EVALUATION OF THE HEALTH ASPECTS OF SULFITING AGENTS
AS FOOD INGREDIENTS

1976

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D. C.

Contract No. FDA 223-75-2004

LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
9650 Rockville Pike
Bethesda, Maryland 20014
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NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee 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 are approved and transmitted to FDA by the Executive Director of FASEB.

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

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

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I. INTRODUCTION

This report concerns the health aspects of using sulfiting agents as food ingredients. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1972.* To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 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; recent literature searches by the Toxicology Information Response Center, Oakridge, Tennessee; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, an announcement was made in the Federal Register of January 7, 1977 (42 FR 1519 to 1521) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information and views on the health aspects of using sulfiting agents as food ingredients. The Select Committee received no requests for such a hearing on sulfiting agents.

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 121.1, revised April 1, 1976, that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. This section of the Code also indicates that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA recognizes further (21 CFR 121.3) that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document (PB-221 217/3) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Select Committee, 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. While the Select Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Select Committee, there are insufficient data upon which to base a conclusion. The Select Committee, aware that biological testing is dynamic, bases its conclusions on information now available; it cannot anticipate the results of experiments not yet conducted or those of tests that may be reconduted, using new technologies. These conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances 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.

II. BACKGROUND INFORMATION

The sulfiting agents used in food include sulfur dioxide and several inorganic sulfites that liberate sulfur dioxide under conditions of use. Such substances have been employed for centuries in food processing as sanitizing agents for food containers and fermentation equipment, as preservatives to reduce or prevent microbial spoilage of food, as selective inhibitors of undesirable microorganisms in the fermentation industries, and as antioxidants and inhibitors of enzyme-catalyzed oxidative discoloration and non-enzymic browning during preparation, storage, and distribution of many foods (2, 3).

The following sulfiting agents are listed in the Code of Federal Regulations (4) as chemical preservatives that are GRAS [21 CFR 121.101(d)(2)] provided that they are not used in meats or food recognized as a source of vitamin B\textsubscript{1}: potassium bisulfite (KHSO\textsubscript{3}), potassium metabisulfite (K\textsubscript{2}S\textsubscript{2}O\textsubscript{5}), sodium bisulfite (NaHSO\textsubscript{3}), sodium metabisulfite (Na\textsubscript{2}S\textsubscript{2}O\textsubscript{5}), sodium sulfite (Na\textsubscript{2}SO\textsubscript{3}), and sulfur dioxide (SO\textsubscript{2}). Potassium or sodium bisulfite, potassium or sodium metabisulfite, and sulfur dioxide are also regulated under 21 CFR 31.1(a)(10) of the Code as ingredients of nonalcoholic beverages; and sodium sulfite and sodium metabisulfite are regulated under 21 CFR 121.1088 as boiler water additives.
Food Chemicals Codex specifications for the food grades of four of these compounds are given in Table I (5). The Codex provides no specifications for food grade potassium bisulfite or sulfur dioxide.

### TABLE I

**Specifications for Food Grade Sulfites (5)**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Assay</th>
<th>Arsenic</th>
<th>Heavy metals (as lead)</th>
<th>Iron</th>
<th>Selenium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium metabisulfite</td>
<td>K₂S₂O₅</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>Sodium bisulfite</td>
<td>58.5 and 67.4% SO₂</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>Sodium metabisulfite</td>
<td>Na₂S₂O₅</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>Sodium sulfite</td>
<td>Na₂SO₃</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
</tbody>
</table>

*≥10 ppm of Pb

Choice among the various sulfiting agents depends in large measure upon the stability required for the intended use. The sulfites tend to oxidize during storage, thus decreasing the amount of available sulfur dioxide; the metabisulfites are more stable than the bisulfites which are, in turn, more stable than the sulfites. The functional effects of the sulfiting agents are related to their sulfur dioxide content. When dissolved in water the sulfites form sulfurous acid and sulfite and bisulfite ions, the relative proportions of each depending upon the pH of the solution (3). In much of the published literature, concentrations of the sulfiting agents are given in terms of sulfur dioxide equivalents; for this reason such equivalents have often been calculated for the amounts of the various sulfiting agents referred to in this report.

