr>
<td></td>
<td>Added Residual</td>
<td>Added Residual</td>
</tr>
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</table>

| CONDIMENTS                   |                               |                              |                              |
| Olives \(^12\)               |                               |                              |                              |
| Unspecified \(^12\)          | 200                           | 2                            |                              |
| Pickles/Relishes \(^13\)     | 30                            |                              |                              |
| Salad dressing mix (dry)     | 50                            | 10                           | 0.7                          |
| Vinegar \(^15\)              |                               |                              |                              |
| Malt Vinegar                 | 10                            |                              | 0.06                         |
| Wine vinegar                 | 150                           | 75                           | 0.24                         |

| DAIRY ANALOGS                |                               |                              |                              |
| Filled milk \(^16\)          | 185                           | 200                          | 0.15                         |

| FISH and SEAFOOD \(^17\)    |                               |                              |                              |
| Dried cod                    | 200                           |                              |                              |
| Fabricated, frozen           | 200                           |                              |                              |
| Uns pecified fish product    |                               |                              |                              |
| Shrimp, fresh (U.S.)         | 0-295                         | 4-36                         | 0.6                          |
| Shrimp, fresh (foreign)      |                               |                              |                              |

| FRESH FRUIT \(^18\)         |                               |                              |                              |
| Fruit salad                  | 500                           | 5                            | 42.6                         |
| Grapes                       | 7-20                          |                              | 3.9                          |
| Unspecified                  | 15                            |                              |                              |

See footnotes at end of table.
Table 1. Levels of Sulfiting Agents (Sulfur Dioxide Equivalents, SDE) in Foods and Estimated Per Capita Daily Intake (continued)

<table>
<thead>
<tr>
<th>Food Category and Subcategory</th>
<th>Level in Product as Processed ppm</th>
<th>Level in Product as Consumed ppm</th>
<th>Food Intake g/capita/d</th>
<th>SDE Intake mg/capita/d</th>
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<tr>
<td></td>
<td>NRC 1977 Survey</td>
<td>FDA/LSRQ</td>
<td>Reported</td>
<td>Estimated</td>
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<td>Added</td>
<td>Residual</td>
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<td>Residual</td>
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<td><strong>DRIED FRUIT</strong>&lt;sup&gt;19&lt;/sup&gt;</td>
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<td>Apples</td>
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<tr>
<td>Cake w/dried apple</td>
<td>200</td>
<td>1400</td>
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<tr>
<td>Raisins (golden)</td>
<td>1100</td>
<td>110</td>
<td>0-20</td>
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<td>Fruit filling, apple</td>
<td>35</td>
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<td>Fruit filling (unspec.)</td>
<td>500</td>
<td>&gt;4000</td>
<td>&gt;2000</td>
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<tr>
<td></td>
<td>1500</td>
<td>575</td>
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<td><strong>FRUIT JUICES</strong>&lt;sup&gt;20&lt;/sup&gt;</td>
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<td>CANNED &amp; BOTTLED</td>
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<tr>
<td>Apple concentrate,</td>
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<td>42.6</td>
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<td>Imported</td>
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<tr>
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<td>10</td>
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<td>0.002</td>
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<td>3</td>
<td>3</td>
<td>0.009</td>
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<td>Grape, concentrate,</td>
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<tr>
<td>domestic</td>
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<tr>
<td>Grape, white</td>
<td>70-105</td>
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<tr>
<td>Grape, white,</td>
<td>60-125</td>
<td>85</td>
<td>0.3</td>
<td>0.025</td>
</tr>
<tr>
<td>sparkling</td>
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<tr>
<td>Grape, red,</td>
<td>60-100</td>
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<td></td>
</tr>
<tr>
<td>sparkling</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Grape, concentrate,</td>
<td>800</td>
<td>800</td>
<td>0.12</td>
<td>0.096</td>
</tr>
<tr>
<td>imported</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemon, non-frozen</td>
<td>160</td>
<td>160</td>
<td>0.04</td>
<td>0.016</td>
</tr>
<tr>
<td>Dietetic</td>
<td>1000</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Unspecified</td>
<td>100</td>
<td>85, 125, 0</td>
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</tbody>
</table>

See footnotes at end of table.
<table>
<thead>
<tr>
<th>Food Category and Subcategory</th>
<th>Level In Product as Processed</th>
<th>Level In Product as Consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td></td>
<td>NRC 1977 Survey</td>
<td>Rprtd. to FDA/LSRO</td>
</tr>
<tr>
<td>FROZEN FRUIT&lt;sup&gt;21&lt;/sup&gt;</td>
<td>NRC 1977 Survey</td>
<td>Rprtd. to FDA/LSRO</td>
</tr>
<tr>
<td>Unspecified</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>CANNED FRUIT&lt;sup&gt;22&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maraschino Cherry</td>
<td>50</td>
<td>50</td>
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<tr>
<td>FRESH VEGETABLES&lt;sup&gt;23&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mushrooms</td>
<td>560</td>
<td>13</td>
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<tr>
<td>Salad Bar</td>
<td>950</td>
<td></td>
</tr>
<tr>
<td>Unspecified</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>CANNED VEGETABLES&lt;sup&gt;24&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Pickled cocktail onions</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Pickled peppers</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Sauerkraut (in glass)</td>
<td>610</td>
<td></td>
</tr>
<tr>
<td>Unspecified</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>DRIED VEGETABLES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Instant mashed</td>
<td>240,475-500</td>
</tr>
<tr>
<td></td>
<td>Sliced</td>
<td>250-300</td>
</tr>
<tr>
<td></td>
<td>Hash brown</td>
<td>470-560</td>
</tr>
<tr>
<td></td>
<td>Hash brown</td>
<td>470-560</td>
</tr>
<tr>
<td></td>
<td>Unspecified</td>
<td>50,75</td>
</tr>
<tr>
<td></td>
<td>Unspecified</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Unspecified</td>
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</table>

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Table 1. Levels of Sulfiting Agents (Sulfur Dioxide Equivalents, SDE) In Foods and Estimated Per Capita Daily Intake (continued)

<table>
<thead>
<tr>
<th>Food Category and Subcategory</th>
<th>Level In Product as Processed ppm</th>
<th>Level In Product as Consumed ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NRC 1977 Survey(^\text{1})</td>
<td>Rprt(_\text{d}^\text{2}) to FDA/LSRO(^\text{2})</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>FROZEN VEGETABLES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes(^\text{26})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>French Fries,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsaturated</td>
<td>11, 11</td>
<td>5, 35 ave., 50 max</td>
</tr>
<tr>
<td>VEGETABLE JUICES(^\text{27})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sauerkraut juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsaturated</td>
<td>&lt;800</td>
<td></td>
</tr>
<tr>
<td>SUGAR(^\text{28})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown sugar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, granulated, cane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White granulated, beet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWEET SAUCES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn syrup(^\text{29})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High fructose corn syrup(^\text{29})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit topping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maple syrup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molasses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancake syrup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsaturated</td>
<td>900</td>
<td>30-40</td>
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</tbody>
</table>

**Footnotes:**

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Table 1. Levels of Sulfiting Agents (Sulfur Dioxide Equivalents, SDE) in Foods and Estimated Per Capita Daily Intake (continued)

<table>
<thead>
<tr>
<th>Food Category and Subcategory</th>
<th>Level in Product as Processed ppm</th>
<th>Level in Product as Consumed ppm</th>
<th>Food Intake(^4) g/capita/d</th>
<th>SDE Intake(^5) mg/capita/d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NRC 1977 Survey(^1)</td>
<td>Rprt. to FDA/LSRO(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Added Residual</td>
<td>Added Residual</td>
<td>Reported(^2)</td>
<td>Estimated(^3)</td>
</tr>
<tr>
<td>JAMS &amp; JELLIES(^30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domestic</td>
<td>&lt;185</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
</tr>
<tr>
<td>Imported</td>
<td>&lt;200</td>
<td>&lt;100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>SOUPS(^31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry mix</td>
<td>&lt;10, &lt;20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOUPS(^31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GELATIN(^32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavored mix</td>
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<tr>
<td>Unflavored mix</td>
<td>40</td>
<td></td>
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<tr>
<td>SNACK FOODS</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Apple bits(^33)</td>
<td>275</td>
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<td></td>
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<tr>
<td>Crackers, filled(^34)</td>
<td>75</td>
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<tr>
<td>Corn based snacks(^35)</td>
<td>&lt;1</td>
<td></td>
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<td></td>
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<tr>
<td>Pretzels(^35)</td>
<td>&lt;1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PROTEIN ISOLATES, SOY(^36)</td>
<td>36</td>
<td>40-100</td>
<td>2-5</td>
<td>0.25</td>
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</tbody>
</table>

See footnotes at end of table.
<table>
<thead>
<tr>
<th>Food Category and Subcategory</th>
<th>NRC 1977 Survey(^1)</th>
<th>Reported(^2)</th>
<th>Estimated(^3)</th>
<th>Food Intake(^4) g/capita/d</th>
<th>SDE Intake(^5) mg/capita/d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added</td>
<td>Residual</td>
<td>Added</td>
<td>Residual</td>
<td></td>
</tr>
<tr>
<td>NUT PRODUCTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coconut</td>
<td>25</td>
<td>5</td>
<td>5</td>
<td>0.5</td>
<td>0.003</td>
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<tr>
<td>Unspecified</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GRAVIES, SAUCES(^3)</td>
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<tr>
<td>Meat, tomato, milk,</td>
<td>112</td>
<td>45</td>
<td>75</td>
<td>3</td>
<td>0.225</td>
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<tr>
<td>buttery, and</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>specialty</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Unspecified</td>
<td>90</td>
<td></td>
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<td>BEVERAGES, NONALCOHOLIC(^4)</td>
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</tr>
<tr>
<td>Cola and pepper</td>
<td>4</td>
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<td>3.3</td>
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<tr>
<td>Orange</td>
<td>1</td>
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<tr>
<td>Root beer</td>
<td>4</td>
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<tr>
<td>Ginger ale</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grape</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juice-containing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carbonated beverage</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>1</td>
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</table>

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Table 1. Levels of Sulfiting Agents (Sulfur Dioxide Equivalents, SDE) in Foods and Estimated Per Capita Daily Intake (continued)

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<thead>
<tr>
<th>Food Category and Subcategory</th>
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<th>Level in Product as Consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td></td>
<td>NRC 1977 Survey(^1) Rptd., to FDA/LSRO(^2)</td>
<td>Reported(^2) Estimated(^3)</td>
</tr>
<tr>
<td></td>
<td>Added Residual</td>
<td>Added Residual</td>
</tr>
<tr>
<td>BEVERAGES, ALCOHOLIC(^4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td>10, 15</td>
<td>80</td>
</tr>
<tr>
<td>Wine, table</td>
<td>150, 150</td>
<td>180</td>
</tr>
<tr>
<td>Wine, red</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine, white</td>
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<td></td>
</tr>
<tr>
<td>Vermouth</td>
<td>59, 87</td>
<td>200</td>
</tr>
<tr>
<td>Wine, fortified</td>
<td>120, 120</td>
<td>133</td>
</tr>
<tr>
<td>Wine, sparkling</td>
<td>140, 175</td>
<td>149</td>
</tr>
<tr>
<td>Wine, pop/fruit</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Wine, nonsulfited</td>
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</tr>
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</table>


2. Values reported to FDA subsequent to the proposed order on the GRAS status of sulfiting agents published in the Federal Register, July 9, 1982 and/or to LSRO after July 9, 1984.

