Final Report on the Safety Assessment of Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite and Potassium Metabisulfite[1]

Sodium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Potassium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are inorganic salts that function as reducing agents in cosmetic formulations. All except Sodium Metabisulfite also function as hair-waving/straightening agents. In addition, Sodium Sulfite, Potassium Sulfite, Sodium Bisulfite, and Sodium Metabisulfite function as antioxidants. Although Ammonium Sulfite is not in current use, the others are widely used in hair care products. Sulfites that enter mammals via ingestion, inhalation, or injection are metabolized by sulfite oxidase to sulfate. In oral-dose animal toxicity studies, hyperplastic changes in the gastric mucosa were the most common findings at high doses. Ammonium Sulfite aerosol had an acute LC_{50} of >400 mg/m^3 in guinea pigs. A single exposure to low concentrations of a Sodium Sulfite fine aerosol produced dose-related changes in the lung capacity parameters of guinea pigs. A 3-day exposure of rats to a Sodium Sulfite fine aerosol produced mild pulmonary edema and irritation of the tracheal epithelium. Severe epithelial changes were observed in dogs exposed for 290 days to 1 mg/m^3 of a Sodium Metabisulfite fine aerosol. These fine aerosols contained fine respirable particle sizes that are not found in cosmetic aerosols or pump sprays. None of the cosmetic product types, however, in which these ingredients are used are aerosolized. Sodium Bisulfite (tested at 38%) and Sodium Metabisulfite (undiluted) were not irritants to rabbits following occlusive exposures. Sodium Metabisulfite (tested at 50%) was irritating to guinea pigs following repeated exposure. In rats, Sodium Sulfite heptahydrate at large doses (up to 3.3 g/kg) produced fetal toxicity but not teratogenicity. Sodium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite were not teratogenic for mice, rats, hamsters, or rabbits at doses up to 160 mg/kg. Generally, Sodium Sulfite, Sodium Metabisulfite, and Potassium Metabisulfite were negative in mutagenicity studies. Sodium Bisulfite produced both positive and negative results. Clinical oral and ocular-exposure studies reported no adverse effects. Sodium Sulfite was not irritating or sensitizing in clinical tests. These ingredients, however, may produce positive reactions in dermatologic patients under patch test. In evaluating the positive genotoxicity data found with Sodium Bisulfite, the equilibrium chemistry of sulfurous acid, sulfur dioxide, bisulfite, sulfite, and metabisulfite was considered. This information, however, suggests that some bisulfite may have been present in genotoxicity tests involving the other ingredients and vice versa. On that basis, the genotoxicity data did not give a clear, consistent picture. In cosmetics, however, the bisulfite form is used at very low concentrations (0.03% to 0.7%) in most products except wave sets. In wave sets, the pH ranges from 8 to 9 where the sulfite form would predominate. Skin penetration would be low due to the highly charged nature of these particles and any sulfite that did penetrate would be converted to sulfate by the enzyme sulfate oxidase. As used in cosmetics, therefore, these ingredients would not present a genotoxicity risk. The Cosmetic Ingredient Review Expert Panel concluded that Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are safe as used in cosmetic formulations.

INTRODUCTION

This report is a compilation of data concerning the safety of Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite for use in cosmetics. Little information was found regarding Ammonium Sulfite or Ammonium Bisulfite.

CHEMISTRY

Definition and Structure

All seven ingredients are inorganic salts that conform to the formulas presented in Table 1 (Pepe, Wenninger, and McEwen 2002).

Physical Properties

Sodium Sulfite is described as a white or tan to slightly pink, odorless or nearly odorless powder having a cooling, saline, sulfurous taste. It undergoes oxidation in air. Its solutions are alkaline to litmus and to phenolphthalein. It is soluble in water and sparingly soluble in alcohol (National Academy of Sciences 1981).

Potassium Sulfite is described as a white, odorless, granular powder. It undergoes oxidation in air. It is soluble in water

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TABLE 1
Ingredient Formulas and Synonyms

Ingredient/CAS no.	Formula[1]	Synonyms[1,2,3,4]
Sodium Sulfite 7757-83-7[1] 10579-83-6[2]	Na_2SO_3	Sulfurous Acid, Disodium Salt; Anhydrous Sodium Sulfite; Disodium Sulfite; Exsiccated Sodium Sulfite; Sulftech; Natriumsulfit (German); Sodium Sulphite; Sulfurous Acid, Sodium Salt (1:2)
Potassium Sulfite 10117-38-1[1]	K_2SO_3	Sulfurous Acid, Potassium Salt
Ammonium Sulfite 10196-04-0[1]	$(NH_4)_2SO_3$	Sulfurous Acid, Diammonium Salt; Ammonium Hydrogen Sulfite; Ammonium Monosulfite; Monoammonium Sulfite
Sodium Bisulfite 7631-90-5[1]	$NaHSO_3$	Sulfurous Acid, Monosodium Salt; Sodium Hydrogen Sulfite; Sodium Acid Sulfite; Bisulfite De Sodium (French); Hydrogen Sulfite Sodium; Monosodium Sulfite; Sodium Bisulphite; Sodium Sulhydrate
Ammonium Bisulfite 10192-30-0[1]	NH_4HSO_3	Sulfurous Acid, Monoammonium Salt
Sodium Metabisulfite 7681-57-4[1] 7757-74-6[2]	$Na_2S_2O_5$	Disulfurous Acid, Disodium Salt; Sodium Pyrosulfite; Disodium Disulfite; Disodium Metabisulfite; Disodium Pyrosulfite; Disodium Pentaoxodisulfate
Potassium Metabisulfite 16731-55-8[1] 4429-42-9[2]	$K_2S_2O_5$	Disulfurous Acid, Dipotassium Salt; Potassium Pyrosulfite; Dipotassium Disulfite; Dipotassium Metabisulfite; Potassium Disulfite; Pyrosulfurous Acid, Dipotassium Salt; Dipotassium Pentaoxodisulfate

[1]Pepe, Wenninger, and McEwen 2002; [2]RTECS 1998; [3]National Academy of Sciences 1981; [4]FAO/WHO 1994.

and slightly soluble in alcohol (National Academy of Sciences 1981).

Ammonium Sulfite is described as hygroscopic, colorless crystals with an acrid, sulfurous taste (Lewis 1993). It is soluble in water and almost insoluble in alcohol and acetone. In a 0.1 M aqueous solution the pH is 5.5 (Budavari 1989).

Sodium Bisulfite consists of Sodium Bisulfite and Sodium Metabisulfite in varying proportions, but possesses the properties of the bisulfite. It occurs as white or yellowish-white crystals or granular powder with an odor of sulfur dioxide. It is unstable in air and is soluble in water and slightly soluble in alcohol (National Academy of Sciences 1981).

Ammonium Bisulfite is an inorganic salt and is described as a colorless crystal readily soluble in water (Grant 1972).

Sodium Metabisulfite is described as colorless crystals or a white to yellowish crystalline powder having an odor of sulfur dioxide. It is soluble in water and slightly soluble in alcohol. Its solutions are acid to litmus (National Academy of Sciences 1981).

Potassium Metabisulfite is described as white or colorless free-flowing crystals, crystalline powder, or granules, usually having an odor of sulfur dioxide. It gradually oxidizes in air to the sulfate. It is soluble in water and insoluble in alcohol. Its solutions are acid to litmus (National Academy of Sciences 1981).

Ultraviolet (UV) Absorption

Eberlein-König et al. (1993) reported that Sodium Metabisulfite (identified as sodium disulfite) had an absorbance peak at 209 nm. Sodium Sulfite was identified as having a "similar" absorbance pattern.

Formation and Dissociation

The term "sulfiting agents" is used to describe sulfur dioxide (SO_2) and several forms of inorganic sulfite (sulfurous acid [H_2SO_3], Sodium Sulfite, Sodium Bisulfite, Potassium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite) that liberate sulfur dioxide under certain conditions (Taylor, Higley, and Bush 1986). The theoretical yield of sulfur dioxide from sulfiting agents is found in Table 2.

All are quadrivalent sulfur (S^{IV}) substances that exist in a pH-sensitive equilibrium (Gunnison 1981). Sulfur dioxide readily dissolves in water to produce sulfurous acid. Under physiological conditions (pH 7.4 and 37°C), a mixture of sulfite ions (SO_3^{-2}), and bisulfite anions (HSO_3^-) predominates. Acidification will liberate sulfur dioxide vapors; in alkalis, sulfites, bisulfites, and metabisulfites are produced (Green 1976). At concentrations >1 M, bisulfite anions will dimerize with the elimination of water to form metabisulfite ($S_2O_5^{-2}$); at low concentrations

TABLE 2
Theoretical sulfur dioxide yield (Green 1976)

Sulfiting agent	Theoretical yield of SO_2 (%)
Sulfur Dioxide	100.00
Sodium Sulfite, Anhydrous	50.82
Sodium Sulfite, Heptahydrate	25.41
Sodium Bisulfite	61.56
Potassium Bisulfite	53.32
Sodium Metabisulfite	67.39
Potassium Metabisulfite	57.60

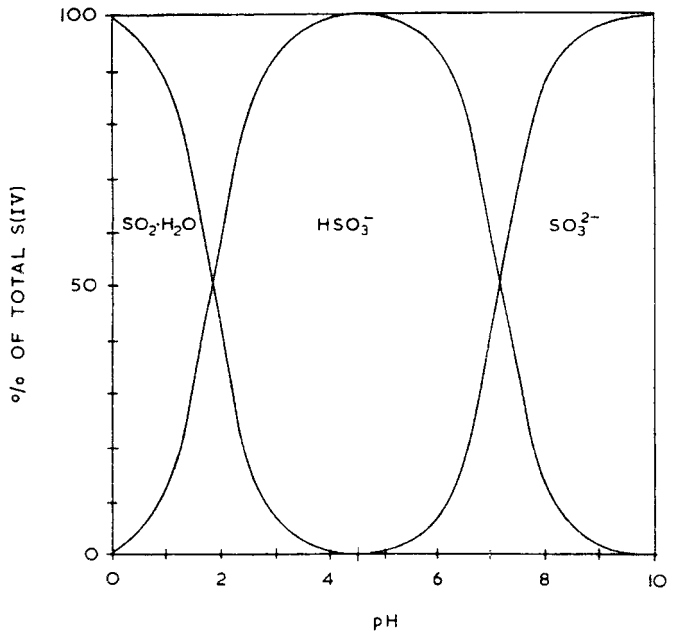

FIGURE 1
Distribution of the species $SO_2 \cdot H_2O$, HSO_3^-, and SO_3^{2-} as a function of pH in dilute solution (Wedzicha 1984).

metabisulfite will hydrolyze to form bisulfite (Shapiro 1983; Gunnison and Jacobsen 1987). Chemical conversions in water under acidic conditions proceed along the following pathway:

Metabisulfite → Bisulfite
→ Sulfite or sulfurous acid,
sulfur dioxide, and water

and are dependent on temperature and ionic strength (Nicklas 1989; Gunnison 1981; Atkinson, Sim, and Grant 1993).

The chemical form as a function of pH is given in Figure 1 (Wedzicha 1984).

According to Fazio and Warner (1989), free sulfite in food is a mixture of sulfur dioxide, bisulfite ion, and sulfite ion in chemical equilibrium dependent on the pH (acidity) of the food.

Reactivity

Hui et al. (1989) reported that sulfites are fairly reactive with reducing sugars, proteins, carbonyl compounds, amino acids, and vitamins.

According to Taylor, Higley, and Bush (1986), the theoretical yields of sulfur dioxide cited in Table 2 would almost never be achieved in food applications because of these reactions. Analytical procedures distinguish between "free" sulfur dioxide (sulfur dioxide and the other inorganic sulfite salts) and "total" sulfur dioxide (free sulfur dioxide plus *some* of the combined forms of sulfite).

Sulfur Dioxide

Sulfur dioxide is one of the species to which these ingredients addressed in this safety assessment may convert. Based on air quality concerns the U.S. Environmental Protection Agency (EPA) has set National Ambient Air Quality Standards (NAAQS) for sulfur dioxide. An annual arithmetic mean of 0.03 ppm (80 $\mu g/m^3$) and a 24-h level of 0.14 ppm (365 $\mu g/m^3$), which are not to be exceeded more than once per year, and a 3-h level of 0.5 ppm (1300 $\mu g/m^3$) have been established (EPA 1994). The 24-h level and annual mean are based on the concerns about both the public health, including the health of asthmatics, children, and the elderly, and the 3-h level is based on the public welfare, including damage to animals, crops, vegetation, and buildings. In addition, the American Conference of Government Industrial Hygienists (ACGIH) has established a workplace threshold limit value (TLV) for sulfur dioxide of 2 ppm averaged over an 8-h day (ACGIH 1987).

USE

Cosmetic

All seven ingredients function as reducing agents in cosmetic formulations. All except Sodium Metabisulfite also function as hair-waving/straightening agents. In addition, Sodium and Potassium Sulfites, Sodium Bisulfite, and Sodium Metabisulfite function as antioxidants (Pepe, Wenninger, and McEwen 2002).

As of January 1998, Sodium Sulfite was used in 911 formulations, Potassium Sulfite was used in 1 formulation, Sodium Bisulfite was used in 58 formulations, Sodium Metabisulfite was used in 348 formulations, and Potassium Metabisulfite was used in 1 formulation (FDA 1998) (Table 3). Of the combined 1319 uses for these five ingredients, 1249 were in hair dyes and colors or hair tints. Ammonium Sulfite was not reported in current use, and was not used in 1984 (see next paragraph).