It is to be noted that because sulfur dioxide is volatile and, like the sulfites, readily oxidized to sulfate, the residual amount of available sulfur dioxide in foods as consumed is less than would be expected on the basis of the amount added during processing. Moreover, good manufacturing practice limits the residual amount in foods to less than 500 ppm (0.05 percent) since 500 ppm of sulfur dioxide is the level which can be taste-detected by most consumers (3).
The years in which sulfiting agents were first used in food by the processors responding in a recent survey (6) are: sulfur dioxide, 1908; sodium bisulfite, 1921; sodium sulfite, 1930; potassium and sodium meta-
bianulfites, 1939. Between 1960 and 1970 there was a 30 to 70 percent
increase (6) in the total amounts of the several sulfiting agents used annually
in the United States, representing a greater increase than would be accounted
for by the population increase of about 12 percent that occurred in this same
period.

III. CONSUMER EXPOSURE DATA

In 1970, a subcommittee of the National Research Council (NRC) sur-
veyed manufacturers concerning the addition of GRAS substances, including
the sulfiting agents, to foods and estimated the possible daily intake of these
substances within various age groups (6). We have since been informed by
the subcommittee* that they have recently reexamined their published data
on the sulfiting agents and do not now regard their intake estimates for these
compounds as typical. Accordingly, alternative data to permit estimation
of daily human intakes have been sought.

Several considerations are to be noted at the outset. While sulfiting
agents are used in relatively low concentrations (about 0.001 to 0.01 percent)
during processing of a number of food products, they are used in higher con-
centrations during the processing of fruits, vegetables, juices, and drinks
other than those that are canned which are seldom sulfited (2). Of these
products, dehydrated, light-colored fruits such as apples, apricots,
bleached raisins, pears, and peaches initially contain the greatest amounts
(up to about 3000 ppm or 0.3 percent SO₂ equivalent on a dry weight basis);
dehydrated vegetables and soup mixes may contain up to 2000 ppm; instant
potatoes may average about 400 ppm (7).

It should be emphasized, however, that such figures as those given
above represent estimates of the sulfur dioxide equivalents added as foods
are processed or present initially in the processed food. Losses through
volatilization can and do occur during processing, in subsequent storage, and
in home preparation. Oxidation of sulfite to sulfate can also occur at all
stages and sulfite can react with a number of food ingredients. All of these
factors tend to lower the amount of available sulfite in foods as eaten.

---

*Letter, dated June 11, 1976, to G.W. Irving, Jr., from D.F.
Dodgen, Food Chemicals Codex, National Academy of Sciences,
National Research Council, Washington, D.C.
Few data are available concerning the extent of this reduction but a recent study of foods in Israel indicates that the reduction is substantial (8). The foods consumed per person daily had 30 mg of SO\(_2\) equivalents added during processing, while actual analysis of these foods showed that only about 9 mg of SO\(_2\) equivalent were present in the foods as eaten. Salem and Hegazi (9) treated the juice of cooked apricots with 1 percent of sodium metabisulfite, sun dried it for three days, and measured the SO\(_2\) content of the dried juice before and after storage for six months. The SO\(_2\) content of the juice was about 6,700 ppm before drying and was 1,625 ppm after drying; after six months' storage it was 1,280 ppm. Studies by Burroughs and Sparks (10, 11) demonstrated that part of the disappearance of available SO\(_2\) in foods processed with sulfites can be accounted for by the formation of adducts with carbonyl compounds such as ketones and aldehydes, naturally present in foods. Wine exposed to atmospheres containing successively increasing concentrations of SO\(_2\) binds increasing amounts of SO\(_2\); at the point of maximum binding, the wine contained 250 ppm of bound SO\(_2\). Closely similar behavior was exhibited by a "model wine" consisting of a solution of several carbonyl compounds in the concentrations that their analytical and isolation studies had shown to be present in unsulfited wine. Evidence is not available concerning the extent to which the sulfite adducts of carbonyl compounds dissociate in foods or after foods are consumed.