3. Estimates made by LSRO staff.

4. Estimates of average values of daily food intake per capita are based on data from the National Food Consumption Survey (Pao et al., 1982; USDA, 1983a, b); USDA statistical sources (USDA, 1983c; 1984a, b); U.S. Imports (USDC, 1984), and information provided by industry associations and companies. Estimates based on food purchased and used in households have been increased by 11% to allow for food eaten in food service establishments (USDA, 1979).
5. Product of reported or estimated SDE level in food as consumed x intake of food.

6. Although reported levels of sulfite in baked products as consumed (baked) were <6 ppm, a value of 5 ppm was selected because of the uncertainty in analytical results at low SDE levels. An exception is cake with dried carrots with a reported residual SDE level of 0-20 ppm.

7. Analyses of commercial products reported to the Life Sciences Research Office, FASEB, by Dr. Steven Taylor, Food Research Institute, University of Wisconsin, Madison, WI.

8. Estimates based on reported intakes of pies and pizzas (Pao et al., 1982) and percent of crust in pies (USDA, 1966).

9. Residual SDE levels as processed and intake data for corn starch and modified corn starch were reported by the Corn Refiners Association, Inc., Washington, DC. Intake level of spinach pasta is based on industry estimates; intake of hominy estimated by LSRO.


11. Proposed maximum level of use.

12. Canned and bottled olives were reported by the California industry not to contain added sulfites. The unspecified olive product(s) could not be identified; to include for sulfite in such products, an average SDE level of 2 ppm was assigned to olives.

13. Some relishes, but not pickles (i.e. pickled cucumbers) are reported to contain added sulfite. Intake is based on industry estimate relative to that of cucumber pickles.

14. An average dilution factor of 5 was used in calculating SDE level as consumed. Salad dressings from dry mixes were estimated to constitute 5% of the intake of all salad dressings reported by Pao et al. (1982).

15. Intakes of malt and wine vinegars are based on industry estimates of sales.

16. Intake is based on 1982 sales of filled milk (USDA, 1984b). According to USDA, filled milk sales largely account for the difference between total sales of fluid milk products and the sum of sales of products listed, or 28 million lb. in 1982.

17. Residual levels in shrimp meat as processed and as consumed after cooking were taken from paper by Wood et al. (1976). Intake data for frozen fabricated products were not available; 10 ppm was selected as the average SDE level for all fish and seafood products.

18. Intake of sulfited fruit salad and the identity of unspecified fresh fruits were not determined. To account for these possible sources of sulfite and also include the contribution of table grapes, 5 ppm was selected as the average SDE level in all fresh fruits consumed.
19. The dried fruit category is discussed in the text. Intake is for dried fruits that have been treated with sulfur dioxide, not all dried fruits.

20. Intake of imported apple and grape juice concentrates are based on 1983 import figures (USDA, 1984). SDE level in imported apple juice concentrate is based on information from Industry. SDE level in imported grape juice concentrate is the level reported in one shipment; intake in Table 1 is for the concentrate, and the calculated SDE intake assumes no loss of sulfite in further processing. Intake of white and sparkling grape juices were estimated to be 10% of total grape juice intake reported by Pao et al. (1982). Intake of nonfrozen lemon juice is based on 1983 consumption of canned and chilled lemon and lime juice (USDA, 1984a). Identity and intake of dietetic fruit juices containing added sulfite were not established.

21. Some cherries and sliced apples are reported to be treated with sulfite prior to freezing but information on quantities and residual SDE levels was not obtained. An average SDE level of 5 ppm was assigned to the reported intake of frozen fruits.

22. SDE level in maraschino cherries as consumed is based on report of Nordlee et al. (1984). Intake is based on production data submitted by National Cherry Growers and Industries Foundation, Inc., Corvallis, OR.

23. The fresh vegetable category is discussed in the text. SDE level in sulfite-treated mushrooms reported by ESS Laboratory Services (1983).

24. Information from Industry indicates that sulfite is added only to sauerkraut packed in glass to prevent browning. The "added" level in Table 1 was taken from the NRC 1970 survey (NRC, 1972). No information was available on added levels in pickled cocktail onions and peppers. Residual levels were assumed to be the same as that reported for relishes (condiment category). Information on the intake of these three products was not available, and an average SDE level of 5 ppm was assigned to the intake reported for all canned vegetables to account for the SDE intake of the foods listed and the unspecified canned foods which may not have been identified.

25. Estimated intake of dried potatoes is based on quantity of potatoes dehydrated adjusted for processing and estimated handling losses.

26. Information from major processors of frozen potatoes indicates that less than 20% of commercially processed frozen products contain added sulfite.

27. Sauerkraut juice is the only vegetable juice identified as containing added levels of sulfite. Identity of juices containing <800 ppm SDE (Table 1) was not established. An average SDE level of 5 ppm was selected for the intake of all vegetable juices.

28. Residual SDE levels in cane and beet sugar were reported by the Cane Sugar Refiners Association, Inc., Washington, DC; the level for dextrose was reported by the Corn Refiners Association, Inc., Washington, DC. Intake of sugar is based on per capita domestic disappearance of cane and beet sugar (USDA, 1983c) and annual consumption of dextrose reported by the Corn Refiners Association, Inc.

29. Residual SDE levels and intake figures are based on data reported by the Corn Refiners Association, Inc., Washington, DC.
30. SDE level in domestic jams and jellies is based on analytical data reported by the International Jams and Jellies Association, Atlanta, GA. The NFCS Intake value includes jams, jellies, fruit butters, fruit topping, pastry filling, pectin, and jellying agents. SDE level in imported jams and jellies is based on the report of Keerling and Thomas (1982) which states that maximum SDE level permitted in English jams is 100 ppm. Intake of imported jams and jellies is taken as 1% of the jam and jelly intake reported by NFCS. This is based on data on imports and domestic production provided by the U.S. Department of Commerce which shows that poundage imported is about 1% of domestic production.

31. Residual values in dry mixes were not reported by all manufacturers. Dilution ratios reported for dry soup mixes ranged from 1:6 to 1:18. The value, 25 ppm max, is a calculated value based on dilution of the dry mix and does not include any reduction resulting from cooking in preparation for serving.

32. Residual levels reported in products processed (column 5) are for dry gelatin mixes. A dilution ratio of 1:5 was assumed in estimating levels in product as served.

33. Included in dried fruits category.

34. Included under crackers in baked goods category.

35. SDE levels are based on information reported by the Potato Chip/Snack Food Association, Laguna Beach, CA.

36. About one-half of estimated production of soy protein isolate is estimated to contain added sulfite. Soy isolate is used as an ingredient in other foods at levels generally less than 5% (SCOGS, 1979) and SDE levels in these foods contributed by soy isolate is 2-5 ppm. Food intake in Table 1 is for soy isolate per se and SDE intake is total for soy isolate not adjusted for processing losses in food formulation.

37. Intake is based on 1983 imports of shredded and desiccated coconut (USDC, 1984).

38. NFCS reports do not separate soups, sauces, and gravies (USDA, 1983a). It is estimated that purchased sauces and gravies are about 5% of total soups, sauces, and gravies.

39. SDE levels in soft drinks (calculated from residual SDE in their ingredients) were reported by the National Soft Drinks Association, Washington, DC. Market share of each type beverage was also reported. Average level (3.3 ppm) in all soft drinks was calculated from these data.

40. Two estimates are included for per capita daily wine intake: 5.4 g from the NFCS and 24.5 g based on the quantity of wine distributed in the U.S. in 1983 (USDA, 1984). Corresponding values for SDE intake are 0.81 mg and 3.68 mg.
reliability of the SDE intake data reported by NRC. There are factors favoring overestimation of intake values. When a processor reported the addition of a sulfite to a single food, the NRC calculation procedure caused all foods in the pertinent NRC food subcategory to be assigned the reported kind and level of added sulfite. Thus, the NRC procedure typically resulted in an overestimation of additive intake, sometimes by a rather substantial margin. Furthermore, food processors often reported the amount of sulfite added to the food rather than the amount remaining at the time of consumption. As explained earlier, sulfites can be lost during processing, storage, distribution, and home preparation, and some sulfite may also be converted to reacted forms. Finally, no correction was made for plate waste.

There are also factors favoring underestimation of intake values. Consumption of alcoholic beverages away from home was probably under-reported in the data on frequency of consumption gathered by Market Research Corporation of America and used by the NRC. Alcoholic beverages, especially wine, can contribute substantially to intake of sulfites. Food service establishments were not included in the 1977 NRC survey and use of proprietary sulfite solutions on salad bar vegetables was not considered. Salad bars can be a substantial source of sulfite intake.

NRC found no way to correct accurately for the factors that contribute to errors in estimation of intakes. Using the NRC data, the Panel has estimated per capita daily sulfite intake (as SDE) including consumption of wine and/or beer to be 20 mg, the 90th percentile intake to be 41 mg, and the 99th percentile 177 mg. These estimates were made in the following manner. The NRC data revealed the per capita daily consumption in SDE to be 7 mg of potassium metabisulfite, 17 mg of sodium bisulfite, 1.2 mg of sodium metabisulfite, 7.8 mg of sulfur dioxide, and 1.0 mg of sodium sulfite. By assuming that use of one of these sulfiting agents in a food subcategory precludes use of others and using only the contribution of the sulfiting agent that accounts for the largest consumption, the figures above can be corrected and become respectively: 0, 11, 1, 1, 7.4, and 0.6 for a total of 20 mg. The 90th percentile daily consumption figures, similarly corrected, are respectively: 0, 19.5, 3.0, 20, and 1.1 for a total of 44 mg; the 99th percentile daily consumption figures, similarly corrected are respectively: 0, 33.1, 8.4, 133, and 2.2 for a total of 177 mg.

Several characteristics and problems in interpreting data in Table 1 should be recognized because they too have bearing on the reliability of the data:

1) In Table 1, there is no relation, necessarily, between the values reported in the "Added" and "Residual" columns for a given food since companies frequently reported values for only one, not both. Exceptions are the values given for the first two
pie doughs and the tortilla shells in the Baked Goods and Baking Mixes Category. Added and residual levels as processed, and level in the product as consumed (after baking the dough) were reported for the same product. For tortilla shells the levels were 25, 25, and 0.3 ppm, respectively. Levels listed for the "Unspecified" subcategory are those designated by reporting companies as used in a general category, e.g., baked goods and baking mixes and thus may apply to one of the subcategories listed in Table 1, or to unidentified subcategories not listed. For most food products, relatively few companies, as compared to the total number manufacturing the food product, reported added or residual levels of sulfites. Thus, the generality of the use of sulfites in various food products manufactured by different companies as well as the representativeness of reported levels of addition may be inaccurate. It is also possible that sulfites are used in foods not reported in Table 1. Answers to such questions were sought by contacts with experts in food science and technology in USDA, universities, industry and industry associations, and from reports in the literature; the estimated sulfite levels in foods as consumed (Table 1) reflect the information obtained.