Concentrations of use are no longer reported to the Food and Drug Administration (FDA) (FDA 1992). Data from 1984 indicated that Sodium Sulfite was used up to a concentration of 5%, Potassium Sulfite was used up to 10%, Ammonium Sulfite was used up to 5%, Sodium Bisulfite was used up to 5%, Ammonium Bisulfite was used up to 50%, and Sodium Metabisulfite was used up to 1% (FDA 1984). Current concentration of use data provided to CIR by the industry (CTFA 1999a, 1999b) are included in Table 3.

Particle sizes of anhydrous hair sprays range from 60 to 80 μ (typically, $<1\%$ are below 10 μ) and pump hair sprays have particle sizes of ≥ 80 μ (Bower 1999). In product categories that contain spray uses, however, sulfites were not used as sprays.

Sodium Sulfite, Potassium Sulfite, and Ammonium Sulfite are listed in Annex VI, *List of Preservatives Which Cosmetic Products May Contain*, of the European Community Directive. These three ingredients are allowed at a maximum authorized concentration of 0.2%, expressed as free SO_2 (Cosmetics Directive of the European Union 1995).

TABLE 3
Product formulation data

Product category (No. Formulations Reported to FDA 1998)	No. containing ingredient (FDA 1998)	Current range of concentrations (CTFA 1999a, 1999b) (%)
Sodium Sulfite		
Bath oils, tablets, and salts (124)	1	—
Other bath preparations (159)	1	—
Hair conditioners (636)	1	—
Permanent waves (192)	2	—
Shampoos (noncoloring) (860)	9	0.01
Hair dyes and colors (1572)	872	0.7–3
Hair tints (54)	19	0.6
Hair lighteners with color (6)	1	—
Other hair-coloring preparations (59)	1	0.5
Basecoats and undercoats (48)	1	—
Bath soaps and detergents (385)	1	—
Other personal cleanliness products (291)	—	0.2
Moisturizing creams, lotions, powders, and sprays* (769)	—	0.1
Other skin care preparations (692)	2	0.4
1998 total for Sodium Sulfite	**911**	
Potassium Sulfite		
Permanent waves (192)	1	—
1998 total for Potassium Sulfite	**1**	
Sodium Bisulfite		
Tonics, dressings, and other hair-grooming aids (549)	—	0.03
Hair dyes and colors (all types requiring caution statements and patch tests) (1572)	—	0.7
Face and neck creams, lotions, powders, and sprays (excluding shaving preparations)* (263)	—	0.05
Paste masks (mud packs) (255)	—	0.1
Other bath preparations (159)	1	—
Hair conditioners (636)	1	—
Shampoos (noncoloring) (860)	1	—
Hair dyes and colors (1572)	49	—
Body and hand skin care (excluding shaving) (796)	1	—
Moisturizing (769)	1	—
Other skin care preparations (692)	4	0.3
1998 total for Sodium Bisulfite	**58**	
Sodium Metabisulfite		
Other bath preparations (159)	8	—
Eye lotion (18)	1	—
Permanent waves (192)	1	—
Shampoos (noncoloring) (860)	2	0.1
Tonics, dressings, and other hair-grooming aids (549)	—	0.1
Wave sets (55)	—	14
Hair dyes and colors (1572)	309	—
Hair color sprays (aerosol) (4)	1	—
Foundations (287)	—	0.15
Basecoats and undercoats (48)	1	—
Deodorants (underarm) (250)	7	0.1

(Continued on next page)

TABLE 3
Product formulation data *(Continued)*

Product category (No. Formulations Reported to FDA 1998)	No. containing ingredient (FDA 1998)	Current range of concentrations (CTFA 1999a, 1999b) (%)
Aftershave lotion (216)	—	0.1
Skin cleansing (cold creams, cleansing lotions, liquids, and pads) (653)	—	0.1
Face and neck creams, lotions, powders, and sprays (excluding shaving preparations)* (263)	—	0.003
Night creams, lotions, powders, and sprays (excluding shaving preparations)* (188)	—	0.003
Body and hand skin care (excluding shaving) (796)	2	0.003
Moisturizing (769)	1	0.003
Other skin care preparations (692)	4	0.4
Indoor tanning preparations (62)	11	0.3
1998 total for Sodium Metabisulfite	**348**	
	Potassium Metabisulfite	
Permanent waves (192)	1	—
1998 total for Potassium Metabisulfite	1	
	Ammonium Bisulfite	
Wave sets (55)	—	32
1998 total for Ammonium Bisulfite	—	

*None of the products that contain sulfites in these product categories are sprays.

According to the Ministry of Health, Labor and Welfare (MHLW) in Japan, Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are not restricted in cosmetic formulations in any manner (MHLW 2001).

Noncosmetic

Food

Sulfiting agents are used primarily to reduce or prevent spoilage and discoloration as well as to bleach food starches, condition dough for some baked goods, control fermentation of wine, and soften corn kernels during the wet-milling process (Fisher 1997; Green 1976). They are found in many foods, especially those that have been fermented. Total sulfur dioxide concentrations of >100 ppm are found in dried fruits (excluding dark raisins and prunes), lemon and lime juices, wine, molasses, and sauerkraut juice. Concentrations between 50 and 100 ppm are found in dried potatoes, grape juice, wine vinegar, gravies, fruit topping, and maraschino cherries. Concentrations between 10 and 50 ppm are found in pectin, fresh shrimp, corn syrup, sauerkraut, pickled foods, corn starch, hominy, frozen potatoes, maple syrup, imported jams and jellies, and fresh mushrooms (Lester 1995).

In 1983, the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization of the World Health Organization (FAO/WHO) established an acceptable daily intake (ADI) level of 0.7 mg/kg body weight. This value was a "group ADI for sulfur dioxide and sulfites expressed as sulfur dioxide, covering sodium and potassium metabisulfite, sodium sulfite, sodium and potassium hydrogen sulfite and sodium thiosulfate" (FAO/WHO 1994). Review articles (Walker 1985; Til and Feron 1992) explained that this level was determined by applying a safety factor of 10^{-2} to the no-effect level of 0.25% Sodium Metabisulfite (equal to 72 mg sulfur dioxide/kg body weight/day) was used by Til, Feron, and DeGroot (1972a) in a three-generation oral-dose study using rats. The study is detailed in the Oral Toxicity section of this report. Critics of using this study noted that in addition to the inadequacy of applying results from rat studies to humans, it was the toxicity of "total sulfite" rather than "free sulfite" that was tested, and free sulfite loss could have been underestimated (Taylor, Higley, and Bush 1986).

Food grade specifications are listed in Table 4.

FDA Requirements

In 1982, sulfur dioxide, Sodium Sulfite, Sodium and Potassium Bisulfite, and Sodium and Potassium Metabisulfite were classified "generally recognized as safe" (GRAS) by the FDA. They were not to be used in meats or foods recognized as sources of vitamin B_1 (thiamine). Their concentration in wines and raw shrimp were respectively limited to 350 ppm (5.5 mM) and 100 ppm (1.6 mM) sulfur dioxide equivalents (Gunnison and Jacobsen 1987). The GRAS status was supported by an evaluation by the Federation of American Societies for Experimental Biology (FASEB). That evaluation used animal studies

TABLE 4
Food grade specifications (National Academy of Sciences 1981)

Requirement	Sodium Sulfite	Potassium Sulfite	Sodium Bisulfite	Sodium Metabisulfite	Potassium Metabisulfite
Assay	95.0% min of Na_2SO_3	90.0% min, 100.5% max of K_2SO_3	58.5% min, 67.4% max of SO_2	90.0% min, 100.5% max of $Na_2S_2O_5$	90.0% min of $K_2S_2O_5$
Heavy metals (as Pb)	10 mg/kg max	10 mg/kg max	10 mg/kg max	10 mg/kg max	10 mg/kg max
Selenium	0.003% max	30 mg/kg max	0.003% max	0.003% max	0.003% max
Iron			0.005% max	0.002% max	10 mg/kg max
Alkalinity (as K_2CO_3)		0.25%–0.45%			

(primarily rat) to estimate a "no observed adverse effect level" of 30 to 100 mg sulfur dioxide for humans. At the time, estimated average per capita consumption was 0.2 mg sulfur dioxide/kg/day, with a high estimate of up to 2 mg/kg/day. The Select Committee concluded that no available evidence suggested a hazard to the public at current practices of use. However, additional data were needed to determine whether a significant increase in consumption would constitute a dietary hazard (FASEB 1976).

By October 1986, FDA had received 767 reports of adverse reactions following ingestion of sulfiting agents used as preservatives on fresh fruits and vegetables, in packaged foods, shrimp, and alcoholic beverages. Several of the reactions occurred after eating at a restaurant salad bar, and others after eating packaged foods prepared at home. Most of the reactions occurred in steroid-dependent asthmatics and many involved respiratory distress or failure, or anaphylaxis. FDA analyzed 22 deaths allegedly associated with sulfite ingestion and determined that 9 fatalities (all severe asthmatics) were probably and 5 fatalities (also asthmatics) were possibly due to sulfite ingestion (FDA 1986).

These instances prompted a reevaluation of the GRAS status (FASEB 1985). The Committee concluded that there was no evidence that sulfiting agents were a hazard "for the majority of the population." However, "for the fraction of the public that is sulfite sensitive," evidence was available to suspect that these agents were a "hazard of unpredictable severity to such individuals when they are exposed to sulfiting agents in some foods at levels that are now current and in the manner now practiced." The Committee was of the opinion that additional labeling requirements alone were not sufficient. The Committee noted that use of sulfites on fresh produce was being voluntarily curtailed by food service establishments and advised that further discontinuance "should be encouraged by appropriate use of the regulatory process."

Based on the new evaluation, FDA required that packaged sulfited foods that contain $\geq$10 ppm sulfite must list it on the ingredient label. GRAS status was revoked for use of sulfiting agents on fresh fruits and vegetables (FDA 1986). The only produce exempt from the ban are precut or peeled (not whole raw) potatoes and grapes (Fisher 1997).

Pharmaceutical Products

Sulfites are used as preservatives in a variety of parenteral and aerosolized drug preparations (Gunnison and Jacobsen 1987). Sodium Bisulfite is used in fade creams at a concentration of 0.5% (CTFA 1999a). They are no longer used in bronchodilators (Lester 1995). Sulfites have to be identified in a warning label on prescription drugs, but are not required to be listed on over-the-counter products (21 CFR 201.22).

Specifications for Sodium Metabisulfite and Potassium Metabisulfite listed in the *National Formulary* are presented in Table 5.

Workplace Exposure Limits

The ACGIH established a TLV time-weighted average of 5 mg/m^3 for Sodium Bisulfite and Sodium Metabisulfite (ACGIH 1987).

GENERAL BIOLOGY

Metabolism

Endogenous Sulfite

Sulfites are generated in the human body by processing of the sulfur-containing amino acids, cysteine and methionine. Endogenous sulfite is maintained at a low, steady-state concentration by a mitochondrial enzyme, sulfite oxidase, that promotes the oxidation of sulfite to sulfate that is excreted in the urine (Gunnison and Jacobsen 1987; Lester 1995).

TABLE 5
National Formulary specifications (Committee of revision of the United States Pharmacopeial Convention 1995)

Requirement	Sodium Metabisulfite	Potassium Metabisulfite
Assay	$Na_2S_2O_5$ equivalent to 65.0% min, 67.4% max of SO_2	$K_2S_2O_5$ equivalent to 51.8% min, 57.6% max of SO_2
Heavy metals	0.002% max	0.001% max
Iron	0.002% max	0.001% max
Arsenic	3 ppm max	3 ppm max

Sulfites can also be metabolized to thiosulfates (enzymatic reaction of sulfite with 3-mercaptopyruvate) or *S*-sulfonate compounds (nonenzymatic reaction with disulfide bonds). Thiosulfate and *S*-sulfonate were detected at very low concentrations in the urine of normal humans or rats, but were excreted in large amounts by those deficient in sulfite oxidase (Calabrese et al. 1981; Taylor, Higley, and Bush 1986).

Human neutrophils released sulfite in response to lipopolysaccharide in a study by Mitsuhashi et al. (1998). Neutrophils isolated from human blood samples were incubated with 100 ng/ml of serum-activated lipopolysaccharides (SA-LPSs). To overcome basal release of sulfites due to neutrophil adherence to plastic culture tubes, poly-HEMA tubes were coated to abolish the adherence. Unstimulated neutrophils released <0.3 nmol/h/10^7. Stimulated neutrophils released 2.5-, 2.4-, and 3.7-fold increases of sulfite at 10, 30, and 60 min. LPS treatment enhanced release up to 1.0 ± 0.12 nmol/h. The glucocorticoid prednisolone and FK506 also were incubated with SA-LPS–treated neutrophils. These immunosuppressive agents completely suppressed sulfite release by stimulated neutrophils to the numbers of unstimulated neutrophils. The enhanced production of sulfite in response to LPS was confirmed in vivo by injecting 1 mg/kg of LPS into Wistar rats and determining the sequential serum sulfite concentration. Before the LPS treatment, rat serum sulfite concentrations were 0.52 ± 0.17 μmol/L. After treatment at 1 h, the response was five times greater.