An expert panel of the Institute of Food Technologists (7), studied sulfites as food additives and pointed out that estimates of "average" daily sulfur dioxide intake are relatively meaningless because of the wide variation in food and beverage preferences. Nevertheless, the panel indicated that the most commonly used figure for per capita daily intake from solid foods and nonalcoholic beverages in the U.S. is approximately 2 mg of SO\(_2\) equivalent, and estimated that U.S. wine and beer consumption figures for 1971 correspond to an additional 5 mg of SO\(_2\) per capita, assuming that these beverages are consumed by 75 percent of the adult population. The panel regarded it as probable that the bulk of the U.S. population consumes no more than 10 to 15 mg SO\(_2\) daily (about 0.2 mg per kg body weight for a 60 kg man), although some individuals may consume as much as 120 mg or more per day, amounting to about 2+ mg per kg body weight. Similar estimates have been made for the food intake of sulfur dioxide in Belgium (12, 13).

Lishmund in 1969 (14) estimated about 74 mg SO\(_2\) equivalent as the potential daily intake from certain major foods and beverages in England, amounting to about 1.2 mg per kg body weight per day. He recognized that his figure did not take into consideration intake from several food sources for which he did not have consumption data. However, his data included such food and beverage items as dehydrated fruits and vegetables and wine that are known to be among those whose content of sulfiting agents tends to be relatively high.
The Codex Alimentarius Commission has established for man a potential daily intake (PDI) of sulfiting agents as SO₂ of 2.1 mg per kg body weight (15). The Joint FAO/WHO Expert Committee on Food Additives established an acceptable daily intake (ADI) of 0.7 mg per kg (16); their earlier established ADI was 0.35 mg per kg (17).

It is reasonable to conclude, therefore, that the daily intake of SO₂ via sulfited foods consumed in the United States does not exceed 2 mg per kg body weight for adults, except in unusual circumstances, and probably is no more than 0.2 mg per kg for the bulk of the adult population. There is no reason to suspect from the data available that SO₂ intakes of infants and children will exceed 0.2 mg per kg daily. The Select Committee agrees, however, with the Codex Committee on Food Additives (15) that actual analysis of foods as consumed by various age groups is desirable if more accurate estimates of daily SO₂ intakes are to be obtained.

IV. BIOLOGICAL STUDIES

Metabolism

The primary detoxication mechanism for sulfite in rats, and presumably in other mammalian species, is by oxidation to sulfate; only a small proportion is converted to organic sulfate (18). In four hours, 55 percent of the sulfur of intubated sodium metabisulfite solution (equivalent to about 1.0 g SO₂ per kg body weight) was recovered as inorganic sulfate in the urine of rats (18). In dogs, 80 mg of sodium bisulfite per kg per hour (corresponding to 49 mg SO₂ per kg per hour) infused for three hours into the splenic vein was completely metabolized to sulfate (19). In rats, mice, and monkeys 70 to 95 percent of the ³⁵S of ingested Na₂⁻³⁵SO₃ (10 to 50 mg SO₂ per kg body weight) was absorbed from the intestine and voided in the urine of all three species within 24 hours (20). Most of the remaining ³⁵S was eliminated in the feces; 2 percent or less was found in the carcass. No free sulfite was detected in the urine of rats even after a single oral dose of sodium bisulfite (400 mg SO₂ per kg) indicating a large capacity to rapidly oxidize sulfite.