2) Reports of the NFCS generally identify the quantity of foods eaten as such by individuals in a 3-day survey period or used by households in a week during the survey year, April 1977 through March 1978. Not included are the quantities of these foods which were consumed as ingredients in processed foods not listed in Table 1. Examples of such foods that are reported to contain sulfites are sugar, corn syrup, dried fruit, corn starch, modified corn starch, and dextrose. The latter three substances are consumed almost entirely as ingredients of processed foods. In order to include the total contribution of the residual sulfite in these substances, per capita daily intake values calculated from total annual consumption of these products are entered in Table 1. Total annual consumption of sucrose and dried fruits was obtained from USDA statistics, and was provided by Corn Refiners Association, Inc. for the corn-derived products.

Daily intake of sugar calculated from total annual consumption in 1983 (sucrose plus dextrose) is 99g/capita as compared with 4.35 g/capita reported for sugar consumption by individuals (Pao et al., 1982). The respective intakes for corn syrup are 26 and 0.30 g capita/day. Using intakes based on total annual consumption of these foods results in some duplication in the calculation of SDE intake. Sugar is a major component of jams, jellies, and gelatin desserts that also are entries in Table 1, and both sugar and corn syrup (high fructose corn syrup) are used as sweeteners in soft drinks, also an entry in Table 1.

3) Sulfites were reported to be added to dried apples, raisins, and unspecified fruits (Table 1). Contact with the dried fruit industry revealed that prunes and dark raisins, which constitute about 70% of all dried fruit, are not treated with sulfur
dioxide. Dried apples, apricots, some figs, peaches, pears, and golden raisins are treated with sulfur dioxide. Household consumption of dried fruits other than prunes and raisins was reported as 0.01 lb/household/week or 0.235 g/capita/day based on an average of 2.76 members per household (USDA, 1983a). However, in addition to that purchased as such, dried fruit is also purchased and eaten as an ingredient of breakfast cereals, baked goods and baking mixes, snacks such as apple bits, and in reconstituted form in some pie fillings. In order to include such uses, daily intake (0.93g/capita/d) was based on per capita consumption of all dried fruits less that of raisins and prunes as reported by USDA (1984a). The SDE level in dried fruits as consumed was estimated to be 275 ppm based on a study in England in which the average SDE content of 164 samples of dried fruit, excluding prunes, was found to be 275 ppm at the point of sale.

4) In estimating the average SDE concentrations in the total intake (39.1 g/capita/day Pao et al., (1983)) of fresh vegetables eaten raw, the contribution of fresh vegetables eaten in restaurants, cafeterias, and other food service establishments was estimated as follows. It was assumed that the percent of fresh vegetables eaten raw in food service establishments was the same as the percent of all vegetables obtained and eaten away from home (15.7%), as found in the NFCS (USDA, 1983b); or that 39.1 x 0.157 = 0.61 g/capita/day is eaten in food service establishments. From a poll of its membership in 1983, the National Restaurant Association found that 3.9% of 17,000 respondents reported the use of sulfites, a decrease from the 20% who reported use of sulfites in 1982 (Neville, 1983). We increased this figure (3.9%) to 10% to allow for possible wider use among nonmembers of the NRA, higher sulfite concentration than that assumed, and a possible disproportionate share of the market held by establishments that use sulfites. An SDE concentration of 950 ppm in cut lettuce was found in experiments with commercial sulfite solutions used according to directions (Taylor, 1984). This value is assumed to be the level in leafy vegetables and also in other fresh vegetables served in restaurants and other food service establishments among the 10% which may use sulfiting agents.

Sulfite consumed in these vegetables is 950 ppm x 0.61 g = 0.58 mg/capita/day. The fraction of mushrooms marketed fresh and treated with sulfites (about 10%) contributes about 0.003 mg SDE/capita/day to the total SDE consumed in fresh vegetables. The average SDE level in all fresh vegetables eaten raw based on the contribution of mushrooms and sulfite-treated vegetables as discussed above is 14.3 ppm. In order to allow for possible unidentified (e.g., unspecified uses, Table 1), the average was increased by about 10% to 16 ppm.

The NFCS provides data on the percentage of the population that drank wine on only one day of the three-day dietary survey, on only two days of the three, and on all three. For the respective groups, the daily intake of SDE from wine is calculated to be
11, 22, and 36 mg based on an average SDE level of 150 ppm. The average daily intake for all three groups, i.e., for those who drank wine on at least one of the 3 days, is 17 mg. Only 4.8% of the population surveyed reported use of wine and per capita daily intake for the total population is calculated to be 5.4 g of wine and 0.81 mg SDE. It appears, however, that consumption of wine was under-reported in the NFCS. Based on USDA reports, wine amounting to 21 g/capita/day entered U.S. distribution channels in 1977. In 1983, 24.5 g/capita/day were distributed (USDA, 1984a); this quantity would contain an estimated 3.68 mg SDE.

Information on other food categories is provided in the footnotes to Table 1.

Based on the estimates in Table 1, the total per capita daily intake of sulfites from food, expressed as sulfur dioxide equivalents (SDE), is 6 mg. Beer provides an additional 0.4 mg/capita/day and wine, based on NFCS intake, 0.8 mg but 3.7 mg based on the total quantity distributed. Thus, it seems reasonable to conclude that the mean daily intake of SDE from food, wine, and beer does not exceed 10 mg. For those individuals who consume wine and/or beer regularly, there would be an additional 30 mg for each 200 ml of wine and 10 mg for each liter of beer consumed. For comparison, the Codex Alimentarius estimated in 1975 that heavy consumers of foods and beverages containing high levels of sulfites (99th percentile consumption) might have a daily intake of 177 mg SDE. For further comparison, the following is an example of a meal that may provide a maximal amount of SDE:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>ppm</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tossed salad</td>
<td>100 g</td>
<td>950</td>
<td>95</td>
</tr>
<tr>
<td>Instant potatoes</td>
<td>160 g</td>
<td>70</td>
<td>11.2</td>
</tr>
<tr>
<td>Shrimp</td>
<td>63 g</td>
<td>30</td>
<td>1.9</td>
</tr>
<tr>
<td>Wine</td>
<td>200 g</td>
<td>150</td>
<td>30</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>138.1 mg</strong></td>
</tr>
</tbody>
</table>

Thus it also seems reasonable to conclude that the 99th percentile intake, including regular consumption of wine and beer, probably does not exceed 180 mg of SDE per day.

Unsuccessful attempts have been made by the Panel to obtain reliable data on the poundage of sulfites currently being used in food per annum and the trend in usage since 1976.

While intakes of sulfites from non-food sources are not to be considered in the present evaluation, approximate levels of intake from such sources should be mentioned for comparative purposes and consideration of cumulative effects. Non-food sources of sulfite intake include the atmosphere and drug solutions.
Sulfur dioxide intake by inhalation can be estimated from standards of air quality (U.S. Clean Air Act Amendments of 1970) which require that average sulfur dioxide concentrations in communities do not exceed 0.14 ppm in any one 24-hour period and do not exceed 0.03 ppm per 24 hours over one year. For industrial environments, a threshold limit value of 5 ppm has been established for an 8-hour day (American Conference of Governmental Industrial Hygienists, 1982). The 0.14 ppm level would result in inhalation of 3.8 mg in 24 hours, the 0.03 ppm level 0.85 mg per 24 hours, and the 5 ppm level 59 mg per 8 hours.

Many injectable and oral drug preparations contain sulfite ranging from 0.25 to 1.0% as SDE (Pulmonary Allergy Drugs Advisory Committee, 1983). Sulfite intake from such solutions, which are administered in small volumes, is quite small compared to that from intravenous infusion and peritoneal dialysis solutions which may also contain sulfites (Metcalf, 1984). Infusion of 500 ml of a solution containing 0.1% sodium bisulfite, for example, would result in an intake of 308 mg SDE.
IV. BIOLOGICAL STUDIES

The report of the Select Committee on GRAS Substances reviewed biological data relevant to the health effects of sulfiting agents available at that time (SCOGS, 1976). Except for information considered necessary to provide perspective and context for recent studies, literature cited in this section of the report represents studies completed since the 1976 evaluation of the GRAS status of sulfiting agents.

Metabolism of Sulfites

The chief pathway for metabolism of sulfite consists of its oxidation to sulfate. At high concentrations of sulfite (1 M sulfite, pH 7) an autooxidation reaction, initiated by superoxide or HSO₃⁻ radicals and enhanced by Mn²⁺ and Fe³⁺ proceeds readily (McCord and Fridovich, 1969). However, at 20 mM sulfite concentration, this route of oxidation is relatively ineffective either in oxidizing sulfite to sulfate or in initiating other biologically significant reactions (Hayatsu, 1976; McCord and Fridovich, 1969; Rajagopalan, 1980). In vivo sulfite oxidation is catalyzed by sulfite oxidase (also called sulfite dehydrogenase or sulfite: ferricytochrome c oxidoreductase, EC 1.8.2.1). The enzyme is located in the intermembranous space of the mitochondria, and it exists as a dimer (molecular weight 120,000 daltons in the human) with subunits containing a molybdenum atom (Mo⁶⁺), a cytochrome b₅-type heme component and a pterin cofactor (Cohen and Fridovich, 1971; Johnson et al., 1980). Sulfite oxidase functions by transferring a pair of electrons from sulfite to molybdenum, heme, and cytochrome c sequentially. Sulfate and other anions inhibit sulfite oxidase activity in vitro; tungstate inhibits the enzyme in vivo and in vitro.

Sulfite oxidase is distributed widely in mammalian tissues with the most significant concentrations in liver, heart, and kidney. Activity of the enzyme has been measured in the livers of many species including rats, dogs, cattle, monkeys, and man (Johnson and Rajagopalan, 1976; MacLeod et al., 1961). Sulfite oxidase activity measurements reported in the literature are difficult to compare because of the localization of the enzyme in the mitochondria and variations in the methods used. However, it can be generally observed that sulfite oxidase activity in the human is slightly less than that of the rhesus monkey and substantially less than any of the other mammals studied except the rabbit. Cohen et al. (1973) estimated that the total activity of sulfite oxidase of the rat is capable of oxidizing 750 mmoles/kg body weight/day of sulfite to sulfate, corresponding to 48 g SDE/kg body weight/day. Human liver has only 5 to 10% of the sulfite oxidase activity of rat liver (Johnson and Rajagopalan, 1976). In biopsy samples from three of four normal subjects, hepatic sulfite oxidase activity was 13.5 Units/g; in the fourth
subject, enzyme activity was 27.5 Units/g (one Unit being equivalent to 0.1316 μmoles of cytochrome c reduced per minute) (Johnson et al., 1980). Sulfite oxidase activity in cultured fibroblasts of normal human subjects was 1.07 mmoles cytochrome c reduced/min/mg protein (range, 0.75-1.76) (Shih et al., 1977) and 2.10 mmoles/min/mg protein (range, 0.75-3.03) (Johnson et al., 1980).

At least seven cases of congenital deficiency of sulfite oxidase in humans have been reported (Gunnison and Jacobsen, 1983). No detectable activity of sulfite oxidase was found in liver or in skin fibroblasts of these patients (Johnson et al., 1980; Shih et al., 1977). Metabolically, the condition is characterized by greatly increased excretion of sulfite, thiosulfate and cysteine-S-sulfonate and by somewhat decreased excretion of sulfate. Clinically, the condition produces dislocated ocular lenses and severe neurological abnormalities resulting in mental and physical retardation.