Exogenous Sulfite

Sulfite that enters the body via ingestion, inhalation, or injection is metabolized by sulfite oxidase to sulfate. Oral dose studies using dogs and rats and intravenous (IV) dose studies using rabbits, rats, and rhesus monkeys, demonstrated rapid metabolic clearance. In all species $\leq$10% of the administered dose was excreted unchanged in the urine. One difference in the metabolism kinetics of exogenous sulfite versus endogenous sulfite is that hepatic oxidation of exogenous sulfite (at least in rats) is diffusion limited. The liver metabolizes a constant fraction of sulfite it receives, but a finite amount will pass through the organ and enter the systemic circulation (Gunnison and Palmes 1976; Gunnison and Jacobsen 1987).

Review articles note that hepatic sulfite oxidase activity was estimated to be 10 to 20 times greater in rats compared to humans (Gunnison, Bresnahan, and Palmes 1977; Walker 1985).

Ji, Savon, and Jacobsen (1995) determined the total serum sulfite concentrations in 41 women and 35 men. Blood was taken and serum sulfite concentrations were analyzed by the separation of sulfite-bimane from thiol-bimanes by reverse-phase high-performance liquid chromatography (HPLC) and quantization of sulfite-bimane fluorescence detection. The intra- and interassay coefficients of variation (CVs) for total serum sulfite at 5.4 μmol/L were 8.1% and 22.0%, respectively. The mean concentrations ($\pm SD$) of total serum sulfite in women and men were 4.63 ± 2.33 and 5.16 ± 2.68 μmol/L, respectively. The reference range for total serum sulfite in normal subjects is 0 to 9.8 μmol/L. There was no correlation between total serum sulfite and total serum cysteine, cysteinylglycine, homocysteine, subject age, serum cobalamin, or serum folic acid.

Antioxidant Activity

Lavoie, Lachance, and Chessex (1994) reported that Sodium Metabisulfite had in vitro antioxidant activity against hydrogen peroxide, *tert*-butyl-hydroperoxide, and cumene hydroperoxide. A follow-up study was conducted to test whether Sodium Metabisulfite reduced spontaneously generating hydroperoxides in pharmaceutical lipid emulsions. Infants requiring total parenteral nutrition received amino acid solutions containing Sodium Metabisulfite (300 mg/L) for a 4-day period. Each infant served as his own control by receiving (also for a 4-day period) amino acid solution not containing Sodium Metabisulfite. The total volume of multiple vitamins was kept constant throughout the study as lipid soluble vitamins can affect lipid peroxidation. A 24-h urine collection was done on the last day of each period. Urine was analyzed for malondialdehyde, a stable end product of lipid peroxidation. Malondialdehyde excretion was lower ($p < .01$) following Sodium Metabisulfite treatment. The investigators noted that the concentration of metabisulfite present is "critical" because unless it is present in excess concentrations it can have oxidant activity.

Cellular Toxicity

Sodium Bisulfite

Seravalli, Lear, and Cottree (1984) reported that Sodium Bisulfite (1.6×10^{-3} to 0.2×10^{-3} M) did not produce cell membrane fusion in murine glial and hepatic cells and human fibroblasts.

Seravalli and Lear (1987) reported a study in which Sodium Bisulfite (tested because it is a component of the local anesthetic chloroprocaine) reduced cell multiplication in human neuroblastoma cells. Colony-forming ability (CFA) was reduced 72% to 92% by a 3-h exposure to one commercial sample of Sodium Bisulfite and 57% to 72% by another commercial sample (both tested at 0.8×10^{-3} M). When exposure time was lengthened to 20 h, both solutions inhibited CFA to the same extent (98%). No difference in the inhibition of CFA was observed between the two samples at $<0.8 \times 10^{-3}$ M.

Sodium Metabisulfite

Eberlein-König et al. (1993) conducted a study in which suspensions of human erythrocytes (from three donors) were each incubated with Sodium Sulfite and Sodium Metabisulfite (identified as sodium disulfite). Each material was tested at 10^{-5}, 10^{-4}, and 10^{-3} mol/L. Erythrocyte-free samples were also incubated with the test materials and used as controls. Following incubation, suspensions were exposed to varying amounts of UVA or UVB light from one of three sources detailed in Table 6. Hemolysis was measured as a function of absorbance of 550-nm light.

TABLE 6
UV sources used (Eberlein-König et al. 1993)

Source (distance of 40 cm)	Emission (nm)	Irradiance (mW/cm^2)	Dose
UVASUN 5000	320–460 nm (max ~375 nm)	42 for UVA	UVA: 25, 50, or 100 J/cm^2
TL 20 W/12 lamp	275–365 nm (max ~315 nm)	1.0 for UVB	UVB: 100, 200, 400, 800, or 1600 mJ/cm^2
		0.4 for UVA	UVA: 37.5, 75, 150, 300, or 600 mJ/cm^2
SOL 3 sunlight-simulating with H2 filter	290–800 (broad max ~400–700 nm)	0.95 for UVB	UVB: 0.45, 2.26, or 4.52 J/cm^2
		10.5 for UVA	UVA: 5, 25, or 50 J/cm^2

A UV dose-dependent increase in hemolysis was noted following exposure to the TL 20 W/12 lamps with the highest dose of both Sodium Sulfite (64.1% hemolysis) and Sodium Metabisulfite (almost 100% hemolysis). Sodium Metabisulfite also induced hemolysis following irradiation with the SOL 3 lamp, but no effect was noted following exposure to the UVASUN 5000 apparatus. The strong response produced by the Metabisulfite was considered a "concentration effect" because the ion separates into two bisulfite ions in aqueous solution. It was noted that most phototoxic substances act in the UVA range. It was also noted that the stronger response was exerted at a lower dose: 1.6 J/cm^2 max from the TL 20 W/12 light source versus 4.5 J/cm^2 from the SOL 3 (Eberlein-König et al. 1993).

ANIMAL TOXICOLOGY

Oral Toxicity

Reviews of oral-dose toxicity studies noted that results of early studies are difficult to interpret because those studies did not recognize either the destruction of thiamine by sulfites, or the instability of sulfite which results in loss during processing and storage due to autoxidation and chemical reactions with other constituents of the preparations (Gunnison and Jacobsen 1987; Taylor, Higley, and Bush 1986; Til and Feron 1992).

Oral-dose toxicity studies from 1920 to 1972 are summarized in the GRAS report (Franklin Institute Research Laboratories 1972). In general, the studies confirmed that sulfite was toxic to animals at 50 mg sulfur dioxide/kg when in a thiamine-deficient diet. When adequate thiamine concentrations were maintained, animals could tolerate up to 300 mg sulfite/kg/day without significant effect on weight gain or feed utilization. Freshly prepared feed containing 400 mg sulfur dioxide/kg reduced growth rates in rats, but the rate was restored with thiamine supplementation. However, a reduced growth rate was observed even with the addition of thiamine when the diet had been stored for ≥75 days (FASEB 1976).

Acute Oral Toxicity

Sodium Metabisulfite

The acute oral LD_{50} was 1131 and 1903 mg/kg for female and male rats, respectively (Eastman Kodak Co. 1980).

Sodium Metabisulfite (25% solution in distilled water) was administered as a single dose (by intragastric intubation) to adult male ChR-CD rats. It was considered "slightly toxic" with an approximate lethal dose (ALD) of 2250 mg/kg body weight (Haskell Labs 1975).

Potassium Metabisulfite

The GRAS report (Franklin Institute Research Laboratories 1972) cited acute oral LD_{50} values of 1040 and 1800 mg/kg in rats.

Short-Term Oral Toxicity

Sodium Metabisulfite

Til et al. (1972a) reported that anemia developed in mice that had been dosed with ≥2% Sodium Metabisulfite for 10 to 56 days; increased hematopoiesis and splenomegaly was observed with doses ≥4%. Hemorrhagic erosions, inflammation, and necrosis of the stomach were observed in rats fed 4%, 6%, or 8% Sodium Metabisulfite. In a review, Walker (1985) noted that the principal finding was local gastric irritant effects without systemic toxicity. Vitamin B_{12} deficiency was considered a possible contributor to the development of anemia.

A 4-week oral toxicity study of Sodium Metabisulfite using Wistar rats determined a no-observed-adverse-effect level (NOAEL) of 5000 ppm and a minimum-observed-adverse-effect level (MOAEL) of 20,000 ppm (details not given). Sodium Metabisulfite was then tested in a 4-week combined toxicity study with seven other chemicals each at their respective MOAELs, NOAELs, 1/3 NOAELs, and 1/10 NOAELs. The other chemicals include Mirex, Loperamide, metaldehyde, di-*n*-octyltin dichloride, stannous chloride, lysinoalanine, and potassium nitrite. As Sodium Metabisulfite was the least toxic of the eight chemicals, it was present in the greatest concentration in the diets. Slightly decreased hemoglobin content and slightly increased relative kidney weight were the only treatment-related adverse effects seen in the group receiving the chemicals at the NOAEL concentration. No treatment-related effects were found in the group receiving chemicals at the 1/3 NOAEL and 1/10 NOAEL concentrations (Jonker et al. 1990).

Subchronic Oral Toxicity

Sodium Bisulfite

Groups of 50 male and 50 female crossbreed white and Wistar mice received doses of Sodium Bisulfite (160 mg/kg/day), benzoic acid (80 mg/kg/day), or Sodium Bisulfite (160 mg/kg/day) and benzoic acid (80 mg/kg/day) by oral intubation for 3 months. Seventy percent of males and 68% of females receiving 160 mg/kg/day of Sodium Bisulfite survived. Survival rates were similar for mice given benzoic acid. However, the survival rate of mice receiving the combination of Sodium Bisulfite and benzoic acid was only 30% for males and 38% for females. After the 3 months, the mice of each treatment group were given a 90% feed reduction. The mortality percentages after 5 days were 57.2% (Sodium Bisulfite group), 85.7% (benzoic acid group), and 83.3% (Sodium Bisulfite and benzoic acid group). Individual mice of the Sodium Bisulfite and the benzoic acid groups were given single doses of carbon tetrachloride (0.1 ml/mouse); 45.0% of the Sodium Bisulfite group and 62.5% of the benzoic acid group died. Ehrlich ascites mouse carcinoma was implanted intraperitoneally into mice after 3 months on test diets. Tumor growth was greatest in mice that had received Sodium Bisulfite (Shtenberg and Ignat'ev 1970).

Sodium Metabisulfite

In a study utilizing sulfite oxidase–deficient virgin female Wistar rats, the endogenous and exogenous toxicity of sulfites was examined (Gunnison et al. 1981). The sulfite oxidase deficiency was achieved through the addition of tungsten and reduced molybdenum. Four control groups, all having normal hepatic sulfite oxidase activity, were fed normal protein diets and two groups were provided with normal tap water. Two of the control groups with normal sulfite oxidase activities received no drinking water supplementation. The other two control groups received tungsten, molybdenum, and Na_2SO_4 (12.5 mM) in their drinking water. The three treatment groups, consisting of sulfite oxidase–deficient animals, received either tungsten, tungsten and $Na_2S_2O_5$ (25 mM), or tungsten and $Na_2S_2O_5$ (50 mM). The mean steady-state sulfite oxidase activity of all treatment groups was about 1 to 2% of normal adult activities. At week 7, all rats were mated with normal males. All rats, including nonpregnant rats, were killed on day 21 of gestation.

A second experiment using normal sulfite oxidase activity female rats was also conducted. These rats were fed 0%, 1%, 2%, or 6% powdered Sodium Metabisulfite. All diets containing Sodium Metabisulfite were supplemented with 50 ppm thiamine.

Toxicity due to decreased feed consumption, reactions with feed constituents of the diet, and irritation of the gut was observed in this study. These effects and anemia were produced by the large concentrations of SO_3^- in the diet or gut; systemic SO_3^- does not appear to be related to any toxicity seen in this study. In the second study, powdered Sodium Metabisulfite in the feed was associated with destruction of thiamine. In the first study, however, no destruction of thiamine was associated with large systemic concentrations of SO_3^-.

The researchers also reported a statistically insignificant incidence of mammary gland adenocarcinoma in young, sulfite oxidase–deficient females. Because these carcinomas occurred in rats less than 5 months old when spontaneous formation is unlikely, the researchers speculated that it was likely that these adenocarcinomas were in fact due to sulfite treatment. The neoplasms, however, were seen in animals not receiving supplementation and a dose-response was not observed in those animals receiving supplemental sulfite; i.e., adenocarcinomas were observed in the 25-mM group, but not the 50- or 75-mM group (Gunnison et al. 1981).

Chronic Oral Toxicity

Sodium Bisulfite

A three-part, 3-year study using the Osbourne-Mendel strain of rats evaluated the chronic toxicity of Sodium Bisulfite (Fitzhugh, Knudsen, and Nelson 1946). All three parts used a balanced incomplete block design method. In the first part of the study, rats were fed either one of four diets: sulfite added, sulfite added with supplemented thiamine, sulfite added with reduced thiamine content, and control. In addition, each of the sulfite-added diets were further divided into groups that received three different concentrations of sulfite: 0.5%, 1.0%, and 2.0%. This part of the study was replicated for males and females (three per sex) for 1 year.