A sulfite oxidase has been isolated from the livers of rats, dogs, and cattle (21, 22). Tissue distribution of the enzyme in the rat shows highest activity in liver, heart, and kidney, with lower activity in several other tissues, and very low activity in lung. It is not known whether human tissues show a similar distribution of sulfite oxidase activity. A congenital deficiency of hepatic sulfite oxidase has recently been described in man (23). In this rare hereditary metabolic disorder, S-sulfocysteine is excreted in the urine. It is noteworthy that Olney et al. (24) have found this compound [COOH-CH(NH₂) CH₂-S-SO₃H] to produce lesions in the retinas and arcuate nuclei of the hypothalamus in 5-day-old rats injected subcutaneously at levels of 80 to 800 mg per kg body weight, but not at a level of 8 mg per kg; similar brain damage also occurred in adult rats injected intracerebrally at a level of 30 mg per kg.
It is possible, based on studies in rabbits, that the small portion of oral sulfite that may not be immediately oxidized could react with disulfide bonds of plasma proteins to form S-sulfonates in the plasma (25, 26). The authors theorize that the formation of plasma S-sulfonates may protect the tissues from exposure to sulfite, but because the chemical reaction is reversible, these products might also serve as carrier forms of sulfite to release it in the tissues at a later time. The action of sulfite on proteins and peptides to produce S-sulfonate derivatives has been reviewed by Meister (27). Such thiosulfate esters are converted to sulfite by reaction with glutathione (28). This conversion is catalyzed reversibly by an enzyme in rat liver for glutathione disulfide and cystine but not for disulfide groups in proteins. S-sulfocysteine is converted to thiosulfate, pyruvate, and ammonia when fed to rats (29).

Sulfites can produce direct effects on tissues presumably as a consequence of disturbance of acid-base balance. Rost and Franz (30) gave dogs up to 1.0 g of sulfite per day for more than a year without evidence of gross or microscopic tissue changes. Larger doses caused vomiting but no other symptoms appeared. Humans receiving 1.0 g of sodium sulfite per day (about 17 mg per kg) had no gastrointestinal symptoms but abdominal pain and vomiting occurred at doses of 4 to 5.8 g per day (about 70 to 100 mg per kg). Lafontaine and Goblot (31) found doses of sulfite in excess of 3.5 mg per kg to induce gastrointestinal irritation leading to vomiting in man and suggested that the vomiting reflex protects against acute toxicity.

Enzymes inhibited by sulfites in vitro include a number that require nicotinamide adenine dinucleotide (NAD) or pyridoxal as cofactors (32, 33), peroxidase (34), and acetylcholine esterase (35); alkaline phosphatase was inhibited in vivo (36). Bisulfite in vitro in excess (118 mg of sodium bisulfite to 5 mg of yeast ribonucleate) has been shown to react over a period of several hours with some nucleic acid components to alter their structure; for example, the deamination of cytosine to uracil (37, 38). However, treatment of calf thymus DNA with excess of bisulfite under similar conditions, even for 72 hours, resulted in no deamination of cytosine (39). Such reactions have not been demonstrated to occur in vivo. However, the authors suggest their findings imply that the mutagenic properties of bisulfite (see p. 12 of this report) are due to its reaction with single-stranded DNA.

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 organs of the teeth (40). Such evidence appeared only at levels of 500 mg of sodium sulfite per kg body weight and higher.

Interpretation of the biological effects of sulfiting agents in many experimental studies is difficult because sulfite reacts with thiamine (41).
Thus, some of the observed effects attributed to sulfiting agents may well be due to the destruction of thiamine and the resulting avitaminosis, rather than to direct action of sulfiting agents on the tissues. The relationship between thiamine content of the food and sulfite content has been studied by Wilmes (42) in rats, and by several others (43-48) in laboratory animals and man. Typically, the animal experiments showed that sulfite was toxic at a level of 50 mg SO₂ per kg to animals on diets deficient in thiamine but when adequate thiamine levels were maintained, animals survived 300 mg of sulfite per kg per day without significant influence on weight or food utilization (44). However, a study by Bhagat and Lockett (49) indicates that some stored sulfited diets may be toxic to rats. Rat diet containing 0.6 percent solid sodium metabisulfite (about 400 mg SO₂ per kg body weight) did not support normal rate of growth when fed as freshly prepared, but normal growth rate was restored by addition of thiamine. When the sulfited diet was stored at room temperature for 75 days or more before feeding, reduced growth rate occurred and normal rate was not restored by addition of thiamine. The authors offered no explanation of this effect.