In four sulfite oxidase-deficient patients, Shih et al. (1977) found that daily urinary sulfite excretion ranged from none to 4.28 mmoles/g creatinine; thiosulfate excretion was increased from 0.79 to 1.97 mmoles/g creatinine. Oral loading of one patient with 0.5 mmole each of methionine and cystine produced an increase in plasma sulfite from an initial level of 15 μM to 120 μM and 60 μM, respectively, after 4 hours. The loading tripled thiosulfate excretion.

Parents and siblings of sulfite oxidase-deficient patients frequently have lower levels of enzyme activity. Shih et al. (1977) and Johnson et al. (1980) reported that these relatives metabolized sulfite normally, confirming the occurrence of a large excess of sulfite oxidase activity in heterozygous as well as normal individuals.

A model system for study of sulfite oxidase deficiency can be produced in the rat by inclusion of 100-200 ppm of sodium tungstate in the drinking water (Johnson et al., 1974). In this early study, hepatic sulfite oxidase activity decreased from 40 to 0.05 Units/mg protein in 28 days. Tungsten competes with molybdenum for the metal binding site on the apoenzyme and sulfite oxidase activity can be diminished to any level by choosing an appropriate tungstate:molybdate ratio.

Sulfite oxidase-deficient rats have markedly increased levels of urinary and tissue endogenous thiosulfate and S-sulfonates in proportion to the decrease in enzyme activity (Gunnison et al., 1981a,b,c). In sulfite oxidase-deficient rats administration of 0.5 mmole/kg of sulfite produced amounts of plasma S-sulfonates equivalent to those produced in normal rats by administration of 10 mmole/kg sulfite. High levels of plasma sulfite also occurred. Intubation of 10 to 20 times as much sulfite into the normal rat was required to produce a systemic sulfite level equivalent to
that of the sulfite oxidase-deficient rat even though the former had 100 times the activity of enzyme (Gunnison et al., 1981a). Probably the limiting factor for sulfite oxidation is the rate of diffusion into the mitochondria (Oshino and Chance, 1975).

Since the oxidation of sulfite by nonenzymatic reactions is minimal in the presence of the normal distribution of sulfite oxidase, sulfate excretion provides an estimate of the metabolism of sulfite. It has been estimated by Gunnison (1981) that endogenous sulfite generation in humans is about 0.3 to 0.4 mmole/kg/day (19.2 to 25.6 mg SDE/kg/day). In the U.S. the mean dietary intake based on information on consumer exposure is calculated to be between 0.17 and 3.0 mg SDE/kg/day for a 60 kg individual. The endogenous sulfite arises in the mitochondria and only needs to diffuse across the inner membrane to contact sulfite oxidase. Such sulfite arises from cystine and methionine in the diet in excess of bodily requirements. Sulfite has not been detected in the plasma or urine of normal humans, nor that of other mammals, unless large quantities of sulfite had been administered (Gibson and Strong, 1973).

Exogenous sulfite enters the body by inhalation of SO₂ and by ingestion via the blood stream. Some sulfite is oxidized in the lung and intestine before it enters the circulation. Pfleiderer et al. (1968) reported that the empty intestine of rats oxidized about 50% of radiolabeled sulfite while filled intestines oxidized even more. This may be due to sulfite oxidase activity in the intestinal wall. No reports of gastric absorption or metabolism have been noted.

Sulfite administered intravenously was cleared rapidly in the rhesus monkey. It has a biological half-life of 10 minutes for doses in the range of 0.3 to 0.6 mmole/kg (Gunnison and Jacobsen, 1983). Hepatic oxidation of infused sulfite in rats has been shown by Oshino and Chance (1975) to be diffusion limited. A constant proportion of the sulfite in the perfusate, up to 1 mM, is removed (mean, 33%, range 24-40%). Since all sulfite is not removed by the liver at first pass of the blood, all tissues will be exposed to a significant proportion of the administered dose. On the basis of data from rats and monkeys, Gunnison and Jacobsen (1983) speculate that the half-life of sulfite in man is 15 minutes. As an example of expected levels of sulfites, approximately 0.25 mg of a 1 mg dose of potassium metabisulfite would remain in body fluids 30 minutes after ingestion. An immediate second challenge of 5 mg of metabisulfite would increase the level to 5.25 mg, declining to 1.31 mg in another 30 minutes. The immediate concentration after injection of 1 mg of potassium metabisulfite would be 2.57 μM for a 70 kg man assuming all the sulfite was confined to the plasma and 0.21 μM if the compound were evenly distributed in total body water.
Because of rapid clearance of sulfite by action of sulfite oxidase, chronically ingested sulfite does not accumulate in the tissues but is rapidly eliminated, primarily as sulfate. In all species tested, Gunnison and Palmes (1976) found that no more than 10% of the administered dose was excreted in the urine as sulfite. Brief exposure of tissues to sulfites does not appear sufficient to maintain sulfite adducts that are unstable. A sufficiently large physiologic equilibrium constant has not been demonstrated for the minor adducts to make them a factor in metabolism. Irreversible reactions, although quantitatively minor, could exert more significant biological effects.

Sulfite persisting in body fluids undergoes a number of reactions with tissue components. Thiosulfate is a product of three enzymatic reactions: 1) the reaction of sulfite with mercaptopyruvate (Sörbo, 1957); 2) metabolism of cysteine-S-sulfonate (Sörbo, 1958); and, 3) the reaction of sulfite and thioctystine (Szczepkowski and Wood, 1967). Söörbo and Ohman (1978) determined the daily excretion of thiosulfate in the urine of normal human subjects to be 31.7 mmol/day ± 12.8 (range 12.4–67.5). These values probably do not represent actual formation of thiosulfate in the body since thiosulfate is metabolized to sulfate and is excreted in the urine (Gunnison et al., 1981c; Skarzynski et al., 1959).

Several nonenzymatic reactions also occur between sulfite and tissue components. Few of the products are observed to form in vivo at the levels of sulfite arising from endogenous formation. Under conditions of sulfite loading however, appreciable amounts of sulfite-derived compounds are produced.

Sulfite lyases disulfide bonds with formation of S-sulfonates and thiols (Cecil, 1963):

\[
\text{RSSR} + \text{SO}_3^- \rightarrow \text{RSSO}_3^- + \text{RS}^-
\]

The reaction is reversible, but the equilibrium lies well to the right for small molecules such as cysteine-S-sulfonate and glutathione-S-sulfonate (Cecil, 1963); the former is found in the urine after sulfite loading (Gunnison and Palmes, 1974). Glutathione-S-sulfonate was also found in bovine lenses (Waley, 1959). Evidently only interchain disulfides of native proteins undergo sulfitolysis (Cecil and Waley, 1962). Protein S-sulfonates slowly release sulfite ions in the presence of sulfhydryl compounds (Swan, 1959).

Little information is available regarding other essential biological disulfide systems. No reports have been noted on modification of the dithiolane ring of lipoxy-containing transacylase cores (e.g., transacetylasyl of pyruvate dehydrogenase multienzyme complex, transsuccinylalysyl of α-ketoglutaric dehydrogenase multienzyme complex or transacylasyl of branched chain α-keto acid dehydrogenase) by sulfiting agents. The same considerations may apply to other essential disulfide systems, e.g., thio-redoxin.
The formation of protein S-sulfonates is concentration-
dependent both in vitro and in vivo. At 2 mmole/kg/day intake of 
sulfite, plasma of rabbits and rhesus monkeys contained measurable 
levels of S-sulfonates while plasma of rats did not. However, 
parenteral administration of 3.2 mmoles of sulfite/kg/day for 5 
consecutive days produced significant increases in plasma S-
sulfonate levels in the rat from 5-10 nmole/ml plasma to 
19-30 nmoles. Plasma S-sulfonate fractions in the rat and rhesus 
monkey had half-lives of approximately 4 and 8 days, respectively 
(Gunnison and Palmes, 1978).

Gunnison and Palmes (1974) determined that the level 
of plasma S-sulfonates was related to exposure in human subjects 
inhaling sulfur dioxide at 0.3, 1.0, 3.0, 4.2, and 6.0 ppm for 
120 hours. For each increment in exposure of 1 ppm, 1.1 ± 0.16 
nmoles of additional S-sulfonate per ml of plasma were found.

Portions of lungs and aortas of rabbits exposed to con-
stant arterial sulfite concentrations of 245, 580 μM for 0.6 to 
6 hours were analyzed for S-sulfonates (Gunnison and Farruggella, 
1979). The kinetics of formation was first order with asymptotic 
concentrations of S-sulfonates of approximately 900 and 9000 nmole/g 
of dry weight of lung and aorta, respectively. Clearance of 
S-sulfonates from both tissues was apparently first order with a 
half-life of 2 to 3 days. No appreciable amounts of S-sulfonates 
were found in liver, kidney, testes, heart, brain, skeletal muscle, 
stomach, ovaries, duodenum, spleen, or eye.

Levels of S-sulfonates in tracheas of rabbits exposed to 
3 ppm sulfur dioxide for 3 and 24 hours were constant at approxi-
mately 53 nmole/g dry weight (Gunnison et al., 1981c). Prelimi-
ary results of subsequent experiments in which rabbits were 
exposed to 10 ppm sulfur dioxide for 1, 3, 10, 24, 48, and 72 
hours showed formation of 9 nmole/ml plasma in 3 hours and 
30 nmole after 24 hours. No exogenous S-sulfonates were detected 
in aortas and scarcely any in the distal portions of posterior 
lung lobes. Thus, the sulfur dioxide absorbed was partially 
metabolized in passing through pulmonary tissues. S-Sulfonate 
concentrations of 107 nmole/g dry weight of trachea were reported 
in animals exposed for 3 hours with a gradual increase to 
163 nmoles in animals exposed for 72 hours; however increase was 
attributed to increased mucus production. During the exposure, 
S-sulfonates in plasma increased from 40 to 70 nmole/ml; sulfite 
was not detected in the plasma. It was concluded that with 
possible exception of heart and lungs, there was no transport of 
inhaled sulfur dioxide to organs some distance away from the site 
of absorption (Gunnison et al., 1981c).

Nasal mucus of four rats exposed to an initial concen-
tration of 5 ppm of 35SO2 for periods of 0.5, 1, 2, and 4 hours 
contained 90% of the inhaled 35S after 30 minutes (Gause and 
Barker, 1978). Later, the ratio of sulfur in mucus to serum 
leveled off at 3:1. After rats were exposed to 5 ppm or 20 ppm
sulfur dioxide continuously for 7 days, electrophoresis of the
glycoproteins from nasal mucus showed the appearance of three to
five new bands from the acidic fraction, a finding interpreted to
indicate an increase in crosslinking in the protein. The effect
first appeared 2 hours after sulfur dioxide exposure. The poly-
merization of glycoprotein molecules could account for the decrease
in flow rate of nasal mucus commonly seen with inhalation of sulfur
dioxide. Habeeb (1971) found that sulfitoysis of seven disulfide
bonds of bovine serum albumin, with concomitant reorganization of
the topography of the molecule, caused an 80% reduction in ability
to react with antiserum. However, Gunnison et al. (1981c) con-
sidered that localized tracheal concentrations of sulfur dioxide
at or near 10 ppm would not be sufficient to produce protein cross-
linking.