The second part compared the effects of sulfite prepared weekly and diets prepared to last 5 to 6 weeks and refrigerated. The 10 different diets are as follows: weekly prepared at both 1.0%, and 2.0% Sodium Bisulfite, aged fed at 0.1%, 0.25%, 1.0% and 2.0%, controls at 1.0% and 2.0%, and two diets with 0.25% and 1.0% sodium sulfate. This part of the study had a duration of $1^1/_2$ years.

The third part of the study was for 2 years and used lower doses of Sodium Bisulfite. Four diets containing 0.0125%, 0.025%, 0.05%, and 2.0% of Sodium Bisulfite were utilized, along with a control and 0.25% and 1.0% sodium sulfide.

Sodium Bisulfite at concentrations of 0.1% (615 ppm as SO_2) or more were added to the diet were toxic to rats. No observed significant effect on growth by Sodium Bisulfite was observed at concentrations less than 1.0% (615 ppm as SO_2). A definite trend toward smaller average weights and smaller gains in weight was observed as the concentration was increased from 1.0% to 2.0%. The addition of thiamine to the diet produced similar growth to that of the control diet. In contrast, removing the thiamine caused the lowest weights and gains in weight. Sodium Bisulfite at a concentration of 0.25% (1538 ppm as SO_2) caused decreased survival time that continued to shorten as the concentrations of sulfite increased. Reduced dietary thiamine sharply decreased the survival time as well. The addition of sulfates or sulfides had no effects on either the survival time, weight gain, or histopathological changes in the rats. The lowest dose of sulfite

that produced histopathological changes was 0.1% (615 ppm as SO_2). From 0.25% (1538 ppm as SO_2) and greater, the following clinical and pathological changes were observed: stunting of growth, clinical polyneuritis, "spectacle" eye, bleached incisor teeth, brown uteri, atrophy of various viscera, calcified renal tubular casts, atrophy of bone marrow and bone, focal myocardial necrosis and fibrosis, and gastric squamous epithelial hyperplasia. Animals fed the aged diet had a greater incidence of lesions of the teeth and uteri with no significant effect on incidences of polyneuritis. It was the opinion of the investigators that the greater amount of deleterious effects caused by sulfites is probably due the destruction of vitamins (Fitzhugh, Knudsen, and Nelson 1946).

The skulls and teeth from 43 rats of the previous study described above were utilized for the study of vitamin deficiencies (Fitzhugh, Knudsen, and Nelson 1946). The teeth were examined macroscopically for degree of pigmentation; the skulls were x-rayed, decalcified, and embedded in paraffin; and central sections of the incisors and molars were stained and impregnated with silver. Small doses of Sodium Bisulfite, up to 0.025%, caused a slight deficiency of pigmentation of the incisor and slight atrophy of the enamel organ; large doses ranging from 0.5% to 2.0% caused a pronounced lack of pigmentation of the enamel, sudden and atypical atrophy of the enamel organ often accompanied by edema, foldings of the dentino-enamel junction, atrophy and disturbed histodifferentiation of the odontoblasts, retardation and disturbance of dentin formation, invasion of the odontogenic epithelium into the pulp, thickening of the fundic alveolar bone, and keratinization of the epithelium of the nasolacrimal duct. Atypical atrophy and edema of the enamel organ were indicative of a vitamin E deficiency, whereas the atrophy of the odontoblasts, invasion of the odontogenic epithelium into the pulp, and metaplasia of the epithelium of the nasolacrimal duct were considered to be specific for a vitamin A deficiency (Irving et al. 1952).

In a study designed to evaluate the effects of preservatives alone, in combination, and with added stress factors, Shtenberg and Ignat'ev (1970) tested the survival rates, reproduction, and tumor incidences in crossbreed white and Wistar mice exposed to Sodium Bisulfite and benzoic acid over a 17-month period. Groups of 25 males and 25 females (initial body weight 10 to 15 g) and groups of 25 males and 25 females (initial body weight 16 to 20 g) received doses of Sodium Bisulfite (80 mg/kg/day), benzoic acid (40 mg/kg/day), and a Sodium Bisulfite/benzoic acid combination (80/40 mg/kg/day). Control groups were given no preservatives other than that present in the feed. After 8 months, the survival rate of mice receiving a combination of Sodium Bisulfite and benzoic acid was 28.5% for females and 44.4% for males in group I, and 55.3% for females and 35.4% for males in group II, compared to 60% for males and 62% for females of the control groups. After 17 months of sulfite treatment, 100% of the feed was restricted as an additional stress factor. None of the mice treated with Sodium Bisulfite (80 mg/kg) died after 5 days on the restricted diet. However, 51.5% of mice treated with the combination of Sodium Bisulfite and benzoic acid (80/40 mg/kg) and 50.0% treated with benzoic acid (40 mg/kg) alone died. These mortality rates were much greater compared to 12.5% for the controls. As for neoplasm incidence, 8/100 mice in the first generation and 1/8 mice in the third generation of the Sodium Bisulfite/benzoic acid group had malignant neoplasms. No neoplasms were reported in the control group. No information was provided on neoplasm incidences in the benzoic acid, the Sodium Bisulfite, or the sorbic acid groups.

Sodium Metabisulfite

In a study by Lockett and Natoff (1960), Sodium Metabisulfite was added to the drinking water (375 and 750 ppm as SO_2) of three generations of rats for 2.5 years. Generation I consisted of three groups: 13 females in each group with group 1 having 5 males and group two having 6 males. The control groups of generation II were produced from the matings of control groups of generation I. Likewise, the sulfite drinking water groups of generation II were produced from matings of the sulfite drinking water groups of generation I. Generation III was derived similarly from generation II. Observations on growth, feed consumption, fluid intake, fecal output, reproduction, lactation, and the incidence of tumors were recorded.

No significant difference was detected among growth rates of the control animals and sulfite drinking animals in any generation. However, each generation experienced an increase in growth compared to the previous one. A marked difference was observed between the generational consumption of feed and water, i.e., the rats of the third generation ate and drank twice as much as the first generation. Throughout the three generations, feed intake was unaffected, and only the sulfite-drinking females of the first generation maintained a greater intake of water as compared to all the other generations and groups. Feces output remained relatively stable among all generations and groups with one exception. The sulfite drinking females of generation III had a mean percentage that far exceeded that of the control's percentage. No significant difference was reported in the number of offspring of either generation I or II, and the proportion surviving to the end of lactation did not differ. Neither weight nor the percentage of weight contributed by various organs was affected. Microscopic examination of various tissues was completed ten months after treatment began. No abnormalities of the spleen, adrenal glands, stomach, ileum, colon, gastrocnemius muscle, sciatic nerve, uteri, testes, and seminal vesicles were observed. Thirty-seven percent of 54 animals had tumors. Incidences were greater among groups of females but unaffected by the addition of sulfite to the water (Lockett and Natoff 1960).

In a three-generation study, groups of 40 rats (20 each sex) received 0.125%, 0.25%, 0.5%, 1.0% or 2.0% Sodium Metabisulfite administered in a thiamine-rich diet beginning shortly after weaning (Til, Feron, and DeGroot 1972a). Diets were prepared frequently to control sulfite loss due to instability. Despite this

precaution, losses of 4.5% to 22% of sulfite and 2.7% to 15.4% of thiamine were measured.

Rats of the F_0 generation were mated during weeks 21 and 34 to produce F_{1a} and F_{1b} generations, respectively. Ten males and 10 females of the F_{1a} generation were selected for further mating. F_0 rats and the selected F_{1a} were fed the same diet for 104 weeks. The selected F_{1a} rats were mated during weeks 12 and 30; pups of the F_{2a} litters were selected for mating. The F_3 litters were discarded; their dams were fed the same diet for 30 weeks. Five males and five females of the F_0 generation were killed at week 52 for interim observations on organ weights and pathological changes. Dams of all generations were killed at the end of the study and necropsied.

A slight growth reduction was observed with 2% sulfite in the F_1 and F_2 generations and was ascribed to lower body weight of offspring. Relative kidney weight was increased in F_2 females of the 2% group but was not accompanied by functional or histopathologic renal changes. At doses of $\geq$1% Sodium Metabisulfite (300 and 600 mg sulfur dioxide/kg/day), inflammatory and hyperplastic changes in the stomach and occult blood in the feces were observed in rates of all three generations. Slight changes in the stomach of F_2 rats of the 0.5% group were observed. The number of F_{2a} pups was significantly reduced in groups fed $\geq$0.5% Sodium Metabisulfite. The no-effect level was 0.25% Sodium Metabisulfite (or 0.215% accounting for the loss of sulfite). The corrected value corresponded to 72 mg sulfur dioxide/kg/day (Til, Feron, and DeGroot 1972a).

The above detailed study was used by the JECFA to establish an ADI. (See Noncosmetic Use section of this report.)

Til et al. (1972b) conducted a similar study using groups of 40 guinea pigs (20 each sex) fed 0.06%, 0.16%, 0.35%, 0.83%, and 1.72% Sodium Metabisulfite. The protocol called for dosing between 0.125% and 2.0%, but the above values represent the calculated concentration present despite precautions to limit sulfite loss. Diets were supplemented with thiamine. After 15 weeks, 14 males and 14 females from each group were killed; the remaining guinea pigs were kept on their respective diets for an additional 33 weeks.

Thiamine concentrations in the urine and liver were markedly decreased in guinea pigs fed $\geq$0.16%; the added thiamine prevented deficiency in all but the highest-dose group. No adverse effects on health or hematological parameters were observed. In contrast to the rat study, occult blood was not detected in the feces. Guinea pigs of the 0.83% and 1.72% groups had decreased growth and decreased feed conversion that were considered due to reduced consumption of the less palatable diets. Organ-to-body weight ratios of the liver, kidneys, heart, and spleen were increased in the 0.83% and 1.72% dose groups; the increase in heart and spleen weights was attributed to the lowered body weights. Inflammatory and hyperplastic changes of the gastric mucosa were observed in several guinea pigs of the 0.83% and 1.72% groups. A black pigmentation of the cecal mucosa that resembled pseudomelanosis coli was also observed, but was not considered toxicologically significant. The no-effect level was 0.35% Sodium Metabisulfite in the diet for 48 weeks (Til et al. 1972b).

In a subsequent study, Feron and Wensvoort (1972) found hyperplastic and inflammatory changes in the nonglandular stomach of rats after feeding Sodium Metabisulfite at 0.5% to 8% for 10 to 56 days or 0.125% to 2% for up to 2 years. Diets were supplemented with thiamine and prepared and stored to minimize sulfite loss. Mild atrophic gastritis developed in some rats treated with 2% metabisulfite for 2 years. The no-effect level was 0.5%.

A more recent study by Hui et al. (1989) was designed to represent human exposure to sulfites. Sodium Metabisulfite and a bound form, acetaldehyde hydroxysulfonate, were added to the drinking water of female Sprague-Dawley rats. Rats in some groups were made sulfite oxidase deficient by the addition of tungsten to the drinking water. Six groups of eight animals (three enzyme-deficient groups and three normal groups) received Sodium Metabisulfite in the drinking water; another six groups received the bound form. The three sulfite doses (measured as sulfur dioxide equivalents) were 7 or 70 mg/kg/day for 8 weeks, or 350 mg/kg/day for 3 weeks followed by 175 mg/kg/day for the remaining 5 weeks. Doses were selected to be 10 to 500 times the ADI established by the WHO. Two control groups (one normal group containing three rats, and one group made enzyme deficient) received untreated water. Diets were fortified with thiamine.

Enzyme-deficient rats that received the largest dose of Sodium Metabisulfite had significantly reduced body weight at death ($p < 0.05$), although feed consumption for this group was not significantly different from that of other groups. These rats consumed significantly less water, a response to the altered taste resulting from the addition of sulfite and tungsten. Hematological parameters were comparable among rats. Dried blood was observed around the noses of sulfite-treated, enzyme-deficient rats beginning at week 4; lung edema was noted at necropsy. Gastric lesions were noted microscopically in rats of the highest-dose groups (metabisulfite and bound sulfite), and were more severe and numerous in enzyme-deficient rats. The no-effect level for Sodium Metabisulfite was 70 mg sulfur dioxide equivalent/kg/day for both normal and enzyme-deficient rats. Hepatic lesions were observed in rats treated with the bound sulfite and were considered possibly due to the free acetaldehyde. The no-effect level for acetaldehyde hydroxysulfonate was 7 mg/kg/day for enzyme-deficient rats and 70 mg/kg/day for normal rats. Enzyme-deficient rats treated with Sodium Metabisulfite had increased urinary excretion of sulfite and increased plasma *S*-sulfonate concentrations. Enzyme-deficient rats treated with the bound sulfite had increased urinary sulfite excretion but no change in plasma *S*-sulfonate concentrations. Neither substance was considered "very toxic"; the toxicity of bound sulfite was equivalent to that of Sodium Metabisulfite (Hui et al. 1989).

Potassium Metabisulfite

For 20 months, two groups of rats, 40 male and 40 female, were fed the same diet, and received either 1.2 g/L of Potassium Metabisulfite or distilled water. No differences between the groups in mortality, weight, feed intake, and organ weights were observed. However, an increase in leukocytes of males and an increase in the weight of the spleen of females were observed. The two successive generations produced a smaller number of young per litter and a smaller number of males than the control groups. However, growth was similar to that of the F_0 generation (Clauzan, Causeret, and Hugot 1965).

