Safety Assessment of Sulfites as Used in Cosmetics

Status: Draft Amended Report for Panel Review
Release Date: May 15, 2020
Panel Date: June 8-9, 2020

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst, CIR.

1620 L STREET, NW, SUITE 1200 ◊ WASHINGTON, DC 20036-4702 ◊ PH 202.331.0651 ◊ FAX 202.331.0088 ◊ CIRINFO@CIR-SAFETY.ORG

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst, CIR
Date: May 15, 2020
Subject: Draft Amended Report on the Safety Assessment of Sulfites

The Expert Panel for Cosmetic Ingredient Safety (Panel) first reviewed the safety of Sulfites in 2003. The Panel concluded that Ammonium Bisulfite, Ammonium Sulfite, Potassium Metabisulfite, Potassium Sulfite, Sodium Bisulfite, Sodium Metabisulfite, and Sodium Sulfite are safe as used in cosmetic formulations.

In accordance with Cosmetic Ingredient Review (CIR) Procedures, because it has been at least 15 years since the safety assessment was published, the Panel considered whether the safety assessment of Sulfites should be reopened at the September 2019 Panel meeting. At the conclusion of their discussion, the Panel voted in favor of re-opening the published final report on Sulfites. This decision was based on the following concerns relating to this group of ingredients: 1) increased ingredient use frequency; 2) reports of contact sensitization; 3) the need for clarification of enhanced asthmatic responses to dust mites; and 4) the need for clarification of mutagenic effects in the published literature.

The attached Draft Amended Report (*sulfit062020rep*) contains data from the published final report (indicated by *italicized text*) as well as studies dated 1998 forward. The original report is included for your reference (*sulfit062020orig*). Of the more recent data in this safety assessment are 2020 ingredient use frequency data from FDA (*sulfit062020FDA*).

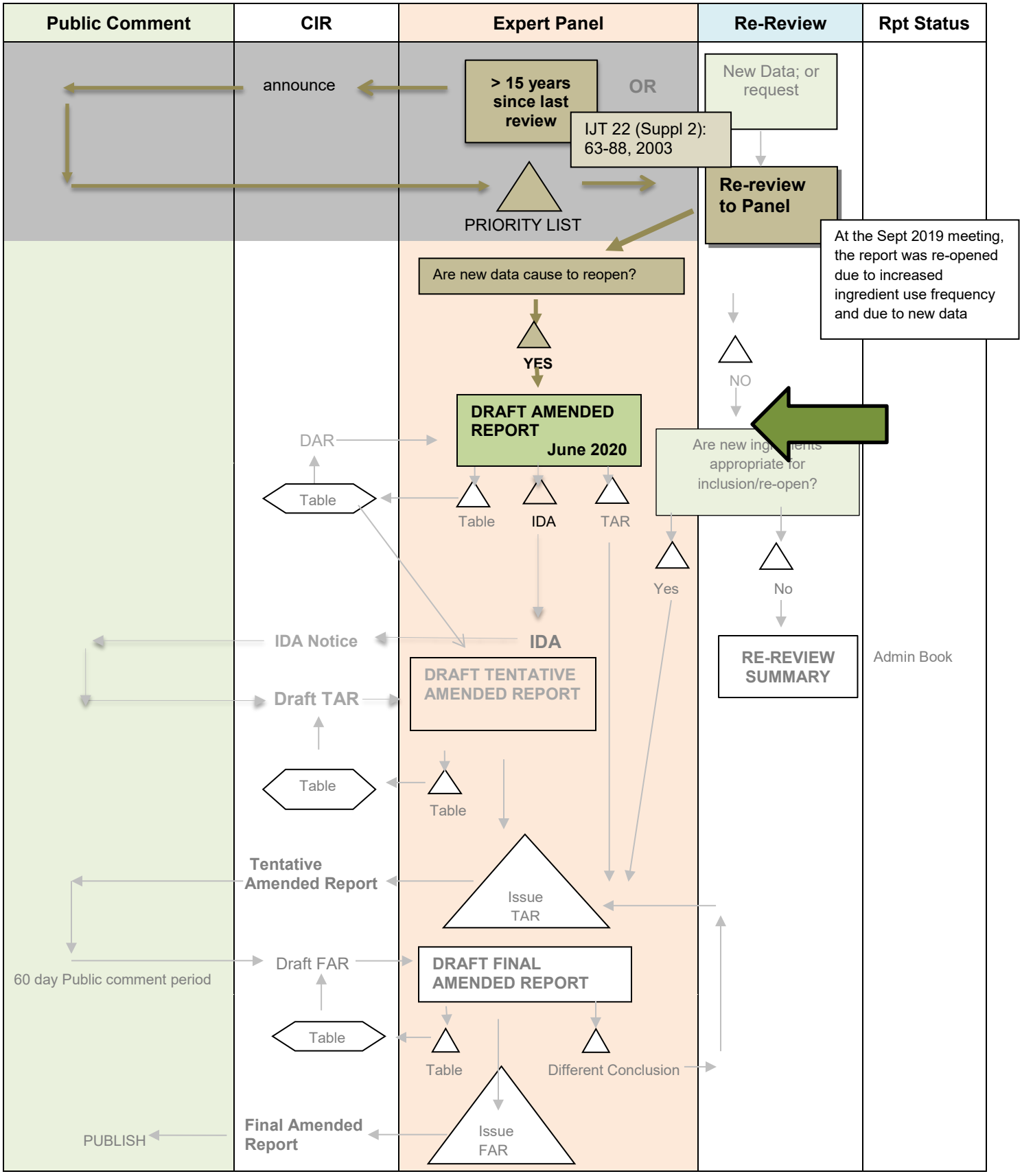
Also included in this package for your review are the report history (*sulfit062020hist*), flow chart (*sulfit062020flow),* literature search strategy (*sulfit062020strat),* 2020 FDA VCRP data (*sulfit062020FDA),* meeting minutes relating to the original review as well as the Panel's decision to re-open the safety assessment (*sulfit062020min)*, and the ingredient data profile (*sulfit062020prof*). This profile identifies information from the original report as well as any new information that was identified since that original report was issued. Comments that were received from the Council prior to the September 2019 Panel meeting are also included in this package (*sulfit062020pcpc*). These comments have been addressed.