Metabolic Effects of Sulfites

The cleavage of thiamin by sulfite was reviewed by Dwivedi
and Arnold (1973). The reaction involves a nucleophilic attack by
sulfite on the quaternary nitrogen of the thiazole ring to yield
pyrimidine sulfonic acid and 4-methylhydroxethyl thiazole. At
pH 5 and 25°C, the half-life of 10 µM thiamin in the presence of
1.0 mM sulfite is approximately 13 hours (Dwivedi and Arnold,
1973).

It has not been demonstrated that destruction of thiamin
by sulfite in vivo is sufficiently rapid and specific to deplete
reserves of thiamin or that the symptoms of bisulfite toxicity
coincide with thiamin avitaminosis (Gunnison et al., 1981a). In
three rats fed a diet containing 6% sodium metabisulfite and
supplemented with 50 ppm thiamin for 21 days, the hepatic thiamin
values were 8.8 ± 1.2 µg/g wet weight while the control values
were 10.1 ± 0.7 µg/g. Although the test animals were severely
anemic (hemoglobin, 9.5 ± 2.6 g/100 ml blood), Gunnison et al.
(1981a) concluded that the systemic effect of sulfite was not due
to destruction of thiamin. Activity of heme synthetase in brain
and liver of male rats 3 months of age was also reduced after
consumption of drinking water containing 900 ppm sodium sulfite
for 7 weeks (Savela-Nen and Tenhunen, 1982).

Destruction of cyanocobalamin in the diet or in the gut
was considered a possible mechanism in the production of anemia
(Gunnison et al., 1981a). The cobalamins form photolabile complexes
with sulfites (Gunnison and Jacobsen, 1983). During photolysis,
sulfite is oxidized aerobically to sulfate. Cyanocobalamin is
reported to be an effective blocking agent for sulfite-induced
asthma (Jacobsen and Simon, 1984). The relation of complex
formation to the blocking of sulfite-induced asthma by cyano-
cobalamin has not been explained.
Menadione (vitamin K₃) adds sulfite to the 2,3-double bond of the naphthoquinone ring. The reaction is reversible and the sulfite adduct can be used by chicks as a source of vitamin K (Nir et al., 1978).

Sodium sulfite added to the diet of rats and fed for periods up to 1.5 years produced evidence of vitamin E deficiency as measured by effects on the enamel of the teeth Irving et al., 1952. Such evidence appeared only at levels of 500 mg of sodium sulfite per kg body weight and higher.

Amino acids are oxidized in the presence of sulfites. Methionine is oxidized aerobically to its sulfoxide by free radicals in the presence of sulfite and Mn²⁺ (Yang, 1970); similarly, tryptophan is destroyed by sulfite and Mn²⁺-induced free radicals (Yang, 1973).

Sulfite combines reversibly with aldehydes such as glucose and ketones such as fructose to form hydroxysulfonate derivatives (Petering and Shih, 1975). Compounds such as pyruvate and α-ketoglutarate form hydroxysulfonates which are somewhat more stable than the hydroxysulfonate of glucose, but would not be expected to persist under physiological conditions. A potential carbonyl addition is the sulfite adduct of pyridoxal phosphate, an important cofactor of many enzyme systems.

Sulfite adds to the 3,4-double bond of the pyridine ring of NAD. Riboflavin, as it occurs in coenzymes (e.g., FAD, FMN), forms chemical adducts with sulfites. The N⁵ atom of flavin is thereby substituted with a sulfonate group at the active site that accepts hydrogen. The free adducts dissociate readily at low sulfite concentrations, but may show a somewhat enhanced stability in protein combinations. This is the probable mechanism of inhibition of dehydrogenases by sulfite (Massey et al., 1962). A number of flavoprotein oxidase enzymes (e.g., D and L amino oxidases, oxynitrilase, lactate oxidase, and glycolate oxidase) form stable sulfite adducts with dissociation constants from 10⁻³ to 10⁻⁷ M. The flavoprotein dehydrogenases did not form adducts at 20 mM sulfite (Massey et al., 1969; Muller and Massey, 1971).

The catalytic action of several oxidative enzymes such as xanthine oxidase, liver aldehyde oxidase, cytochrome oxidase, lipoxidase, and peroxidase can initiate aerobic oxidation of sulfite by intermediate formation of superoxide and other free radicals (Fridovich and Handler, 1961). Cytochrome oxidase was inhibited 37% by 0.5 mM sulfite at pH 7 (Cooperstein, 1963). α-Glucan phosphorylase was inhibited by 10 to 30 mM sulfite concentrations (Kamogawa and Fukui, 1973). Sulfite was a competitive inhibitor for phosphate in glycogen synthesis and in glycogen degradation. Alkaline phosphatase was inhibited in vivo.
Activity of 2,3-diphosphoglyceric acid phosphatase was enhanced 15-fold by 2.5 mM sulfite (Harkness and Roth, 1969). Sulfite was found to be a potent inhibitor of many sulfatases, e.g., sulfatase A, $K_i$, 0.2 μM (Roy, 1976).

Sulfite adds reversibly to the 5,6-double bonds of cytosine and uracil and their nucleosides and nucleotides (Gunnison, 1981; Shapiro, 1983). The additions are pH- and concentration-dependent and the adduct has a half-life of several hours. At 100 mM sulfite, only 12% of the uridine reacted in 0.5 hours. Uridine is regenerated from its sulfite adduct by removal of sulfite. The sulfonate adducts of cytosine and its derivatives are less stable and a high concentration of sulfite is necessary to maintain them at physiological pH.

The deamination of cytosine to uracil nucleotides in single stranded DNA occurs in bisulfite solutions of 1 M or higher at pH 5 to 6 (Hayatsu, 1976; Shapiro, 1983). Purine bases do not react under these conditions. This reaction has been used in a number of studies with microbial systems to demonstrate the mutagenic property of bisulfite. The concentrations of bisulfite used in chemical studies and microbial mutagenicity tests were about two or three orders of magnitude higher than is observed in biological systems.

The cytosine sulfonate adduct can also be transaminated with primary and secondary amines, including lysine, to produce $N^4$-substituted cytosines and polycytidines (Shapiro and Gazit, 1977). Crosslinking was obtained also with heat-denatured calf thymus DNA to the extent of 0.5% of the strands after 6 days in 0.15 M sodium bisulfite. Double-stranded DNA did not crosslink. However, Lukašová and Paleček (1976) found that treatment of native calf thymus DNA with 2.61 M bisulfite at pH 6.5 for 24 hours caused modification of the polarographic behavior which was explained on the basis of modification of less than 1% of the cytosine, presumably by conversion to uracil. The investigators suggested that cytosine residues on "open" loops in the native DNA were the reactive sites. Such crosslinking has not been demonstrated in vivo in mammalian organisms. In cultures of mouse liver cells, Hela cells, and human embryonic lung cells and lymphocytes and oocytes, significant inhibition of DNA synthesis has been observed at bisulfite concentrations from 0.1 to 10 mM (Shapiro, 1983). From alkaline sucrose density gradient measurements, Hayatsu and Miller (1972) concluded intranucleotide bonds of DNA were cleaved by 1 to 10 mM sodium bisulfite. Since the cleavage at pH 7 was enhanced by Mn$^{2+}$ and inhibited by hydroquinone, it was considered to result from free radical formation.

Kaplan et al. (1975) found that low concentrations of bisulfite (0.5 mM) induced oxidation of corn oil emulsified in 1.5% polysorbate solution. The effect was concentration-dependent.
to 10 mM. The initial oxidation products presumably were peroxides. Since it was inhibited by Mn$^{2+}$ and antioxidants, sulfite autooxidation was not considered a significant factor in the oxidation process. Inoye et al. (1978) reported similar results in liver homogenates.

Incubation of unsaturated membrane lipids with a large excess of bisulfite produced significant changes in the behavior of such lipids on silica gel, suggesting the addition of bisulfite to fatty acid double bonds (Akogoram and Southerland, 1980). Such addition, if occurring in vivo, would change the topography of the membrane and could account for the toxic and irritating effect of sulfur dioxide on tissues.

In brains of guinea pigs exposed to 10 ppm sulfur dioxide for 1 hour daily for 21 days, total lipids and free fatty acids were decreased in all regions, but changes in other fractions varied among regions of the brain (Haider et al., 1981). The rates of peroxidation and the activity of lipase were increased significantly in all regions of the brain.

From the information on the metabolism of sulfiting agents in the current literature, the ad hoc Panel finds no reason to presume that individuals with the usual levels of sulfite oxidase activity and adequate nutritional status should develop adverse health effects from consumption of sulfite in foods at the levels currently used. However, there is sufficient information in the current literature to conclude that some individuals or a select subpopulation with a deficiency of sulfite oxidase activity would metabolize sulfite more slowly and may be subject to adverse effects from sulfite at the current level of intake.

**Acute Oral Toxicity**

No animal feeding studies of acute oral toxicity of sulfiting agents published after 1976 were located for consideration by the ad hoc Review Panel. Acute exposure seems to be a factor in hypersensitivity reactions associated with ingestion of foods or beverages containing sulfiting agents (see following section), but this reaction appears to be separate and distinct from other manifestations of acute oral toxicity.

**Hypersensitivity Reactions**

Reactions to sulfite salts or sulfur dioxide have been described in some asthmatic individuals following ingestion of foods or beverages containing sulfites, inhalation of sulfur dioxide, and parenteral administration of medications containing sulfites. The most often reported sign following exposure to sulfites or sulfur dioxide has been bronchial hyperreactivity.
(bronchoconstriction and bronchospasm) although reactions resembling anaphylaxis (shock, gastrointestinal disturbances, and urticaria/angioedema) as well as flushing, hypotension, and tingling sensations have also been reported (NIAID, 1984).

In 1973, Kochen reported that a mildly asthmatic child experienced acute, transient asthmatic reactions following ingestion of freshly opened sulfite-containing foods. However, challenge testing was not carried out to determine if sulfites were the causative agents in this case. Subsequently, Prenner and Stevens (1976) presented a case report of anaphylaxis occurring in a 50-year-old male who consumed a restaurant meal that included a green salad sprayed with a product containing bisulfite. Oral challenge with sodium bisulfite (10 mg total dose) resulted in erythema, itching, nausea, warmthness, coughing, and bronchoconstriction for about 1 hour. Lung function measurements were not made nor was a placebo administered as a part of the challenge.

Thirty of 272 asthmatic patients interviewed reported exacerbations of asthma following ingestion of orange drinks made with sodium bisulfite and sold in England (Freedman, 1977). Fourteen of the 30 patients allowed challenge tests with a single dose of a sodium metabisulfite solution containing 25 mg SDE in a weakly acidic solution (sulfur dioxide concentration 100 ppm). Within 2 to 25 minutes eight of the 14 patients challenged gave a positive response, defined as a drop of at least 12% in FEV₁ (forced expiratory volume at 1 second). However, because of the experimental conditions, no placebo was given.