Acute Inhalation Toxicity

Sodium Sulfite

Noting the lack of inhalation studies available for forms of sulfur dioxide other than sulfurous acid, Chen et al. (1987) studied a Sodium Sulfite aerosol with a mass median aerodynamic diameter (MMAD) of 0.36 μm. Guinea pigs were exposed head-only for 1 h to 474, 669, and 972 $\mu g/m^3$ Sodium Sulfite aerosol. Respiratory mechanics were measured in unanesthetized animals before, during, and after exposure. Dose-related increases in resistance (50% increase at highest dose) and decreases in compliance (19% decrease at highest dose) were observed. Changes were present 1 h after exposure ended. Another group of guinea pigs was exposed whole-body to the same aerosol at 204, 395, and 1152 $\mu g/m^3$. After exposure, lung volume, diffusion capacity for carbon monoxide, and wet lung weight were evaluated in anesthetized, tracheotomized animals. Compared to controls, total lung capacity, vital capacity, functional residual capacity, residual volume, and diffusion capacity for carbon monoxide were all decreased in exposed guinea pigs. A dose-related increase in wet lung weight was found (Chen et al. 1987).

Ammonium Sulfite

Groups of eight guinea pigs were exposed head-only for 1 h to an ammonium sulfite/ammonium sulfate aerosol at concentrations of 50, 250, and 450 mg/m^3. The aerosol had an MMAD of approximately 2 to 3 μm and the pH was greater than 5; chemical composition was 60% to 80% sulfite with the remainder being sulfate. Sulfur dioxide concentrations were monitored and never exceeded 1 ppm; chamber ammonia gas concentrations exceeded 50 ppm throughout the study and occasionally reached 150 ppm. All guinea pigs survived the exposure. The median lethal concentration (LC_{50}) for ammonia sulfite exceeded 400 mg/m^3 (Rothenberg et al. 1986).

Beagle dogs (five female and three male) were exposed nose-only for 1 h to 1 mg/m^3 of aerosolized ammonium sulfite mixed with sulfate. Sulfur dioxide and ammonia gas concentrations were monitored and were less than 0.5 and 5 ppm, respectively. No significant difference was observed between preexposure and postexposure tracheal mucous clearance rates. Citing results of other studies, the investigators noted that ammonium sulfite seemed to be less toxic than sulfuric acid on an equivalent mass basis. The investigators also noted that ammonium sulfite was rapidly oxidized in air, thereby lessening its environmental health effects (Rothenberg et al. 1986).

Short-Term Inhalation Toxicity

Sodium Sulfite

Groups of six male Sprague-Dawley rats were exposed for 3 days to Sodium Sulfite aerosols at concentrations of 0.1, 1, 5, or 15 mg/m^3 (sulfur dioxide equivalents of 0.2 to 2.7 ppm). The particle size was $\sim$1 μm. Two control groups were exposed to either 15 mg/m^3 sulfate aerosol or filtered air. Responses were measured as follows: tracheal explants were cultured to measure glycoprotein secretion rates, lung homogenates were analyzed for protein, DNA and RNA concentrations, and the wet weight to dry weight ratios of the right apical lung lobes were determined. Increased glycoprotein secretion was observed in rats dosed with $\geq$5 mg/m^3, and increased wet to dry weight ratios of right apical lobes were observed in rats dosed with $\geq$1 mg/m^3. The investigators concluded that the rats responded with "mild pulmonary edema." Exposure to $\geq$5 mg/m^3 resulted in an irritation response by the tracheal epithelium. The investigators emphasized that their aerosol generation technique produced "well-characterized sulfite aerosols containing little or no contaminating [sulfur dioxide]." Earlier studies of sulfur dioxide gas were considered inadequate to evaluate sulfites, bisulfites, and metabisulfites because sulfur dioxide was removed by the upper respiratory tract and did not penetrate to the deep lung (Last, Dasgupta, and Etchison 1980).

Chronic Inhalation Toxicity

Sodium Metabisulfite

Eight male beagle dogs were continuously exposed to a 1 mg/m^3 metabisulfite aerosol for 290 days (Takenaka et al. 1990). The generation of the aerosol was detailed by Karg et al. (1988), who specified an MMAD of 0.63 μm. The extrapulmonary airway was examined microscopically following treatment. Three unexposed dogs were also examined. Hyperplastic foci were observed in the respiratory region of the posterior nasal cavity in seven exposed dogs. Changes included a thickened epithelial layer due to epithelial proliferation, loss of secretory material, and moderate mononuclear cell infiltration. One of three control dogs had slight focal secretory cell proliferation with mononuclear cell infiltration. Laryngeal changes characterized by a focal loss of cilia and slight subepithelial mononuclear cell infiltration were observed in four exposed dogs. Focal disappearance of ciliated cells in the transitional region between cartilaginous and membranous trachea was observed in exposed and control dogs. However, an increased number of nonciliated cells was also noted in the membranous portion of the trachea of exposed dogs and was not observed in control dogs. The tracheal changes, as observed in electron micrographs, were likely caused by a disorder in epithelial cell development rather than

by cell degeneration. Sulfite aerosols were considered to have adverse effects on the extrapulmonary airways of beagle dogs.

Dermal Irritation

Sodium Bisulfite

Sodium Bisulfite (0.5 ml of a 38% solution) was applied to the clipped backs of six albino rabbits. The material was applied under a gauze pad and the trunk of each rabbit was loosely wrapped with rubber sheeting for a total exposure time of 4 h. Sites were then washed and observations were made 24 and 48 h after initial application. Sodium Bisulfite was not corrosive (Haskell Labs 1973).

Sodium Metabisulfite

Sodium Metabisulfite (0.5 ml of an undiluted solution) was applied to the clipped backs of six albino rabbits. The material was applied under a gauze pad and the trunk of each rabbit was wrapped with a nonabsorbent binder for a total exposure time of 4 h. Sites were evaluated according to the Draize scale at the time of dressing removal, and 24 and 48 h later. Sodium Metabisulfite did not produce a primary irritation response (Hazleton Labs 1973).

Sodium Metabisulfite (solid, 5 g) was applied to clipped but intact sites on the trunk of six male albino rabbits. The material was applied under a gauze pad and the trunk of each rabbit was wrapped with rubber sheeting for a total exposure time of 24 h. Sites were evaluated at the time of patch removal and 24 h later according to the regulations of the *Federal Hazardous Substances Act*. Sodium Metabisulfite did not produce an irritation response (Haskell Labs 1974a).

Ten applications of a 50% Sodium Metabisulfite solution (0.5 ml) to the clipped backs of guinea pigs "moderately exacerbated the irritative response." Blackened or secondary eschars formed on all animals by the 10th day (no further details provided) (Eastman Kodak Co. 1980).

Ocular Irritation

Sodium Metabisulfite

Sodium Metabisulfite (100 mg) was placed into the right conjunctival sac of each of two rabbits. Twenty seconds later, one treated eye was rinsed with tap water for 1 min. The treated eye of the other rabbit was not rinsed. The cornea, iris, and conjunctiva were examined with a hand-slit lamp at 1 and 4 h and at 1, 2, 7, and 14 days. A biomicroscope and 5% aqueous fluorescein stain were used at the 1-day observation. A small area of mild corneal opacity, transient moderate congestion of the iris, and mild conjunctivitis was observed in the unrinsed eye. The opacity was reversible and the cornea was normal within 14 days, but mild conjunctival irritation persisted. Slight, reversible corneal opacity and mild conjunctivitis, but with no iritic involvement, were observed in the rinsed eye and that cleared within 3 days. The investigators recommended copious flushing with water following ocular contact with Sodium Metabisulfite (Haskell Labs 1974b).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Oral

Several oral-dose teratogenicity studies have been reported in which Sodium Sulfite, Bisulfite, and Metabisulfite or Potassium Metabisulfite were given to pregnant animals on certain gestation days (GDs). These studies are summarized in Table 7.

Sodium Sulfite

Groups of 12 pregnant Wistar rats were fed diets containing 0.32%, 0.63%, 1.25%, 2.5%, or 5% Sodium Sulfite heptahydrate ($Na_2SO_3 \cdot 7H_2O$) on GDs 8 to 20. Average daily intake of Sodium Sulfite heptahydrate was 0.3, 1.1, 2.1, and 3.3 g/kg. Maternal toxicity evidenced by decreased feed consumption and body weight gain was observed in rats of the 5% group. A significant ($p < 0.05$) reduction in fetal body weight was observed in all pups except females of the 2.5% group. The numbers of live fetuses, intrauterine deaths, or sex ratios of fetuses were comparable between treated and controls. External, skeletal, or internal malformations of the fetus were not observed at any dose. Fetal skeletal variations such as lumbar rib, hypoplastic rib, and delayed ossifications were noted in all treated groups, except the 1.25% group; these skeletal variations were not significant compared to controls. A slight increase in delayed ossification was observed with increasing doses. Fetuses with dilation of the renal pelvis and lateral ventricle were observed but the findings were not dose dependent. Postnatal body weights of offspring 3 weeks after birth indicated no evidence of growth retardation or other signs of toxicity. The investigators considered the administration of Sodium Sulfite heptahydrate to produced signs of fetal toxicity but not teratogenicity (Itami et al. 1989).

Sodium Bisulfite

Sodium Bisulfite was not teratogenic for mice, rats, hamsters, or rabbits at doses of 150, 110, 120, and 100 mg/kg, respectively (Food and Drug Research Labs 1972a, 1974a).

Sodium Metabisulfite

Sodium Metabisulfite was not teratogenic for mice, rats, hamsters, or rabbits at doses of 160, 110, 120, and 123 mg/kg, respectively (Food and Drug Research Labs 1972b, 1974b).

It was also negative in sulfite oxidase–deficient rats when tested at doses up to 3.5 mmol/kg (Dulak, Chiang, and Gunnison 1984).

Potassium Metabisulfite

Potassium Metabisulfite was not teratogenic for mice at 125 mg/kg or rats at 155 mg/kg (Food and Drug Research Labs 1975).

Groups of at least 21 pregnant Wistar rats received 0.1%, 1%, or 10% potassium metabisulfite on GDs 7 to 14. Some rats from each group were killed on day 20; the remaining were allowed to deliver and the offspring were reared until week 15. Maternal

TABLE 7
Sulfites, Bisulfites, and Metabisulfites oral-dose teratogenicity studies

Animal	Dosing protocol	Findings	Reference
		Sodium Sulfite (heptahydrate)	
Groups of 12 pregnant Wistar rats	0.3, 1.1, 2.1, and 3.3 g/kg in feed on GDs 8–20	Signs of fetal toxicity but not teratogenicity (see text)	Itami et al. 1989
		Sodium bisulfite	
Groups of at least 21 pregnant CD-1 mice	2, 7, 32, or 150 mg/kg in a water solution via oral intubation on GDs 6–15; caesareans on day 17	No adverse findings*	Food and Drug Research Labs 1972a
Groups of at least 22 pregnant Wistar rats	1, 5, 24, or 110 mg/kg on GDs 6–15; caesareans on day 20	No adverse findings*	Food and Drug Research Labs 1972a
Groups of at least 21 pregnant golden hamsters	1, 6, 26, or 120 mg/kg on GDs 6–10; caesareans on day 14	No adverse findings*	Food and Drug Research Labs 1972a
Groups of at least 11 Dutch-belted rabbits were artificially inseminated	1, 4.64, 21.6, or 100 mg/kg on GDs 6–18; caesareans on day 29	No adverse findings*	Food and Drug Research Labs 1974a
		Sodium Metabisulfite	
Groups of at least 21 pregnant CD-1 mice	2, 7, 34, or 160 mg/kg in a water solution via oral intubation on GDs 6–15; caesareans on day 17	No adverse findings*	Food and Drug Research Labs 1972b
Groups of at least 23 pregnant Wistar rats	1, 5, 24, or 110 mg/kg on GDs 6–15; caesareans on day 20	No adverse findings*	Food and Drug Research Labs 1972b
Sulfite oxidase–deficient rats (females treated with a high-tungsten–low-molybdenum diet to induce steady-state hepatic enzyme activity that was 1%–2% of levels in untreated rats)	Drinking water supplemented to achieve 25 or 50 mM sulfite concentrations; treated continuously from week 3 prior to mating and continued to GD 20. Highest daily intake was 3.5 mmol/kg	No treatment-related teratogenic changes compared to nonexposed rats with normal enzyme activity. A pilot study noted treatment-related anophthalmia in enzyme-deficient rats, but no intergroup differences were found in the teratogenicity study	Dulak et al. 1984
Groups of at least 20 pregnant golden hamsters	1, 6, 26, or 120 mg/kg on GDs 6–10; caesareans on day 14	No adverse findings*	Food and Drug Research Labs 1972b
Groups of at least 12 Dutch-belted rabbits were artificially inseminated	1.23, 5.71, 26.5, or 123 mg/kg on GDs 6–18; caesareans on day 29	No adverse findings*	Food and Drug Research Labs 1974b
		Potassium Metabisulfite	
Groups of at least 21 pregnant CD-1 mice	1.25, 5.47, 26.9, or 125 mg/kg via oral intubation on GDs 6–15; caesareans performed on GD 17	No adverse findings*	Food and Drug Research Labs 1975
Groups of at least 20 pregnant Wistar rats	1.55, 7.19, 33.4 or 155 mg/kg on GDs 6–15; caesareans performed on GD 20	No adverse findings*	Food and Drug Research Labs 1975
Groups of at least 12 pregnant Wistar rats	0.1%, 1%, or 10% on GDs 7–14; some rats from each group killed on day 20, remaining allowed to deliver, offspring reared until week 15	Fetal body weight significantly lower in 10% group, placental weight significantly lower in 1% group. No significant adverse teratogenic effects	Ema et al. 1985

*No adverse findings defined as "Neither adverse effects in maternal or fetal survival nor a significant increase in fetal abnormalities in either soft or skeletal tissues was noted in any of the animals." In these studies, positive controls in mice, rat, and hamster studies received aspirin and positive controls in rabbit studies received 6-aminonicotinamide; negative controls were sham-treated (Food and Drug Research Labs 1972a, 1972b, 1974a, 1974b, 1975).

feed intake and body weight gain were reduced in the 10% group but no other signs of toxicity were observed. Fetal body weight was significantly reduced in the 10% group, and placental weight was significantly lower in the 1% group. No significant teratogenic effects were observed (Ema, Itami, and Kanoh 1985).