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Amended Report with a safe as used, safe with qualifications, or unsafe conclusion, and Discussion items should be identified. If the available data are insufficient, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein.

RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Sulfites

MEETING June 2020



*If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

CIR History of:

Sulfites
Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite,
Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite,
and Potassium Metabisulfite

Draft Report, Teams: December 2, 1998

The Teams issued the following Informal Data Requests:

(1) Concentration of use
(2) Irritation and sensitization
(3) Contact urticaria

Draft Report, Full Panel: March 4, 1999

The Panel voted unanimously in favor of issuing an insufficient data announcement with the following data request on Sodium Bisulfite:

(1) A 2-year dermal carcinogenicity study on Sodium Bisulfite according to NTP methods

The Panel did not request data on any of the remaining ingredients, listed as follows, that are included in this review: Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite.

Draft Tentative Report, Full Panel: September 10, 1999

The Panel voted unanimously in favor of tabling the report, pending presentations to the Panel on sulfite vs. bisulfite chemistry and the mixed mutagenicity test results on Sodium Bisulfite.

Draft Tentative Report, Full Panel: December 21, 1999

The Panel learned from Dr. Warner's (with FDA) presentation that a very rapid bioconversion of sulfites to sulfates occurs in vivo, to a greater extent in rats than in humans. With this in mind, Dr. Belsito said that the Panel recognized the need to review the genotoxicity data on bisulfites again. Upon completion of this review, it was noted that all of the in vivo genotoxicity studies were negative, whereas, the positive genotoxicity studies were in vitro studies. Dr. Belsito said that this was thought to have been an effect of bioconversion, though bioconversion was not studied in either of the genotoxicity tests.

The Panel voted unanimously in favor of issuing a Tentative Report with the conclusion that Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are safe as used in cosmetic formulations.

Draft Final Report, Full Panel: May 19, 2000

The Panel concluded that Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are safe as used in cosmetic formulations, and voted unanimously in favor of issuing a Final Report on this ingredient group.