One case of bronchospasm in an asthmatic patient following ingestion of (canned crabmeat salad with a vinegar dressing) was reported by Baker et al. (1981). Oral challenge of this patient with sodium metabisulfite (dose not reported) resulted in severe bronchospasm within 30 minutes; no reaction was observed after ingestion of the canned crabmeat alone when given as a clinical challenge. In a second patient whose asthma was provoked by wine, a single-blind oral challenge with a capsule containing 500 mg sodium metabisulfite caused a drop in peak flow rate from about 440 l/min before challenge to 100 l/min 30 minutes after challenge. Challenge with a placebo capsule (lactose) did not produce a significant pulmonary change in the second patient.

Clinical presentation of vague, general symptoms were reported following oral challenge with metabisulfite in two patients who developed dizziness, weakness, nausea, chest tightness, tachycardia, and dyspnea associated with restaurant meals (Schwartz, 1983). Pulmonary function studies during an oral metabisulfite challenge showed no changes in FVC (forced vital capacity), FEV₁, FEF 25–75 (forced expiratory flow at 25 to 75% vital capacity), FEF 50, VTG (volume thoracic gas), Rₖₐₖ (airway resistance), or SRₖₐₖ (specific airway resistance).
Clinical investigation of four patients with histories of severe bronchoconstriction and anaphylaxis associated with consumption of restaurant meals was reported by Stevenson and Simon (1981). Single-blind oral challenges were administered to these patients in the fasting state and while they were taking their usual medications. Placebo capsules containing lactose were administered orally every 30 minutes on the first morning of testing and capsules containing 1, 5, 10, 25, or 50 mg potassium bisulfite were given sequentially every 30 minutes on the second day. FEV\(_1\) values were measured at 30-minute intervals on both days. All four patients reacted to bisulfite challenges, developing asthmatic symptoms 10 to 15 minutes after ingestion of a provocative dose (10, 25, or 50 mg). FEV\(_1\) decreased maximally (34 to 49%) at 30 to 90 minutes after provocation. Systemic symptoms including flushing, tingling, and/or faintness occurred in all subjects (Stevenson and Simon, 1981). Subsequent oral challenge of six sulfite-sensitive asthmatic (SSA) patients with sulfite solutions produced reactions equal to the reactions observed after oral capsule challenge, but at levels approximately one-half of the provocative capsule dose (Goldfarb and Simon, 1984). Fifteen additional asthmatic patients with a history of increased asthmatic reactions associated with consumption of food and beverages were serially challenged with capsules containing 5, 10, 25, and 50 mg sodium metabisulfite (Keene and Selner, 1982). Only one of these patients had a significant reaction to the challenge: administration of 5 mg sodium metabisulfite produced a fall of 28% in FEV\(_1\) in 2 minutes.

Twelve patients with idiopathic anaphylaxis, nine of whom had a history of reactions associated with restaurant meals, and ten control subjects were challenged with increasing oral doses (1, 5, 10, 25, 50, 100, and 200 mg) of sodium metabisulfite dissolved in lemonade (Sonic and Patterson, 1985). A similar extent of mild nonspecific irritant and subjective symptoms were reported in both groups of patients. No anaphylactic reactions occurred in the 12 patients with idiopathic anaphylaxis. No bronchospasm occurred, although pulmonary function testing was abnormal in three of these patients (Sonic and Patterson, 1985). Capsule challenge with 1, 5, 10, 25, 50, 100, and 200 mg sodium bisulfite of 32 patients, 14 with recurrent idiopathic anaphylaxis, 8 with systemic mastocytosis, and 10 with instances of allergic reactions to specific foods resulted in anaphylactic episodes in two of the patients with idiopathic anaphylaxis. However, these two patients also reacted in the same way to placebo challenge (Metcalf, 1984).

FEV\(_1\) values did not decline and no manifestations of hypersensitivity were reported following administration of bisulfite to five steroid-dependent asthmatic patients without histories of reactions associated with restaurant meals (Stevenson and Simon, 1981). Of 61 additional asthmatic patients not having
a history suggestive of sulfite sensitivity, five individuals (8.2%) had a fall in FEV$_1$ of at least 25% following single-blind oral challenge of metabisulfite (Simon et al., 1982). In this study, patients were challenged with capsules containing increasing concentrations (10, 25, 50, 100 mg) of bisulfite at 30-minute intervals. If no reaction occurred to these capsules, patients were further challenged by ingestion of acidic solutions containing 1 or 10 mg of metabisulfite. Those reacting positively to metabisulfite challenge were subsequently given a placebo challenge. Although the data were not presented, Simon et al. (1982) reported that the reactions of these five patients were milder and required larger provocative challenges than the reactions in six SSA patients having histories of reactions associated with restaurant meals. Administration of atropine, cromolyn, doxepin, or vitamin B$_{12}$ prior to sulfite challenge of six SSA patients partially or fully blocked asthmatic reactions to sulfite (Simon et al., 1984). Mean activity of sulfite oxidase was 0.15 nmole/min/mg protein in skin fibroblasts of four SSA patients (Jacobsen et al., 1984). This level of enzyme activity is below that reported in skin fibroblasts of normal persons (Johnson et al., 1980; Shih et al., 1977); however, it remains to be shown whether the levels of sulfite oxidase activity in skin fibroblasts is representative of tissues containing the most significant concentrations of the enzyme (liver, heart, and kidney).

Pulmonary function was evaluated in 25 asthmatic patients (history of food or beverage related reactions not specified) and 25 nonasthmatic individuals consuming 112 ml of commercial white wine containing 140 mg/l of sulfur dioxide (15.7 mg sulfur dioxide total dose) (Seyal et al., 1984). A decrease in FEV$_1$ greater than 12% was recorded in one nonasthmatic subject and in five asthmatic patients during or following ingestion of the wine. Two of the asthmatic responders were challenged with two model solutions: the first was reported to contain all ingredients found in wine except metabisulfite, the second was a metabisulfite solution. FEV$_1$ was decreased in both of these subjects following ingestion of the metabisulfite solution and in one subject ingesting the model wine solution without metabisulfite.

Bronchoconstriction and bronchospasm reactions have been reported much more frequently to inhalation of sulfur dioxide than to ingestion of sulfiting agents. Both asthmatic and nonasthmatic individuals react to inhalation of sulfur dioxide (Koenig et al., 1982; Schacter et al., 1984; Sheppard et al., 1980). Mildly asthmatic individuals inhaling sulfur dioxide through a mouthpiece developed bronchoconstriction at a lower concentration of sulfur dioxide (1 to 3 ppm) than healthy nonasthmatic control subjects, and their bronchoconstriction was more severe (Sheppard et al., 1980). However, exposure to a maximum concentration of 1 ppm sulfur dioxide in an environmental chamber (oronasal breathing) did not result in respiratory effects in asthmatic or nonasthmatic.
individuals (Schacter et al., 1984). Inhalation of bronchodilators containing sulfites has also been associated with bronchospasm and anaphylactic reactions (Kaepke et al., 1984; Twarog and Leung, 1982).

Inhalation of sulfur dioxide was reported to induce falls in FEV\textsubscript{1} greater than 25\% at concentrations 1/10 to 1/100 of the concentrations of ingested sulfites in six known SSA patients (Goldfarb and Simon, 1984). One patient with a history of asthmatic reactions related to ingestion of certain foods and beverages began wheezing after breathing from a bag of dried apricots (Werth, 1982). Subsequent oral challenges of this patient with capsules containing potassium metabisulfite to a dose of 50 mg produced no change in peak flow rate. Inhalation of sulfur dioxide probably does not play a role in asthmatic reactions to capsule challenge of sulfite salts, as sulfur dioxide is not likely to be eructated to a noticeable extent after swallowing of the capsules (Metcalfe, 1984). The contribution of inhaled sulfur dioxide to the food and beverage associated episodes of bronchoconstriction and bronchospasm in asthmatic patients is less certain. Freedman, in 1977, cited an estimate that the 3 cm air space above a water solution containing 70 ppm sulfur dioxide contained 1 ppm sulfur dioxide at room temperature, a concentration causing bronchoconstriction in some mildly asthmatic patients (Sheppard et al., 1980). Sonin and Patterson (1985) also considered the possibility that the changes in pulmonary function observed in their studies might have been caused by irritant reactions to inhalation of sulfur dioxide from the acidic lemonade solution administered. Administration of a lemonade solution without sulfites was not reported as a placebo control for this study. Acid fumes are known airway irritants and the pulmonary effects reported could result from exposure to acid fumes as well as to sulfur dioxide. Definitive work remains to be done concerning release of sulfur dioxide from beverages and foods during consumption.

Intravenous infusion of a solution of glucose, theophylline, and dexamethasone which also contained bisulfite severely worsened an asthmatic episode in one patient who had previously shown a large decrease in peak flow rate following an oral capsule challenge of 500 mg sodium metabisulfite (Baker et al., 1981). Intravenous injection of metaclopramide which contained metabisulfite also resulted in bronchospasm in this patient. Injection of a dose of lidocaine hydrochloride containing 0.9 mg sodium metabisulfite was followed by development of plantar pruritis in an individual who had experienced similar symptoms after ingestion of such food items as chili soups, sandwiches, salads, jalapeños, pizza, Chinese pickled green turnips, and dried shrimp (Huang and Fraser, 1984).
Patients receiving total parenteral nutrition (TPN) solutions receive much greater quantities of sulfiting agents than the general population. Administration of TPN solutions containing bisulfites as an antioxidant may result in infusion of about 950 mg/day (Metcalfe, 1984). Although one report of excretion of abnormal sulfur metabolites in a patient receiving TPN for 18 months (Abumrad et al., 1981) led to speculation that tachypnea in this patient might be related to abnormal sulfite metabolism (Gunnison and Jacobsen, 1983). No reports of hypersensitivity reactions to administration of TPN solutions were found in the literature. Similarly, no hypersensitivity reactions attributed to sulfites in TPN solutions have been recognized by the Pharmacy and Therapeutics Committee at the National Institutes of Health (Metcalfe, 1984).

Experience with oral challenge testing of bisulfite has led to differing opinions concerning the extent of hypersensitive reactions to sulfiting agents. Based upon their clinical work with a group of asthmatic patients, Simon et al. (1982) estimated that 5 to 10% of the 10,000,000 asthmatic patients in the U.S. may be sensitive to orally ingested sulfiting agents. In contrast, failure to identify clinical sensitivity to sulfites among an extensive clinic population has led other investigators to conclude that sulfite sensitivity is a minor problem (Patterson, 1984). Data regarding the clinical significance of reactions to sulfiting agents have been extrapolated from fewer than 100 asthmatic patients scattered throughout the U.S. There are no data available on the distribution of asthmatic patients sensitive to sulfiting agents according to age, sex, race, genetic traits, ethnicity, and other variables.

The Food and Drug Administration has received more than 300 letters reporting various types of adverse reactions occurring after consumption of foods containing sulfiting agents. A review of these reports has established that many are accounts submitted by individual consumers (both asthmatic and nonasthmatic persons), some are thorough case reports submitted by physicians, and some include investigations by FDA field investigators. These reports indicate an association of adverse reactions with ingestion of meals including foods containing sulfiting agents. Indeed, life-threatening reactions and deaths of four persons diagnosed as asthmatic individuals have been reported following ingestion of restaurant meals that included foods treated with sulfiting agents.