Intraperitoneal (IP)

Sodium Bisulfite

A cytotoxicity study was conducted in which Sodium Bisulfite was given to adult male Swiss mice in either a single IP injection (500, 600, 700, 800, 900, or 1000 mg/kg), or repeated IP doses (20, 30, and 40 doses of 200 or 400 mg/kg in 28, 42, and 56 days, respectively). The total dose in the long-term study ranged from 4 to 16 g/kg. Mice were killed 1 to 3 days after the last dosing. The testes were dissected and the tunica was fixed and stained in periodic acid Schiff and counter stained with Ehrlich's acid hematoxylin. Different types of spermatogonia and preleptotene spermatocytes were scored on the basis of nuclear cytology and frequency of each stage of the tubules. Sodium Bisulfite did not alter the population of various types of spermatogonia. At 1000 mg/kg, 80% of the mice died within 24 h post treatment (Bhattacharjee, Shetty, and Sundaram 1980).

Sodium Metabisulfite

A sperm-shape abnormality assay was conducted using male inbred albino Swiss mice (Pal and Bhunya 1992). Groups of four mice received five IP doses of Sodium Metabisulfite each given 24 h apart. Total doses were 200, 300, or 400 mg/kg. Mice were killed 35 days after the first injection and the caudae epididymides and vas deferens were dissected and prepared into a suspension. Slides were prepared and stained and sperm abnormalities were categorized. A dose-dependent response was observed.

GENOTOXICITY

Genotoxicity studies cited in this section are detailed in Table 8. No studies were found regarding the Ammonium ingredients.

Sodium Sulfite

Sodium Sulfite was negative in plate and suspension tests using *Saccharomyces cerevisiae* and *Salmonella typhimurium* (Litton Bionetics 1975) and did not interfere with mitotic division of oocytes in mice (Jagiello, Lin, and Ducayen 1975).

Sodium Bisulfite

Under in vitro conditions, bisulfite deaminates the nucleoside cytosine to uracil in single-stranded DNA. The reaction proceeds rapidly at pH 5 to 6, with bisulfite solutions of $\geq$1 M (which are not normal physiological conditions) (Hayatsu et al. 1970; Shapiro 1983). Because the action is specific for cytosine and not other nucleosides, directed mutagenesis techniques using Sodium Bisulfite have been developed for use in the laboratory (Shortle and Botstein 1983; Merlo and Thompson 1987).

At lower concentrations, bisulfite can catalyze transaminations which lead to cross-linking of proteins with nucleic acids, or bisulfite can damage DNA by generating free radicals (Pagano and Zeiger 1987; Shapiro 1983).

Under acidic conditions, Sodium Bisulfite can induce mutations in *S. typhimurium* that contain *his* G46 (base-pair substitution sensitive) and *his* D6610 mutations (De Giovanni-Donnelly 1985; Pagano and Zeiger 1987), lambda phage (Hayatsu and Miura 1970), and some *Escherichia coli* strains (Mukai, Hawryluk, and Shapiro 1970; Kunz and Glickman 1983). At lower concentrations and neutral pH, Sodium Bisulfite was not mutagenic to *S. typhimurium* (SRI international 1978a) or *E. coli* (Mallon and Rossman 1981).

Sodium Bisulfite induced transformation (DiPaolo, DeMarinis, and Doniger 1981; Tsutsui and Barrett 1990) and sister-chromatid exchanges (SCEs) (MacRae and Stich 1979), but not chromosomal aberrations (Tsutsi and Barrett 1990) in hamster embryo or ovary cells. Sodium Bisulfite did not induce mutations in two loci in Chinese hamster V70 cells (Mallon and Rossman 1981; Tsutsui and Barrett 1990). It failed to increase DNA metabolism (which would have indicated DNA repair and mutagenesis) but did reduce the number of functioning replicons (Doniger, O'Neill, and DiPaolo 1982). The results suggested that Sodium Bisulfite induced hamster cell transformations through mechanisms other than mutation (DiPaolo, DeMarinis, and Doniger 1981; Doniger, O'Neill, and DiPaolo 1982).

Sodium Bisulfite induced SCEs and chromosomal aberrations in human lymphocytes (Beckman and Nordenson 1986; Meng and Zhang 1992).

Sodium Bisulfite was negative in all in vivo studies using mammalian systems (Generoso, Huff, and Cain 1978; Litton Bionetics 1972; SRI International 1979).

Sodium Metabisulfite

Sodium Metabisulfite was negative in an Ames/microsome assay (SRI International 1978b). It was negative in the host-mediated assay using mice to test mutagenicity against bacteria and yeast, the cytogenetic assay using rats (Litton Bionetics 1972), and a cytogenetic assay using sulfite oxidase–deficient hamsters and mice (Renner and Wever 1983). Results of one dominant lethal assay using rats indicated further testing was needed (Litton Bionetics 1972); another assay was negative (SRI International 1979).

Potassium Metabisulfite

Potassium Metabisulfite was negative for induction of chromosomal aberrations or SCEs in Chinese hamster cells. The highest dose, 1 mM, did produce an increase in SCE frequency but a twofold increase over control values was needed to be considered positive (Abe and Sasaki 1977).

TABLE 8
Mutagenicity studies on Sodium Sulfite, Bisulfite, and Metabisulfite

Assay	Method	Results	Reference
	Sodium Sulfite		
Salmonella typhimurium TA 1535, TA 1537, TA 1538	0.028% Sodium Sulfite tested ± activation	Negative	Litton Bionetics 1975
Suspension test with *S. typhimurium*	Bacteria mixed with 0.014% and 0.028% Sodium Sulfite ± activation for 1 h at 37°C, aliquots plated	Negative	Litton Bionetics 1975
Suspension test with *Saccharomyces cerevisiae* strain D4	Yeast cultures mixed with 2.5% and 5.0% Sodium Sulfite ± activation for 4 h (at 37°C with activation and 30°C without), aliquots plated	Negative	Litton Bionetics 1975
Induction of abnormality in mouse oocyte during preovulatory period (in vivo)	5 mg IV dose given to six mice during induced follicular enlargement and meiotic maturation (mice received pregnant mare's serum before and human chorionic gonadotropin after). Mice killed 38 h after oocytes removed	Negative (structural chromosomal damage noted in in vitro studies where bisulfite was incubated with mouse oocytes)	Jagiello et al. 1975
	Sodium Bisulfite		
Lambda phage with *c* gene mutation	1.5 h exposure; 3 M Sodium Bisulfite (pH 5.6)	Positive	Hayatsu and Miura 1970
S. typhimurium TA 98, TA 100, TA 1535, TA 1537, and TA 1538 and *E. coli* WP2	33.3, 100.0, 333.3, 1000.0, 3333.3, and 10,000.0 μg Sodium Bisulfite/plate (in a neutral buffer) ± activation	Negative; toxicity observed in some strains at highest doses	SRI International 1978a
S. typhimurium G46, TA 98, TA 100, TA 1535, and TA 1538	64000 μg Sodium Bisulfite/ml (pH 5.9) no activation	Positive in G46	Münzner 1980
S. typhimurium LT2 with *his* G46 mutation (base pair substitution)	1 M Sodium Bisulfite in pH 5.2 sodium acetate	Positive (results strongest in bacteria with WT DNA repair)	DeGiovanni-Donnelly 1985
S. typhimurium his strains, D6610, G46, G428, C3076, and D3052	5120 μg Sodium Bisulfite/ml (pH 5–6)	Positive in G46 D6610; negative in others	Pagano and Zeiger 1987
E. coli K12 and 15	30 min exposure; 1 M Sodium Bisulfite (pH 5.2)	Positive	Mukai et al. 1970
E. coli B cells (repair proficient)	15 min exposure; 0.1 M Sodium Bisulfite (unknown pH)	Negative	Mallon and Rossman 1981
*E. coli lac*I system (repair-proficient) and ung$^-$, dcm$^-$, recA, and repair-deficient strains	1 M Sodium Bisulfite at pH 5.2-6.0	Negative (toxicity observed)	Kunz and Glickman 1983
Transformation of hamster embryo and C3H/10T-1/2 mouse cells	0.5, 2.5, 5, and 100 ppm Sodium Bisulfite (pH not reported)	Negative for transformation	Borek et al. 1985
Transformation of SHE cells	15 min exposure to 1, 5, 10, or 20 mM Sodium Bisulfite (neutral pH)	Positive, dose dependent (60% lethality observed with 20 M)	DiPaolo et al. 1981

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TABLE 8
Mutagenicity studies on Sodium Sulfite, Bisulfite, and Metabisulfite *(Continued)*

Assay	Method	Results	Reference
Transformation and metaphase chromosome analysis of SHE cells (ouabain resistance and HGPRT loci observed)	Cells treated for 15 min or 48 h with 5–20 mM Sodium Bisulfite (neutral pH).	Positive for transformation (dose-dependent increase); negative for induction of gene mutation; SCEs noted at 48 h	Tsutsui and Barrett 1990
SCE in CHO cells	2 and 24 h exposure; 3×10^{-5} M to 7.3×10^{-3} M Sodium Bisulfite (neutral pH)	Positive	MacRae and Stich 1979
Chinese hamster V79 cells (ouabain resistance and HGPRT loci observed)	15 min exposure to 10 or 20 mM Sodium Bisulfite or 48 h to 1 and 5 mM Sodium Bisulfite (neutral pH)	Negative	Mallon and Rossman 1981
Inducement of DNA repair responses associated with DNA damage and mutagenesis in SHE cells	15 min exposure; 20 or 50 mM Sodium Bisulfite (neutral pH)	Negative (failed to induce detectable levels of repair replication or DNA strand breaks, but functioning replicons were decreased in number)	Doniger et al. 1982
Human lymphocytes measured for CA and SCE	25 μg/ml	Positive	Beckman and Nordenson 1986
Human lymphocytes measured for CA, SCE, MN	Test of sulfur dioxide used a Sodium Bisulfite: Sodium Sulfite solution in a 1:3 ratio 5×10^{-5} M to 2×10^{-3} M (neutral pH)	Positive: dose-dependent increase in SCE and MN, induced mitotic delays and decreased mitotic index. Low doses produced chromatid-type aberrations; high doses produced both chromatid and chromosome-type aberrations.	Meng and Zhang 1992
Host-mediated assay using mice and testing mutagenicity against *S. typhimurium* TA 1530 and G-46 and *S. cerevisiae* D3	Groups of 10 mice received either a single dose (acute assay) or daily doses for five days (subacute assay) of Sodium Bisulfite (1.5, 15.0, and 150.0 mg/kg) by oral intubation. Following dosing, mice received an IP dose of bacteria and yeast. Mice were killed, saline was introduced IP, fluid was aseptically removed from the peritoneal cavity, and the recovered bacteria and yeast were diluted and plated.	Negative (an in vitro was also done and Sodium Bisulfite increased recombinant frequencies in the yeast)	Litton Bionetics 1972
Cytogenetic assay using male albino rats	Single dose (acute assay) or daily doses for five days (subacute assay) of Sodium Bisulfite (1.5, 15.0, and 150.0 mg/kg) by gastric intubation. Colcemid administered IP prior to killing (to arrest bone marrow cells in metaphase). Cells were analyzed for chromatid and chromosome gaps and breaks and other aberrations	Negative (also negative in an in vitro study of anaphase chromosomes of human tissue cell cultures)	Litton Bionetics 1972

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TABLE 8
Mutagenicity studies on Sodium Sulfite, Bisulfite, and Metabisulfite *(Continued)*