An experiment involving 12 human volunteers (6 female, 6 male) was particularly significant with respect to evaluating sulfite tolerance (43). After a normal diet for 15 days, all subjects were placed on a thiamine deficient diet (120 μg thiamine per 1000 kcal) for another 15 days. Six of the subjects then received, dissolved in beverages, 400 mg of SO₂ per day (50 mg as sodium bisulfite, 350 mg as sodium glucose sulphonate) for 25 days. Six of the subjects received the same beverages without added SO₂ for 25 days. Sulfite administration was then discontinued for 10 days and all 12 subjects were given 100 mg of thiamine orally each day for two days. All volunteers were examined clinically, including neurophysiological examination of motor conduction and reflex action, before, during, and after the experiment and no clinical changes could be detected. Tests for activity of a number of enzymes, and measurements such as serum electrophoresis, thymol turbidity, hematocrit, and erythrocyte count were conducted and the data exhibited no sign of any disturbance caused by sulfite.

It has been shown by Thomas and Berryman (50) that the usual acceptable amounts of sulfite present in foods such as dehydrated fruits and vegetables which are normally sulfited, do not cause significant destruction of the thiamine content of a mixed meal including meat and other sources of thiamine. It should be noted that the addition of sulfiting agents to meat or other foods recognized as substantial sources of dietary thiamine is prohibited by regulation (4).

Acute and chronic oral toxicity

Acute LD₅₀ values for sulfiting agents by several routes of administration and in several species are given in Table II.
### TABLE II

**LD$_{50}$ of Sulfiting Agents**

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Chemical</th>
<th>LD$_{50}$</th>
<th>SO$_2$ equiv.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg/kg</td>
<td>mg/kg</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>i.p.</td>
<td>Sodium bisulfite</td>
<td>675</td>
<td>416</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>Sodium bisulfite</td>
<td>130</td>
<td>80</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>Sodium sulfite</td>
<td>155</td>
<td>79</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>Sodium sulfite</td>
<td>175</td>
<td>89</td>
<td>51</td>
</tr>
<tr>
<td>Rat</td>
<td>p.o.</td>
<td>Sulfur dioxide$^a$</td>
<td>1040</td>
<td>1040</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>Sulfur dioxide$^b$</td>
<td>2000</td>
<td>2000</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>Sodium bisulfite</td>
<td>650</td>
<td>400</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>Sodium bisulfite</td>
<td>115</td>
<td>71</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>Potassium metabisulfite</td>
<td>1800</td>
<td>1037</td>
<td>53</td>
</tr>
<tr>
<td>Rabbit</td>
<td>i.p.</td>
<td>Sodium bisulfite</td>
<td>300</td>
<td>185</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>Sodium bisulfite</td>
<td>65</td>
<td>40</td>
<td>51</td>
</tr>
<tr>
<td>Hamster</td>
<td>i.v.</td>
<td>Sodium bisulfite</td>
<td>95</td>
<td>58</td>
<td>51</td>
</tr>
<tr>
<td>Dog</td>
<td>i.p.</td>
<td>Sodium bisulfite</td>
<td>244</td>
<td>150</td>
<td>19</td>
</tr>
</tbody>
</table>

$^a$As a 6.5 percent aqueous solution.

$^b$As a 3.5 percent aqueous solution.

Sodium bisulfite fed to groups of rats for a year at various levels (0.0125 to 2.0 percent of the diet) led to the following conclusions: on diets containing less than 0.1 percent bisulfite no significant effect on growth occurred; on diets containing more than 0.1 percent bisulfite, growth rate and animal size decreased as SO$_2$ concentration increased; on diets containing 0.25 percent bisulfite or more, pathological changes observed were polyneuritis, bleached incisors, visceral organ atrophy, renal tubular casts, bone marrow atrophy, stunting of growth and spectacle eyes; the level of bisulfite at which histological changes began to appear was estimated to be about 0.1 percent (about 62 mg SO$_2$ per kg body weight)(54).