However, there are few data regarding hypersensitivity reactions, life-threatening events or other sequelae on the part of nonasthmatic individuals ingesting foods treated with sulfiting agents. Despite the availability of the clinical reports and limited data on asthmatic patients, the Panel can not draw rigorous scientific conclusions regarding the role of foods containing sulfiting agents in hypersensitivity reactions of nonasthmatic
individuals. Therefore, the Panel is basing its consideration of these reactions primarily on the published clinical studies and case reports of hypersensitivity reactions to sulfites by asthmatic patients. The analysis of the available published case reports of hypersensitivity reactions to sulfiting agents indicates to the ad hoc Review Panel that at least six different patterns of response have been described. These include:

1. Asthmatics who experience acute exacerbations of severe asthma after eating in restaurants. They respond with bronchoconstriction on oral challenge with 5-100 mg of metabisulfite.

2. Asthmatics with no clear-cut history of bronchial reactions following ingestion of sulfiting agents who respond with bronchoconstriction on oral challenge to 10-500 mg of metabisulfite.

3. Asthmatics with other allergic-type symptoms who have systemic anaphylactic reactions historically associated with ingestion of sulfites. These individuals respond with systemic symptoms after purposeful challenge with 10 mg of metabisulfite.

4. Asthmatics who have acute asthma after parenteral administration of pharmaceutical agents containing sulfiting agents. They may, in addition, also belong to classes 1-3 above. The acute bronchoconstriction has been reproduced in these individuals by oral challenge with from 50-500 mg of metabisulfite.

5. Nonasthmatics who may not necessarily have a history of other allergic-type reactions, but who have a history of anaphylaxis following ingestion of sulfiting agents. These individuals develop anaphylaxis after purposeful oral challenge with 10 mg of metabisulfite.
6. Normal subjects given challenges of inhaled sulfur dioxide (9-21 ppm) who develop bronchoconstriction with subsequent tachyphylaxis on repeated challenge. This is in contrast to the general asthmatic population who develop bronchoconstriction following inhalation of 1-5 ppm of sulfur dioxide.

As a result of the examination of the available data, questions have arisen regarding the prevalence of reactions to sulfiting agents used as food ingredients and the proportion of the susceptible population at risk of life-threatening reactions. While it is clear that challenge with sulfiting agents may induce acute allergic-type reactions in sensitive individuals, there is no scientific evidence to demonstrate that these same agents incorporated into foods, as they are usually ingested, can induce the same reactions. In addition it remains to be shown whether specific foods containing sulfites influence these reactions.

Short- and Long-Term Toxicity Studies

Early investigation of the chronic toxicity of sulfite was complicated by development of thiamin deficiency, resulting from the destruction of dietary thiamin by sulfite (Fitzhugh et al., 1946). In studies such as those of Cluzan et al. (1965) and Lockett and Natloff (1960), long-term administration of sulfites in drinking water (dosage ranges of 29-40 to 190 mg SO₂/kg body weight/day) was not associated with effects on feed consumption, growth rate, reproduction, hematology, organ weight and histology, or mortality. In the 1976 evaluation of the health aspects of sulfiting agents as food ingredients, the Select Committee considered the thorough investigation on ingestion of sulfites in thiamin-supplemented diets (Til et al., 1972a,b) as substantive evidence for levels of sulfiting agents required to produce chronic toxic effects in rats and pigs. In pigs fed diets containing sodium metabisulfite for 48 weeks, the no observed adverse effect level (NOAEL) was 3500 ppm, corresponding to dosage ranges of 42 to 179 mg SDE/kg body weight/day. In rats, the NOAEL for sodium metabisulfite was 2150 ppm, equivalent to an intake of 72 mg SO₂/kg body weight/day. Based upon the data reported by Til (1972a), the Joint FAO/WHO Expert Committee on Food Additives (1974) established an acceptable daily intake (ADI) of 0.7 mg SDE/kg body weight.

Few reports were found of short- or long-term studies of sulfiting agents published after 1976. Gastric lesions were reported in several chronic feeding studies (Feron and Wensvoort, 1972; Til et al., 1972a,b). The types of gastric lesions induced in Cpb:Wu (Wistar Random) rats by feeding of sodium metabisulfite
were examined by enzyme histochemistry and light and electron microscopy (Beems et al., 1982). Animals were fed thiamin-supplemented diets containing 0, 4, and 6% sodium metabisulfite for 8 or 12 weeks in a subchronic study or 0 and 6% sodium metabisulfite for 4, 7, 14, 21, or 28 days in a time-sequence study. Fundic mucosa of the rats fed sodium metabisulfite contained scattered hyperplastic glands lined with enlarged hyperactive gastric chief cells containing large numbers of pepsinogen granules, but no fat, glycogen, or mucus. Findings from the time-sequence study suggested that preexisting chief cells were transformed to hyperactive chief cells having proliferative capabilities. While it is considered unusual that chief cells of this type should be so involved rather than mucous cells, the pathogenesis of the lesions remains to be resolved (Beems et al., 1982).

Subchronic toxicity of sulfite was examined in female rats with low levels of sulfite oxidase activity induced by feeding of low molybdenum diets containing tungstate for 9 weeks (Gunnison et al., 1981a). Beginning on day 21, drinking water for two groups of animals also included 25 or 50 mM SO$_3^{2-}$. This treatment resulted in levels of hepatic sulfite oxidase activity 1/100 that of untreated rats and 1/10 that of normal humans. The authors reported that the general health of the rats appeared normal and differences in weight gains and organ weights of pregnant and nonpregnant animals were not correlated with exogenous sulfite levels. Nonpregnant animals given sulfite did not develop anemia and hematologic measurements in pregnant animals showed changes normally associated with pregnancy. Erythrocyte glutathione concentrations and prothrombin times were not affected and hepatic thiamin concentrations were not significantly reduced by administration of sulfite to rats with low levels of sulfite oxidase activity. S-Sulfonate concentrations in aorta, pinna, and plasma were elevated in animals with low levels of sulfite oxidase activity. Administration of sulfite produced additional increments in S-sulfonate levels in aorta. Trends appeared similar, but data were incomplete for pinna and plasma concentrations of S-sulfonates.

The Select Committee (SCOGS, 1976) concluded that "the level of sulfite that produces no observed toxic effects varies from about 20 to 100 or more mg of SO$_2$ per kg body weight per day, depending on the species and experimental conditions." Upon review of the information available to the Select Committee and in the absence of additional data conflicting with the reports considered by the group, the ad hoc Review Panel concurs with the conclusion of the Select Committee regarding the no observed adverse effects level of sulfiting agents for chronic toxic effects. Moreover, it is evident from examination of experimental protocols that the no observed adverse chronic effect level is probably nearer the top than the bottom of the range.
Teratogenicity

Reproductive performance was studied in female Wister-derived rats with induced sulfite oxidase deficiency and exposed to 25 or 50 mM sulfite (160 or 280 mg/kg/day) as sodium metabisulfite in drinking water from 3 weeks prior to mating until day 20 of gestation (Dulak et al., 1984). No treatment related trends were reported in reproductive performance or malformations after exposure to sulfite of animals having deficient or normal levels of sulfite oxidase activity.

Inhalation of sulfur dioxide in filtered room air for 7 hours/day by CF-1 mice (25 ppm on days 6 through 15 of gestation) and New Zealand rabbits (70 ppm on days 6 through 18 of gestation) resulted in no evidence of maternal toxicity except for decreased food consumption by both species during the first few days of exposure (Murray et al., 1979). An increase in minor skeletal variants in both species was associated with maternal exposure to sulfur dioxide, but no teratogenic effects were observed in either species (Murray et al., 1979).

It is the opinion of the ad hoc Review Panel that these data in addition to the studies considered by the Select Committee do not provide evidence of teratogenic effects of sulfiting agents.

Mutagenicity and Carcinogenicity

It was reported that bisulfite (10 mM; pH not specified) induced mutations in Micrococcus aureus and that bisulfite (5 mM) induced mutations at pH 3.6 but not 5.5 in Saccharomyces cerevisiae (Shapiro, 1983). Bisulfite (0.1 M) did not cause mutations in Escherichia coli (Mallon and Rossman, 1981). In a Bacillus subtilis test system, concentrations of sulfur dioxide similar to those found in wines (150 ppm, pH 3.0 to 6.5) did not elicit any mutagenic activity (Khoudokormoff and Gist-Brocdes, 1978). Also in a B. subtilis system higher concentrations of a sodium sulfite-bisulfite mixture (0.1 M to 0.5 M; pH 7) showed mutagenic effects; whereas a lower concentration of this mixture (0.05 M; pH 7) caused no mutagenic activity. Cells treated with adducts of sodium bisulfite and cytidine monophosphate or uridine monophosphate demonstrated mutagenic effects at concentrations of 0.25 M and 0.5 M (Chang and Chung, 1977).

Mutagenic effects have also been reported in plant cells, specifically in barley seeds treated with bisulfite (7–10 mM) (Kak and Kaul, 1979). Uptake of $^3$H-thymidine was inhibited in Vicia faba root meristems in the presence of 1000 ppm sodium sulfite (Njagi and Gopalan, 1982). Toxic levels (greater than 5000 ppm)
caused excessive chromosomal condensation that resulted in breakage of anaphase bridges or their fusion; however, lower concentrations (5 to 100 ppm) produced no mutagenic effects in the meristem system.

Transformation of Syrian hamster embryo cells treated with 1, 5, or 10 mM bisulfite for 24 hours at neutral pH was increased in a dose-dependent fashion (DiPaolo et al., 1981); however, the authors suggested this transformation might not occur by a mutagenic mechanism because bisulfite in combination with UV irradiation did not synergistically increase transformation. Further work in this system indicated that bisulfite caused no detectable DNA damage and may have decreased the rate of DNA replication per cell by blocking operation of part of the functioning replicons (Doniger et al., 1982). In Chinese hamster ovary cells, dose- and time-dependent inductions of sister chromatid exchange were shown following exposure to 0.08 mM to 7.3 mM concentrations of bisulfite for 2 or 24 hours (McRae and Stich, 1979). In contrast, Chinese hamster cells (V79) exposed for 15 minutes to 10 and 20 mM bisulfite, exhibited no mutations to ouabain resistance (Mallon and Rossman, 1981). Likewise, exposure for 15 minutes to 10 mM bisulfite produced no mutations to 6-thioguanine resistance. Long-term exposure of Chinese hamster V79 cells (exposed continually and recultured for 8 weeks in complete growth medium containing 5 mM bisulfite) also failed to induce ouabain resistant mutations (Mallon and Rossman, 1981). Cultures of lymphocytes from human peripheral blood exhibited chromosomal abnormalities (clumping) and decreases in DNA synthesis, cell growth and mitotic indices after a single exposure to 100 ml of 5.7 ppm sulfur dioxide in air on days 0 or 1 of incubation but not on days 2 or 3 (Schneider and Calkins, 1970).