Assay	Method	Results	Reference
Dominant lethal assay using random bred rats	Groups of 10 male rats received either a single dose (acute assay) or daily doses for 5 days (subacute assay) of Sodium Bisulfite (1.5, 15.0, or 150.0 mg/kg) by oral intubation. Males were mated with nondosed virgin female rats. Females were then killed and the uterus examined for deciduomata, late fetal deaths, and total implantations	Negative	Litton Bionetics 1972
Dominant lethal using Sprague-Dawley rats	Male rats fed Sodium Bisulfite (4.5, 15.0, or 45.0 mg/kg/day) for 10 weeks. Mated for 7 days with two groups of two nondosed females. Females killed and pregnancy parameters measured	Negative (body weight gain significantly lower for high-dose males)	SRI International 1979
Translocation and dominant-lethal studies using (101 × C3H) F_1 mice	Male mice treated with IP dose of 300 or 400 mg/kg/day of Sodium Bisulfite for a total of 38 and 20 doses, respectively. In translocation study, mice were mated with two sets of two females. Male progeny were weaned and tested for translocation heterozygosity. In dominant-lethal study, males were mated with females at various intervals up to 14.5 days after last injection. This assay was also conducted using females that had received a single injection of 550 mg/kg Sodium Bisulfite and were mated with untreated males within 4.5 days after treatment	Negative in translocation study, no evidence of partial sterility in 858 male progeny	Generoso et al. 1978
	Sodium Metabisulfite		
Ames: *S typhimurium* TA 1535, TA 1537, TA 1538, TA 98, and TA 100; *E. coli* WP2	0.3, 3.3, 33.3, 100, 333, 1000, 3333 and 10000 μg Sodium Metabisulfite/plate (in a neutral buffer) (±) activation	Negative. Toxicity observed in TA 1535 and TA 100 and in one assay on WP2	SRI International 1978b
Host-mediated: mice used to test *S. typhimurium* G46 and TA 1530 and S. *cerevisiae* D3	30 mg/kg, 0.7 g/kg, 1.2 g/kg Sodium Metabisulfite (acute and subacute dosing; protocol not included)	Negative	Stanford Research Institute 1972
Cytogenetics using rats	30 mg/kg, 0.7 g/kg, 1.2 g/kg Sodium Metabisulfite (all doses tested at 6, 24, and 48 h, and a single subacute dosing test done; protocol not included)	Negative (in vitro testing of 2.5, 25, and 250 μg/ml on human embryonic lung cells found mitotic inhibition and damage to anaphase cells)	Stanford Research Institute 1972
Sulfite oxidase–deficient Chinese hamsters and NMRI mice, tested for SCE, CA, and MN	330 or 600 mg/kg Sodium Metabisulfite given as a single or double oral dose (both dose levels) in solution or juice, or by repeated SC injection up to the MTD	Negative	Renner and Wever 1983

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TABLE 8
Mutagenicity studies on Sodium Sulfite, Bisulfite, and Metabisulfite *(Continued)*

Assay	Method	Results	Reference
Bone marrow CA assay using adult inbred albino Swiss mice	(a) 400 mg/kg given IP; killed at 6, 24, and 48 h (b) 200, 300, or 400 mg/kg given IP; killed at 24 h (c) 400 mg/kg given IP/SC/PO; killed after 24 h (d) five IP doses of 80 mg/kg; 24 h between doses; killed at 120 h after first dose	Greatest effect seen in IP dosed group; least seen in PO group. Most CA observed at 24 h; least observed at 6 h. fractionated dose produced less effect than acute	Pal and Bhunya 1992
MN test using adult inbred albino Swiss mice	Two IP doses (200, 300, or 400 mg/kg) 24 h apart; killed at 6 h after 2nd dose; Bone marrow PCEs and NCEs analyzed	Non–dose-dependent results; frequency of MN was greatest in PCEs and lowest in NCEs (except at 200 mg dose)	Pal and Bhunya 1992
Dominant lethal using rats	30 mg/kg, 0.7 g/kg, 1.2 g/kg Sodium Metabisulfite (single and multiple doses, protocol not included)	No consistent differences compared to negative controls at $p < .01$, .05, and .1, but significance noted at $p < .20$, further testing advised	Stanford Research Institute 1972
Dominant lethal using rats	125, 416.7, or 1250 mg Sodium Metabisulfite/kg in the feed for 10 weeks. Thiamine added to diet and diets prepared weekly. Males mated with two sets of two nondosed females. Females killed and uteri examined	Negative	SRI International 1979
	Potassium Metabisulfite		
SCE in Chinese hamster cells	0.1, 0.5, and 1 mM	Negative "dosage effect"—dose increase did not produce twice as many CAs or SCEs as controls. Significant increase (at 5% level) in SCE with 1 mM. Mitotic inhibition increased to more than 50% of control values with doses >0.5 mM	Abe and Sasaki 1977

SC, subcutaneous(ly); IP, intraperitoneal(ly); PO, oral(ly); MTD, maximum tolerated dose; WT, wild type; HGPRT, hypoxanthine guanine phosphoribosyltransferase; SHE, Syrian hamster embryo; CHO, Chinese hamster ovary; SCE, sister chromatid exchange; CA, chromosomal aberration; MN, micronuclei; PCEs, polychromatic erythrocytes; NCEs, normochromatic erythrocytes.

Comutagenicity

Sodium Sulfite (1 to 20 mM) added to cell cultures prior to the addition of anti-BPDE (the carcinogenic form of benzo[a]pyrene, B(a)P) enhanced the mutagenic activity of the diol epoxide in *S. typhimurium* TA98 and TA100 (Reed, Ryan, and Adams 1990) and in Chinese hamster V79 cells (Reed and Jones 1996). DNA binding of ^{3}H-anti-BPDE demonstrated that Sulfite increased the efficiency of processes leading to DNA modification by the diol epoxides.

Mallon and Rossman (1981) reported that Bisulfite was comutagenic with UV against Chinese hamster V79 cells.

The combined effect of Sodium Bisulfite and a nitrogen nucleophile, i.e., semicarbazide, methoxyamine, or hydroxylamine was investigated. Hayatsu (1977) reported that Sodium Bisulfite and a nitrogen nucleophile chemically modify cytosine significantly faster than using either of the reagents alone. Inactivation and mutation of bacteriophage lambda was also observed when treated with Sodium Bisulfite and a nitrogen nucleophile. It was

concluded that mutation and inactivation of a bacteriophage is the result of a cooperative action of the reagents upon DNA and not a result of the interaction between reagents.

Antimutagenicity

In various *S. typhimurium* strains without the addition of metabolic activation, Sodium Sulfite, Sodium Bisulfite, and Potassium Metabisulfite suppressed the mutagenicity of Maillard reaction products (Kim et al. 1991) and instant and freshly brewed coffee (Suwa et al. 1982). Sodium Bisulfite "effectively" inhibited the mutagenic activity of *N*-methyl-*N'*-nitro-*N*-nitro-soguanidine (MNNG) but had no effect on the mutagenicity of *N*-acetoxy-2-acetylaminofluorene (Rosin and Stich 1979). Sulfites also prevented the induction of lambda prophage, and suppressed the mutagenicities of 1,2-dicarbonyls (Suwa et al. 1982).

In mammalian cell systems, Sodium Bisulfite suppressed the mutagenicity of coffee in hamster lung cells (Nakasato et al. 1984), the mutagenicity of B(a)P or x-ray irradiation in C3H/10T-1/2 mouse cells (Borek, Ong, and Mason 1985), the induction of SCEs by coffee in AUXB1 cells (Tucker et al. 1989a), and the induction of SCEs and the proportion of endoreduplicated cells (ERCs) by glyoxal, methylglyoxal, kethoxal, and diacetyl in Chinese hamster ovarian (CHO) cells (Tucker et al. 1989b).

CARCINOGENICITY

A review of sulfur dioxide, Sodium Sulfite, Sodium Bisulfite, and Sodium and Potassium Metabisulfites by the International Agency for Research on Cancer (IARC) (1992) concluded that there is inadequate evidence for the carcinogenicity in humans of sulfur dioxide, sulfites, bisulfites, and metabisulfites, there is limited evidence for the carcinogenicity in experimental animals of sulfur dioxide, and there is inadequate evidence for the carcinogenicity in experimental animals of sulfites, bisulfites, and metabisulfites. The overall evaluation: Sulfur dioxide, sulfites, bisulfites, and metabisulfites are not classifiable as to their carcinogenicity to humans (group 3).

In reaching this conclusion, IARC considered the oral dose carcinogenicity and cocarcinogenicity studies detailed in this report. In addition, IARC also evaluated inhalation studies that tested sulfur dioxide. A significant increase in lung adenomas and carcinomas developed in female LX mice following exposure to 500 ppm sulfur dioxide (1310 mg/m^3) for 5 min per day, 5 days per week for life compared to nonexposed control females. Two rat studies established a cocarcinogenic relationship between sulfur dioxide and B(a)P. In these studies groups were exposed to sulfur dioxide alone (at lower doses than in the mouse study) and no lung carcinomas were found in these rats.

IARC also reviewed several epidemiological studies that evaluated occupational exposure in copper smelters and sulfite pulp mills. These studies could not establish a clear relationship between sulfur dioxide exposure and cancer risk. No study was available regarding risk associated with sulfites, bisulfites, or metabisulfites (IARC 1992).

Sulfur Dioxide

In a study conducted by Meng and Zhang (1990), SO_2 gas produced significantly greater incidences of chromosomal aberrations and SCEs in peripheral blood lymphocytes among factory workers compared to nonexposed SO_2 subjects. It was noted, however, that the time of service in the factory and the aberrations or SCE had no direct correlation.

Oral

Sodium Metabisulfite

In the three-generation study detailed in the Oral Toxicity section of this report, no evidence of carcinogenicity was found in rats that were fed up to 2% Sodium Metabisulfite (Til, Feron, and DeGroot 1972a).

Potassium Metabisulfite

Groups of 100 ICR/JCL mice (50 each sex) received 1% or 2% Potassium Metabisulfite in the drinking water for 24 months. A control group received distilled water. The 2% dose was the maximum tolerated dose determined by subacute toxicity testing. Mice were necropsied at death or at the termination of the study. Ninety-nine of the mice of the control group survived beyond 180 days; 96 mice of the 1% group survived, and 94 mice of the 2% group survived. No significant difference in tumor incidence was observed between treated and control mice. Total tumor incidence was 14.1% for the control group, 14.6% for the 1% dose group, and 17.0% for the 2% dose group (Tanaka et al. 1979).

Parenteral

Sodium Bisulfite

Popescu and DiPaolo (1988) reported that hamster fetal cells that had been transformed by Sodium Bisulfite produced tumors in nude mice after subcutaneous (SC) inoculation. The latency period was 15 to 20 days. Tumorigenic cell lines were chromosomally abnormal (numerical and structural alterations). Three developing tumors preserved the karyotypic pattern of the inoculated transformed cells (with secondary alterations associated with tumor progression). Citing results of mutagenicity studies, the investigators noted, "despite this lack of or limited DNA-damaging potential, all bisulfite-transformed lines had structural rearrangements common for (hamster fetal cells) transformed by potent clastogenic carcinogens." The chromosomal abnormalities were not directly attributed to Bisulfite, but inhibition of DNA replication by Bisulfite (reported by Doniger, O'Neill, and DiPaolo 1982) was considered a contributing factor. Sodium Bisulfite was considered a nonclastogenic carcinogen.

Sodium Bisulfite caused neoplastic transformation of Syrian hamster fetal cells and was associated with qualitative and quantitative polypeptide changes. Seven malignant lines had four polypeptide changes: two polypeptides shifted slightly to the acidic side, one new polypeptide was observed, and one polypeptide was absent. Transformed bisulfite lines differed

from controls in that 10% to 25% and 2% to 4% of the polypeptides had differences in expression greater than two- and four-fold respectively. Twenty-one specific polypeptides in all transformed lines had coordinate quantitative changes. No differences were found in the polypeptides of controls and bisulfite treated expressed immediately or 48 h after the treatment. The lack of differences was attributed to the fact that Sodium Bisulfite does not induce detectable DNA damage or early post-treatment polypeptide changes. All changes in polypeptide expression were observed after transformation (Wirth et al. 1986).

COCARCINOGENICITY

Oral

Potassium Metabisulfite

In a two-stage stomach carcinogenesis experiment, male outbred Wistar rats were given MNNG in the drinking water and sodium chloride in the feed for 8 weeks. They then received drinking water containing 1% Potassium Metabisulfite (or other test substances) for 32 weeks. Animals were killed for necropsy and tissue was collected. Potassium Metabisulfite significantly ($p < 0.05$) increased the incidence of adenocarcinoma of the pylorus of the glandular stomach after initiation with MNNG and sodium chloride compared to controls (initiated rats that had not received treated water). No carcinomas developed in rats given Potassium Metabisulfite without MNNG or sodium chloride. Potassium Metabisulfite was considered to exert tumor-promoting activity in the rat glandular stomach (Takahashi and Hasegawa 1985; Takahashi et al. 1986).

Sodium Bisulfite

In a study performed by Dinerman and Ignat'ev (1966), 365 mice of both sexes were divided into five groups with one control. Four of the groups received doses of the food preservatives, Sodium Bisulfite (0.4%), benzoic acid (0.2%), Sodium Bisulfite with benzoic acid, and sorbic acid at concentrations similar to those consumed by humans. For 3 months, the test animal ingested the preservatives, and then were injected intraperitoneally with Ehrlich's ascites carcinoma. The observation period of all the mice was 53 to 66 days; afterwards surviving mice were killed for necropsy, and the amount of ascitic fluid and blood content was determined. The group of mice receiving the 0.4% dose of Sodium Bisulfite had the greatest incidence of tumors, the shortest survival time, and the greatest volume of ascitic fluid. These data led to the conclusion that "the addition of Sodium Bisulfite and Benzoic Acid to the rations of the mice facilitated a more intensive development of Ehrlich's ascites carcinoma."