It is to be noted that while no toxicological studies of sulfite adducts of carbonyl compounds have come to the attention of the Select Committee, experiments in humans receiving 350 mg of sodium glucose sulfonate per day for 25 days revealed no sign of any disturbance caused by this compound (43).
Long-term studies

One-month-old male and female Wistar rats were given potassium metabisulfite in drinking water (700 mg SO₂ equivalent per liter) for 20 months. The dosage level of the metabisulfite was 190 mg SO₂ per kg body weight during the first 15 days, 58 to 78 mg SO₂ per kg at the end of 60 days and 29 to 40 mg SO₂ per kg at the end of 20 months. There were insignificant differences between the control and experimental animals in mortality, rate of growth, food consumption, clinical signs, and in the weight and histological appearance of heart, kidneys, adrenals, and gonads. Leucocyte count was slightly elevated in experimental males and splenomegaly occurred in some females. There were 19 and 10 percent reductions, respectively, in the reproduction rates in the F₁ and F₂ generations of the metabisulfite-treated F₀ females, and significantly fewer F₁ and F₂ males born in the treated group. There were no malformations in the young (55). Such reproductive effects were not observed by Lockett and Natoff (56) who gave sodium metabisulfite solution containing 750 ppm of SO₂ equivalent per liter to rats as drinking water over three generations. Daily dosage varied from 75 mg SO₂ per kg body weight to 170 mg per kg, depending on volume of solution consumed and animal weight. No differences were found in growth rates between the experimental and control animals. There were no effects on fertility, postnatal survival, lactation, behavior or general health. There was no significant difference in body weight or organ weight between test and control rats, and no carcinogenesis could be attributed to the SO₂. Gastric mucosal hyperplasia was not observed.

More recently, Til et al. (57) mixed sodium metabisulfite in the diet of rats (given added thiamine) and fed them for more than two years and more than three generations. They found the "no observed adverse effect" level to be 0.215 percent (equivalent to 72 mg SO₂ per kg body weight per day determined by analysis of the diet as fed). At a level of 2 percent metabisulfite there was slight growth retardation in the F₁ and F₂ generations. At levels of 1 percent or higher, occult blood was present in the feces, and hyperplastic changes were noted in the gastric mucosa, but there was no evidence of carcinoma. At high metabisulfite levels, 6 to 8 percent, the glandular portion of the stomach showed ulcers, papillomatous elevations, erosions, and inflammatory changes. Anemia occurred at 2 percent metabisulfite or above, and splenic enlargement with marked hematopoiesis took place with levels of 4 percent or above. No dose-related effects of metabisulfite on reproductive factors such as litter size, female fertility, birth weight or mortality of the young were found, but there was a significant reduction in the number of F₂a-generation offspring at sulfite levels of 0.5 percent or above.
The same authors (58) studied the effects of feeding sodium metabisulfite to pigs for up to 48 weeks. Diets were supplemented with extra thiamine to avoid deficiency of the vitamin. They found the no observed adverse effect level of sodium metabisulfite to be 0.35 percent (equivalent to about 120 mg SO$_2$ per kg body weight at the beginning of the experiment and about 28 mg per kg at the end); growth was decreased at the 0.83 percent level (due to lowered palatability of the diet as indicated by paired-feeding), and mild inflammatory and hyperplastic changes in the stomach were noted in several animals fed 0.83 or 1.72 percent sulfite. Except for some pigmentation of the cecal mucosa resembling pseudomelanosis coli, no histopathological changes were noted in any of the tissues.

Cattle (herd of 600) consuming daily silage containing 45 g of sodium metabisulfite (67 mg SO$_2$ per kg body weight per day) showed no adverse effects after more than five years of feeding. A cow in her third month of pregnancy receiving a dosage of about 100 mg SO$_2$ per kg per day for 180 days showed no adverse effects, calved normally, and the calf was normal (59). It should be noted, however, that experience with ruminants should not be equated with monogastric animals since the former appear to tolerate considerably larger amounts of sulfites (60).

From the foregoing acute and chronic administration studies it is evident that the level of sulfite that produces no observed toxic effects varies from about 30 to 100 or more mg of SO$_2$ per kg body weight per day, depending on the species and experimental conditions. This wide range may be related to the variable amounts of thiamine used in the experimental diets of reported studies. The FAO/WHO considers 35 mg SO$_2$ per kg body weight per day as the no observed adverse effect level in the rat (17).