While chromosomal aberrations have been observed in response to sulfites in in vitro systems, mutagenic effects have not been reported in intact animals exposed to sulfur dioxide or sulfites. Dominant-lethal mutations were not increased in 10- to 12-week-old (C3H x 101) F₁ female mice given one intraperitoneal injection of 550 mg/kg sodium bisulfite and mated with untreated (101 x C3H) F₁ males within 4.5 days of treatment (Generoso et al., 1978). In the same study neither heritable translocations nor dominant-lethal mutations were detected when (101 x C3H) F₁ male mice were mated with (C3H x C57BL) F₁ females after intraperitoneal injections of 400 mg/kg sodium bisulfite 20 times during a 26-day period or with 300 mg/kg 38 times during a 54-day period. Chromosomal aberrations were not found in oocytes of female Camm mice given one intravenous injection of 1.0, 2.5, or 5.0 mg sodium sulfite although structural damage was reported during meiosis when cultures of Camm mouse oocytes were treated with sodium sulfite (Jagiello et al., 1975). The influence of low levels of hepatic sulfite oxidase activity on induction of cytogenetic effects was recently studied in Chinese hamsters and
NMRI-mice made sulfite oxidase-deficient by feeding of a low molybdenum diet plus administration of sodium tungstate in drinking water (Renner and Wever, 1983). No cytogenetic effects (measured by sister chromatid exchange, chromosomal aberration, and micronucleus tests) were induced in bone marrow cells of either species in response to subcutaneous or intraperitoneal injection or oral administration of sodium metabisulfite solution, although the authors noted that control animals tolerated higher doses of sulfite than those made sulfite oxidase-deficient (Renner and Wever, 1983).

Carcinogenic potential of potassium metabisulfite and sulfur dioxide has also been examined. Male and female ICR/JCL mice administered 1 and 2% potassium metabisulfite in drinking water for 24 months had no increased incidence of tumors over control animals, suggesting that potassium metabisulfite is not carcinogenic in mice (Tanaka et al., 1979). Exposure to sulfur dioxide by inhalation was associated with an approximate doubling of lung tumors, but no increases in hepatomas or lymphoid tumors (Peacock and Spence, 1967). The increased frequency of lung tumors was not statistically significant. Inhalation of sulfur dioxide in combination with benz(a)pyrene resulted in an increased incidence of tumors in rats over exposure to either substance alone; rats exposed only to sulfur dioxide had no tumors at the end of the experimental period (Laskin et al., 1976).

An unexpected finding of mammary adenocarcinomas after 9 weeks of treatment was reported by Gunnison et al. (1981) in 4 of 149 female rats (two pregnant, two nonpregnant) with low activity of sulfite oxidase induced by feeding of a low molybdenum diet containing tungstate; no tumors were found in rats with normal levels of sulfite oxidase activity. The difference was not statistically significant.

Effects on peripheral blood cells of workers exposed to sulfur dioxide were examined in two Swedish studies. Nordenson (1980) observed an increase in chromosomal aberrations (mostly gaps and chromatid-type breaks) in 7 of 19 workers who were exposed to other inhalants as well as in a sulfite pulp factory. However, peripheral blood lymphocytes of eight workers whose individual mean daily exposure to sulfur dioxide in an aluminum foundry was estimated to be 1.0 ± 0.85 ppm had no greater incidence of chromosomal aberrations or sister chromatid exchange than eight age-control subjects (Sorsa et al., 1982). Average daily sulfur dioxide exposures in the aluminum foundry were estimated to range from 0.2 to 3.0 ppm and mean employment time for the exposed workers was 19.5 years (Sorsa et al., 1982).

Possible synergistic effects of sulfur dioxide and sulfites with other compounds have been examined in an effort to determine whether sulfiting agents might act as comutagens or cocarcinogens. Mutation frequency was approximately doubled in
UV-irradiated Chinese hamster V79 cells exposed to 10 mM bisulfite either during or after irradiation exposure and tryptophan rever-
tants were increased by more than eight-fold in UV-treated E. coli
cells exposed to 75 mM bisulfite (Mallon and Rossman, 1981). Trea-
tment of bacteriophage-λ with bisulfite-amine mixtures (1 M
bisulfite plus 1 M semicarbazide, hydrazine, methoxyamine, or
hydroxylamine) produced increases in clear mutation (plaque-
forming activity) over treatment with bisulfite alone (Hayatsu
and Kitajö, 1977). Combination of bisulfite (150 ppm) with nitrite
(100, 200, or 400 ppm) was reported to produce a weak mutagenic
effect after 2 weeks in B. subtilis (Khoukorkoff and
Gist-Brocades, 1978). Mutagenic effects of coffee on Salmonella
typhiurium strains TA 100 and TA 98 without S9 mix were com-
pletely inhibited by addition of 300 ppm sulfiting agents
(sulfite, bisulfite, or metabisulfite) and phage-inducing activity
of coffee (prophage-λ induction test) was suppressed by 300 ppm
sulfite ion (Suwa et al., 1982). Sodium sulfite was also a weak
inhibitor of mutagenic effects induced by benz(a)pyrene in S.
typhiurium strain TA 98 (Calle and Sullivan, 1982). Bisulfite
concentrations relevant to use in foods (0.5, 2.5, and 5.0 μg/ml)
inhibited transformation of C3H 10T 1/2 cells by x-rays and
benz(a)pyrene; pretreatment of hamster embryo cells with 100 ppm
bisulfite inhibited transformation by x-rays (Borek, 1984).

Upon consideration of these studies as well as the data
available to the Select Committee in 1976, the ad hoc Review Panel
concludes that sulfites are mutagenic to several microorganisms
and plants, and may produce chromosome damage to mammalian cells
in vitro. However, sulfites also inhibit mutagenic effects of
some known mutagens and carcinogens. Sulfites are not carcino-
ogenic or mutagenic in vivo to rats and mice.
V. OPINION

An accurate measure of the poundage of sulfites currently being used and the trend in the use of sulfiting agents in food since 1975 cannot be made from information now available. Steps need to be taken to obtain these data.

The problems involved in making a precise estimate of per capita daily consumption have been elaborated earlier. Taking into consideration all relevant factors, the Panel has estimated that at the present time the mean per capita daily consumption of sulfur dioxide equivalents (SDE) from food, wine, and beer is 10 mg/day which is about the same as the intake estimate in the Select Committee's 1976 report. It has been estimated further that 99th percentile consumption, reflecting that of the most frequent consumers of the more highly sulfited foods, is no more than 180 mg/day. The Panel believes, however, that determination of the level of consumption of sulfiting agents should be based on direct analyses of foods as they are prepared for consumption. Analytical methods will need to be improved to measure both free and total sulfite as presently defined and also to measure the level of irreversibly bound sulfite compounds remaining in food that may possibly induce adverse reactions when ingested. The firm data obtained in this way for sulfite-treated foods would obviate the need to make approximations based on the amount added to food and presumptions concerning the amount lost or reacted in processing, storage, and preparation for the table.

The sulfite generated endogenously and the metabolism of both exogenous and endogenous sulfite needs to be considered. The amount of endogenous sulfite is estimated to be generally several orders of magnitude greater than that from exogenous sources. Virtually all of the sulfite is metabolized by sulfite oxidase in the mitochondria of various organs and tissues and the rate is probably diffusion limited. Thus, a surge of sulfite that could occur from an exogenous source may have a different effect than the same amount generated normally in the body or introduced over a longer period of time.

On the basis of chronic toxicity tests in animals, excluding tests for allergy and hypersensitivity in humans, the no observed adverse effect level (NOAEL) of the sulfiting agents is estimated to be in the range of 30-100 mg SDE/kg/day. The margin between the amount of SDE ingested by high intake consumers and the lowest estimated NOAEL is 10-fold and between the mean per capita daily consumption and the lowest NOAEL 180-fold. There is no evidence, including that in recent scientific literature, to suggest that sulfiting agents are teratogenic, mutagenic, or carcinogenic. However, there is some information in the current literature that suggests that two aspects of the metabolism of sulfites require additional study: 1) there is an unknown number
of individuals with reduced levels of activity of sulfite oxidase whose slower metabolism of sulfite may, but not necessarily will, subject them to adverse effects at current levels of intake; and 2) since in laboratory animal studies sulfiting agents are mixed with the diet or added to the drinking water, there is minimal opportunity for reaction between sulfite and food ingredients such as occurs regularly in foods during processing and preparation for human consumption and, as a result, reported chronic toxicity studies reveal little about the possible effects of the irreversibly bound sulfites that can be present in sulfited foods. These potential physiologically relevant phenomena need to be evaluated.

There is accumulating evidence that certain individuals, particularly but not exclusively asthmatics, may be hypersensitive to sulfite, experiencing acute allergic-type reactions following doses that are apparently considerably smaller than the lowest chronic NOAEL noted above. The prevalence of sulfite sensitivity is unknown in both the asthmatic and nonasthmatic population and it is not known whether reactors will react to sulfites contained in food as well as to challenge with sulfites themselves. However, this type of adverse reaction must be assumed to be real at this stage of our limited knowledge. While it is clear that the vast majority of individuals is not comparably sensitive, appropriate steps need to be taken to alleviate the problem for those who are. Clear and prominent labeling and restaurant notices where sulfited foods are being served and labeling of foods in the market place would be steps in that direction, enabling those who are sensitive to sulfites to avoid foods that contain them.

Answers to the following questions are needed: 1) while challenge with sulfiting agents may induce acute allergic-type reactions in sensitive individuals, do sulfited foods, as they are usually ingested, induce the same reactions; 2) does the nature of the food in a meal containing sulfiting agents influence the reactions of sensitive individuals; 3) what is the prevalence of allergic-type reactions to sulfiting agents and what proportion of the susceptible population is at risk of life-threatening reactions?

Such information may lead to alternative solutions to the sulfite sensitivity problem.

In view of the foregoing, the ad hoc Panel concludes that:

While no evidence in the available information on potassium bisulfite, potassium metabisulfite, sodium bisulfite, sodium metabisulfite, sodium sulfite, and sulfur dioxide demonstrates a hazard to those who are not hypersensitive to these substances when they are used at levels now current and in the manner now practiced, uncertainties exist requiring that additional studies be conducted.
Pending completion of these additional studies, appropriate identification of foods containing sulfiting agents should be instituted to enable those sensitive to these substances to avoid them.
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APPENDIX*

Evaluation Statements Used by the Select Committee on GRAS Substances

In reaching its conclusions, the Select committee on GRAS Substances relied primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. A conclusion, based on reasoned judgment, was expected even in instances where the available information was qualitatively or quantitatively limited. It was recognized at the outset that there needed to be agreement on the language of the conclusions so that there would be minimal opportunity for misunderstanding the Committee's intent when translating evaluation statements into appropriate regulatory action.

Accordingly, four conclusion statements were agreed upon as likely to cover all cases. However, in utilizing the four conclusions, it was soon recognized that there were instances where there were insufficient data upon which to base a conclusion, making it necessary to add a fifth statement. With very few exceptions, the Committee found that its views could be expressed in one or another of the following five conclusion statements:

1. There is no evidence in the available information on _______ that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future.

2. There is no evidence in the available information on _______ that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, it is not possible to determine without additional data, whether a significant increase in consumption would constitute a dietary hazard.

* From SCOGS (1982), with permission.
3. While no evidence in the available information on ________ demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.

4. The evidence on ________ is insufficient to determine that the adverse effects reported are not deleterious to the public health when it is used at levels that are now current and in the manner now practiced.

5. In view of the deficiency of relevant biological (and/or other) studies, the Select Committee has insufficient data upon which to base an evaluation of ________ when it is used as a food ingredient.