CLINICAL ASSESSMENT OF SAFETY

Sulfite Sensitivity

Many asthmatics with bisulfite sensitivity have negative allergy skin tests suggesting a nonatopic nature. Twarog and Leung (1982) reported that immunoglobulin E (IgE), total eosinophil counts, and histamine concentrations were normal during acute reactions, suggesting the lack of an IgE mechanism.

One case study reported by Pirila, Kajanne, and Salo (1963) discussed a 46-year-old carpenter exposed to sulfur dioxide gas. The patient complained of eruptions on his forearms that, within 5 days, spread to all his extremities; also, his eyelids were swollen. The patient was diagnosed with symmetrical exanthema. Two positive exposure tests confirmed the reactions were due to sulfur dioxide. Approximately 2% to 5% of asthmatics are estimated to be sulfite sensitive; most sulfite-sensitive individuals are asthmatics. Sulfite-sensitive asthmatics react to ingestion or parenteral administration of sulfites. Asthmatics in general are more sensitive to inhaled sulfur dioxide (tested as Sodium Metabisulfite) than are nonasthmatic normal subjects (Koepke, Staudenmayer, and Selner 1985; Wright et al. 1990), but inhalation sensitivity alone is not considered indicative of sulfite sensitivity (Gunnison and Jacobsen 1987). In the majority of instances, manifestations include dermatologic signs and symptoms such as urticaria, angioedema, hives and pruritus, flushing, tingling, and swelling. Respiratory signs and symptoms include dyspnea, wheezing, and bronchoconstriction, and gastrointestinal symptoms include nausea and gastric cramps. Bronchoconstriction is a common reaction in steroid-dependent asthmatics. Less common are hypotension, cyanosis, diaphoresis, shock, and loss of consciousness. Clinical management involves avoidance of sulfited food and beverages and pharmaceuticals by people at high risk (Jamieson et al. 1985; Simon 1986; Lester 1995).

Yang, Purchase, and Rivington (1986) reported that results of skin tests, provocative oral challenge test, and passive transfer tests suggested that some metabisulfite-sensitive reactions can be IgE mediated.

Corder and Buckley (1995) studied a tertiary-referral clinic population to estimate safe exposure doses for use in epidemiological studies of acute versus allergic reactions. A positive response was defined as a 15% decrease in the amount of air expired in 1 s following ingestion of the substance. The median effective molar dose for Sodium Metabisulfite was 34.4 mg (0.19 mM). The most sensitive persons (5% of population) might respond to 4.6 mg Sodium Metabisulfite and practically all (95%) susceptible persons might respond to 255.8 mg.

Oral Toxicity

Sodium Bisulfite

Twelve volunteers (six males, six females) were placed on a thiamine deficient diet for 15 days. Six of the volunteers then received 400 mg of sulfur dioxide per day in beverages (50 mg as Sodium Bisulfite, 350 mg as sodium glucose sulfonate) for 25 days. The other six received beverages without added sulfur dioxide. Sulfite administration was then discontinued for 10 days and all subjects were given 100 mg thiamine orally on each of 2 days. Neither clinical changes (including neurophysiological

changes in motor conduction, and reflex action) nor changes in blood serum parameters (thymol turbidity, hematocrit, and erythrocyte count) was noted (Hötzel et al. 1969).

Contact Dermatitis

Sodium Sulfite

Petersen and Menné (1992) patch tested 1762 dermatologic patients with Sodium Sulfite 1% petrolatum (pet.). Following 2 days of occlusive exposure, positive reactions were observed in 25 patients (1.4% incidence). Seven of the 25 tested positive only to Sodium Sulfite (the European standard series was also tested). Only 3 of the 25 patients had previous contact with ketoconazole cream (contains Sodium Sulfite). The investigators did not consider it worthwhile to routinely patch test with Sodium Sulfite because the "clinical relevance of the positive reactions to sodium sulfite remains to be established."

A hair-coloring agent with 0.64% Sodium Sulfite was used in a repeat insult open patch test involving 100 participants. The panelists recieved 0.2 ml or 0.2 g of the test material directly onto a designated area of the back. The procedure was repeated until nine consecutive applications had been made for every Monday, Wednesday, and Friday for 3 consecutive weeks. Reactions were scored just before the next application. The panelists were then allowed a 10- to 14-day nontreatment period, after which a challenge or retest application was applied once to a previously unexposed site. Retest doses were equivalent to any of the original nine exposures and were scored 24 and 48 h after application. Comparisons were made between the sensitizing doses and the retest doses. No adverse reactions were observed and according to the investigators, the test material can not considered a primary irritant or primary sensitizer (Combe Incorporated 1996).

Samples of 0.5% Sodium Sulfite in a topical feminine cream were patch tested using 100 panelists. The semiocclusive patch, containing 0.2 ml or 0.2 g of the test material, was affixed directly onto the back and removed after 24 h. The procedure was repeated until nine consecutive applications had been made for every Monday, Wednesday, and Friday for 3 consecutive weeks. Reactions were scored just before the next application. The panelists were then allowed a 10- to 14-day nontreatment period, after which a challenge or retest application was applied once to a previously unexposed site. Retest doses were equivalent to any of the original nine exposures and were scored 24 and 48 h after application. No adverse reactions were observed and according to the investigators, the test material cannot be considered a primary irritant and primary sensitizer (Combe Incorporated 1998).

Sodium Metabisulfite

Vena, Foti, and Angelini (1994) reported the results of patch testing 2894 eczematous patients over a 2-year period. Positive reactions to Sodium Metabisulfite 1% pet. (following a 2-day occlusive exposure) were noted in 50 patients (1.7% incidence). All 50 patients also reacted to Potassium Metabisulfite 1% pet., and to Sodium Bisulfite 1% and 5% pet. Only two reacted to Sodium Sulfite 1% pet. Prick and intradermal tests of 20 patients with a Sodium Metabisulfite solution (10 mg/ml) were negative and oral challenge of five patients with 30 and 50 mg Sodium Metabisulfite did not provoke a flare-up of dermatitis or patch test. The dermatitis was considered occupational in seven cases. Five of the remaining 43 cases were considered allergic contact dermatitis resulting from the use of topical preparations.

Ocular Toxicity

Sodium Metabisulfite

A double-blind study tested the five individual components of an eye drop therapy for glaucoma. Sodium Metabisulfite was tested at 0.075%, the concentration of use in the preparation. The participants were five male patients with elevated intraocular pressures and histories of local sensitivity reactions to dipivalyl epinephrine (the active component of the eye drops). None had positive reactions to initial patch testing with the five components of the eye drops (Sodium Metabisulfite was patch tested at 0.5%). Patients applied two drops of a preparation twice daily for 1 week with a 1-week treatment-free period between application of different solutions. The order of administration of the five preparations was randomly assigned. Patients were instructed to stop using the drops and report to the study ophthalmologists upon development of any adverse ocular reactions. No adverse effects were reported with Sodium Metabisulfite (Petersen et al. 1990).

Intravenous Toxicity

Low pH and the presence of Sodium Bisulfite were considered partially responsible for the prolonged sensory-motor deficits observed in a few patients following large intrathecal doses of certain local anesthetics (Covino 1988).

Published reports described isolated cases of seizures associated with IV administration of large-doses of morphine containing Sodium Bisulfite as a preservative (Gregory, Grossman, and Sheilder 1992; Meisel and Welford 1992).

SUMMARY

Sodium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Potassium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are inorganic salts. All seven ingredients function as reducing agents in cosmetic formulations. All except Sodium Metabisulfite also function as hair-waving/straightening agents. In addition, Sodium Sulfite, Potassium Sulfite, Sodium Bisulfite, and Sodium Metabisulfite function as antioxidants. Ammonium Sulfite was not reported being used in 1998. The other five ingredients were collectively used in 1319 cosmetic formulations. Of these, 1249 uses were in hair dyes and colors or hair tints. It is important to note that none of the sulfites or bisulfites are used in aerosols or sprays.

Sodium Sulfite, Sodium Bisulfite, Potassium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are defined

as "sulfiting agents" because they liberate sulfur dioxide under certain conditions. The presence of sulfur dioxide in the air is regulated by the EPA, and use of sulfiting agents in foods and pharmaceuticals is regulated by the FDA.

Sulfites that enter mammals via ingestion, inhalation, or injection are metabolized by sulfite oxidase to sulfate. The activity of sulfite oxidase is 20 times greater in rats compared to humans.

In oral-dose animal toxicity studies that provided supplemental dietary thiamine and guarded against sulfite loss, the NOAELs were 0.215% to 0.5%. Hyperplastic changes in the gastric mucousa were the most common finding in the rats given the larger doses. A study that used sulfite oxidase–deficient rats reported a NOAEL of 7 g sulfur dioxide equivalent/kg/day for a bound form of Sodium Metabisulfite. The study was designed to represent human exposure to sulfites in foods.

Ammonium Sulfite aerosol (MMAD of 2 to 3 μm) had an acute LC_{50} of >400 mg/m^3 in guinea pigs. A single exposure to low concentrations of a Sodium Sulfite aerosol (MMAD of 0.36 μm) produced dose-related changes in the lung capacity parameters of guinea pigs. A 3-day exposure of rats to a Sodium Sulfite aerosol (particle size of $\sim$1 μm) produced: mild pulmonary edema following exposure to 5 mg/m^3, and irritation of the tracheal epithelium with 15 mg/m^3. Severe epithelial changes were observed in dogs exposed for 290 days to 1 mg/m^3 of a Sodium Metabisulfite aerosol (MMAD of 0.63 μm).

Sodium Bisulfite (tested at 38%) and Sodium Metabisulfite (undiluted) were not irritants to rabbits following occlusive exposures of $\leq$24 h. Sodium Metabisulfite (tested at 50%) was irritating to guinea pigs following repeated exposure.

Sodium Sulfite and Sodium Metabisulfite absorb light at 209 nm. Under in vitro conditions, these two ingredients were considered phototoxic in the UVB range.

Numerous oral-dose reproductive and developmental toxicity studies have been conducted. In rats, Sodium Sulfite heptahydrate at large doses (up to 3.3 g/kg) produced fetal toxicity but not teratogenicity. Sodium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite were not teratogenic for mice, rats, hamsters, or rabbits at doses up to 160 mg/kg.

Generally, Sodium Sulfite, Sodium Metabisulfite, and Potassium Metabisulfite were negative in genotoxicity studies. Sodium Bisulfite produced both positive and negative results. The sulfiting agents could enhance or attenuate the mutagenic action of other chemicals depending on experimental conditions.

IARC concluded that Sodium Sulfite, Sodium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite were not classifiable (group 3) as to their carcinogenicity for humans.

Between 2% and 5% of asthmatics are sulfite sensitive. The FDA established regulations regarding use of sulfiting agents in order to minimize the hazards to this population. Clinical oral and ocular exposure studies reported no adverse effects. The Sodium and Potassium salts produced positive reactions in dermatologic patients under patch test.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel determined that the data provided in this report are sufficient to assess the safety of the tested ingredients: Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite. The Panel recognized that Sodium Bisulfite caused multiple positive results in mutagenicity tests, yet other ingredients were not mutagenic. In an attempt to understand these data, the Panel considered the equilibrium chemistry of sulfurous acid, sulfur dioxide, bisulfite, sulfite, and metabisulfite. At very low pHs, a pH $<$2, the gas sulfur dioxide is emitted and when water is added sulfuric acid predominates. However, as the pH increases and reaches a neutral state, the equilibrium shifts and bisulfite predominates ($\sim$100% at pH 4.5). It is important to note that metabisulfite is the dehydration product of two molecules of the bisulfite ion. So when bisulfite is in a nonaqueous environment or where water is sequestered, metabisulfite is the product. As the pH increases further, more sulfite ions are produced and bisulfite and sulfite are in equilibrium at pH 7.3. Raising the pH further only increases the sulfite form. The sulfite ion is readily bound to an aldehyde to form carbonyl compounds. This reaction is reversible, but at physiological pH acetaldehyde is favored.

The Panel reviewed this information and agreed that the equilibrium chemistry and the genotoxicity data did not give a clear, consistent picture. Only Sodium Bisulfite had positive genotoxic results; Sodium Sulfite and Sodium Metabisulfite had all negative responses. The Panel considered it significant that all in vivo Sodium Bisulfite genotoxicity data were negative; only in vitro studies gave positive results. The mechanism that caused the positive in vitro responses is unclear. In addition, the bisulfite form is used at very low concentrations (0.03% to 0.7%) in most products except wave sets. However in wave sets, the pH ranges from 8 to 9 where the sulfite form would predominate. It is also important to note that mammals have the enzyme, sulfate oxidase, that converts all sulfite to sulfate. The sulfite and sulfate forms are of least concern regarding genotoxicity. In addition the Panel argued that there would be relatively low penetration due the highly charged nature of these particles. As used in cosmetics, therefore, these ingredients would not present a genotoxic risk.

Incidences of change in lung capacity parameters, mild pulmonary edema and irritation of the tracheal epithelium, and changes of the tracheal epithelium were noted in specific inhalation studies using fine aerosols. These fine aerosols contain fine respirable particle sizes that are not found in cosmetic anhydrous aerosol or pump sprays which typically have particle sizes ranging from 60 to 80 μ for anhydrous sprays and $\geq$80 μ for pump sprays. In product categories that contain spray uses, however, sulfites were not used in sprays.

CONCLUSION

The CIR Expert Panel concluded that Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are safe as used in cosmetic formulations.

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