Teratology

Teratologic evaluations of intubated sodium bisulfite, sodium metabisulfite, and potassium metabisulfite have been made in several species (61, 62). The compounds were administered daily on day 6 through day 15 of gestation in mice and rats; and day 6 through day 10 in hamsters. For sodium bisulfite (61) the doses in mg per kg body weight in mice, rats, and hamsters were up to 150, 110, and 120, respectively; for sodium metabisulfite (61) in mice, rats, and hamsters, up to 160, 110, and 120, respectively; for potassium metabisulfite (62) in mice and rats up to 125 and 155, respectively. In no instance were significant effects observed on nidation or on maternal or fetal survival. The number of abnormalities found in either the soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.
When sodium metabisulfite was injected in the air cell of fertilized eggs at 0 hour the calculated LD₅₀ (based on an average egg weight of 50 g) was 19.5 mg per kg, and when injected after 96 hours of incubation the LD₅₀ was 3.4 mg per kg. Injections into the yolk were less toxic; at 0 hour the LD₅₀ was 53 mg per kg, and at 96 hours, 162 mg per kg. Significantly elevated levels of abnormalities attributable to temporary growth retardation were noted, and a low level of structural anomalies involving the head and/or limbs was encountered (63). A second laboratory (64) tested potassium metabisulfite on chick embryos and found it to be quite embryotoxic; yolk treatment was more toxic than air cell treatment; the evidence was inconclusive with respect to teratological effects. A third laboratory (65) found sodium bisulfite to be toxic to chick embryos when injected into either the air cell or the yolk with greater toxicity following air cell administration. No significant teratogenic findings were reported. A fourth laboratory (66) found sodium sulfite to be toxic on air cell injection into eggs at 0 and 96 hours of incubation (LD₅₀ 20.7 and 16.7 mg per kg of egg, respectively) but not significantly toxic on yolk injection. Because there were no serious structural abnormalities compared to untreated or solvent treated controls, it was concluded that sodium sulfite is not teratogenic under these conditions.

Carcinogenicity and mutagenicity

When a great excess of sodium bisulfite is present, the deamination of cytosine to uracil in vitro in single-stranded DNA is optimal at pH 5 (37-39). At higher pH values the rate is considerably reduced, but the authors suggest the possibility that a potential hazard of sulfite acting as a mutagenic agent in vivo may exist based on this model chemical reaction in vitro. In at least four microorganisms (E. coli, γ phage, T₄ phage and a yeast) sulfite has been shown to be a mutagen (67-70), and the nature of the mutation follows the expected pattern predicted by the model chemical reaction. However, there is no evidence of sulfite causing mutations or cancer in mammals in vivo.

Sodium bisulfite was not mutagenic in the host-mediated assay in mice, the dominant lethal assay in rats, or the in vivo cytogenic assay in rats at doses up to 150 mg per kg; it showed no mutagenic activity on human tissue culture cells in vitro at levels up to 200 µg per ml (71). In similar tests conducted on sodium metabisulfite by another laboratory (72), no mutagenic activity was observed in the host-mediated, dominant lethal, or cytogenic assays, but mitotic inhibition and widespread damage to anaphase cells were noted when sodium metabisulfite was added to human embryonic lung cells growing in tissue culture. No mutagenic activity was exhibited by sodium sulfite (73) or potassium metabisulfite (74) in in vitro plate and suspension microbial assays using Salmonella typhimurium, strains TA-1535, TA-1537, and TA-1538, and Saccharomyces cerevisiae, either un-activated or activated with liver, lung, or testis homogenates from mice, rats, and monkeys.
Effects of $SO_2$, sodium sulfite, and sodium bisulfite on three tissue culture cell lines [mouse fibroblast strain L cells (NCTC 929), mouse liver cells (NCTC 1769), and HeLa cells] were studied by Thompson and Pace (75). If the culture media were high in protein, the cells tolerated concentrations of sulfite of 500 ppm with no inhibition and as much as 2000 ppm with only a moderate degree of growth inhibition. If the media contained a low concentration of protein, the toxicity of sulfite was increased nearly tenfold. HeLa cells were more sensitive to sulfite than the mouse cell lines.

Human lymphocyte cells in tissue culture received a single exposure to 100 ml of air containing 5.7 ppm $SO_2$ which was bubbled through the medium after 0, 1, 2, or 3 days of incubation. Cell growth and DNA synthesis were reduced and chromosomal abnormalities, mainly chromosomal clumping, were observed (76). The authors suggest that lymphocyte damage caused by sulfur dioxide exposure may explain the reduced immunological responses of laboratory animals after $SO_2$ inhalation in vivo. Other workers (77) have found that rabbits exposed seven hours per day for 113 days to air containing 8 ppm $SO_2$ showed a reduced formation of agglutinins and conclude that chronic exposure to low $SO_2$ concentrations decreases the effectiveness of the immune response and resistance to infections.

Other considerations

The Select Committee considered the possibility that inhalation exposure would add an additional burden of $SO_2$ that should be considered in discussion of exposure to sulfiting agents in foods. Conditions might exist where the load of inhaled $SO_2$ could be equal to or greater than that of dietary $SO_2$. However, there is evidence to indicate that at prevailing levels, most air-borne $SO_2$ is rapidly oxidized to sulfate before inhalation or in the respiratory tract. Because the sulfuric acid formed has greater irritant properties than sulfurous acid, it is difficult to make dose-response interpretations (78). From the information available, the Select Committee is unable to estimate the contribution made to total $SO_2$ load from that present in inspired air.

V. OPINION

Based upon chronic toxicity tests in animals, primarily in rats, the no observed adverse effect level of $SO_2$ is estimated to be in the range of 30 to 100 mg of $SO_2$ per kg of body weight per day. These values are considerably higher than the estimated average per capita consumption of about 0.2 mg of $SO_2$ equivalent per kg body weight per day, and well above
the estimates of up to 2 mg per kg body weight per day that some individuals may consume if they select foods and beverages relatively high in SO₂ content. The margin of only about fifteenfold between the SO₂ that may be ingested by high-intake consumers and the lowest estimated no observed adverse effect level is relatively narrow. However, consideration of the significance of this difference should recognize the difficulties in estimating with confidence the components which are the basis of the calculated margin.

While the biological effects of sulfiting agents are still incompletely understood, certain conclusions are warranted. There is no reason to believe that the direct, local, irritating effects of sulfite, seen in high-dose acute toxicity tests, constitute a hazard from ingestion of sulfiting agents as they are presently used in foods. Orally administered sulfite is very rapidly oxidized to sulfate in all species studied. The metabolic removal of sulfite appears to be the critical defense mechanism, and this points to the important role of the enzyme, sulfite oxidase. Congenital deficiency of hepatic sulfite oxidase has been described as a rare metabolic disorder in man. There is also a paucity of data on the normal development of this enzyme with age in various species, and on the possible effects of dietary factors and disease on sulfite oxidase activity. Moreover, sulfite is capable of deaminating cytosine in vitro and inhibiting several enzymes requiring NAD or pyridoxal as cofactors which suggests that sulfite might be toxic in vivo if sulfite oxidase activity were sufficiently impaired or this metabolic mechanism were sufficiently overloaded, to prevent rapid oxidation of ingested sulfite to sulfate. Information in these respects would be helpful in assessing any special risk factors that may apply for select subpopulations.

Destruction of thiamine can occur as a result of the sulfiting of foods, but sufficient thiamine is present in usual mixed diets, particularly because use of sulfiting agents is prohibited by regulation in foods known to be major sources of the vitamin.

While there is no evidence that the sulfiting agents are teratogenic, there is evidence that directly added sulfite produces mutations in bacteria by alteration of nucleic acids. None of the available mammalian in vivo studies confirms these observations. Because the same organisms are not affected in the host-mediated assay, it seems reasonable to infer that rapid destruction of sulfite by the host's sulfite oxidase provides protection.

In view of the foregoing, the Select Committee concludes 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.
VI. REFERENCES CITED


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