Re-review, Team/Panel: September 16-17, 2019

The Panel voted in favor of re-opening the published final report on Sulfites determined that the Final Report, based on significantly increased ingredient use, reports of contact sensitization, enhanced asthmatic responses to dust mites that need to be clarified, and mutagenic effects that need to be clarified.

Draft Amended Report, Teams/Panel: June 8-9, 2020

The re-opened safety assessment contains data from the published final report as well as studies dated 1998 forward. The more recent data in the safety assessment include 2019 ingredient use frequency data from FDA and use

concentration data submitted by the cosmetics industry in response to surveys conducted by the Personal Care Products Council (Council) in 2018.

Sulfites Data Profile* – June 8-9, 2020 Panel – Wilbur Johnson, Jr.

	Use				Toxico-kinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	New Rpt	Old Rpt	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Ammonium Bisulfite	**1**						O																						X
Ammonium Sulfite							O			O																			
Potassium Metabisulfite	**1**	**1**		O			O		O						O	OX	X		O										X	X
Potassium Sulfite	**1**	**1**		O			O																							
Sodium Bisulfite	**59**	**58**		O			O					O			O	O	OX		O		O									X
Sodium Metabisulfite	**2**	**348**		O			O		O			OX	OX		OX	OX	O		O		O							O	OX	X
Sodium Sulfite	**1679**	**911**		O			O			O			O		O	OX	OX		O			O			O				OX	X

* "X" indicates that new data were available in this category for the ingredient; "O" indicates that data from the original assessment were available

Sulfites (1998 forward) – 7/23-24/2019;1/31/2020]

Ingredient	CAS #	InfoBase	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	ECETOC
Ammonium Bisulfite	10192-30-0	Yes	9/4	2/1	No	Yes	No Dossier	No	No	No	Yes	No	No	No	No	No
Ammonium Sulfite	10196-04-0	Yes	16/0	3/1	Yes*	Yes	No Dossier	No	No	No	Yes	No	No	No	No	No
Potassium Metabisulfite	16731-55-8; 4429-42-9	Yes	37/7	8/3	Yes**	Yes	No Dossier	No	No	No	Yes	No	No	Yes	Yes	No
Potassium Sulfite	10117-38-1; 23873-77-0	Yes	3/0	2/1	Yes*	Yes	No Dossier	No	No	No	Yes	No	No	No	Yes	No
Sodium Bisulfite	7631-90-5	Yes	796/2	47/4	Yes**	Yes	No Dossier	No	No	No	Yes	No	No	Yes	Yes	No
Sodium Metabisulfite	7681-57-4; 7757-74-6	Yes	302/13	17/3	Yes**	Yes	No Dossier	No	Yes	No	Yes	No	No	Yes	Yes	No
Sodium Sulfite	7757-83-7	Yes	426/15	15/1	Yes*/ **	Yes	No Dossier	No	Yes	No	Yes	No	No	Yes	Yes	No

*Ammonium Sulfite, Potassium Sulfite, and Sodium Sulfite are color additives (for food use) exempt from certification.

** Potassium Metabisulfite, Sodium Bisulfite, Sodium Metabisulfite, and Sodium Sulfite are GRAS as preservatives in food.

ECHA

Ammonium Bisulfite – Dossier evaluation status (ECHA's dossier evaluation process covers compliance checks and the examination of testing proposals.)

Ammonium Sulfite – No Dossier

Potassium Metabisulfite – Dossier evaluation status

Potassium Sulfite – Dossier evaluation status

Sodium Bisulfite – Dossier evaluation status

Sodium Metabisulfite – No Dossier

Sodium Sulfite – Dossier evaluation status

LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - http://www.personalcarecouncil.org/science-safety/line-infobase
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - http://www.ncbi.nlm.nih.gov/pubmed
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – https://toxnet.nlm.nih.gov/ (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true (EAFUS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm (SCOGS database);
http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives (indirect food additives list);
http://www.fda.gov/Drugs/InformationOnDrugs/default.htm (drug approvals and database);
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf (OTC ingredient list);
http://www.accessdata.fda.gov/scripts/cder/iig/ (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - http://ec.europa.eu/growth/tools-databases/cosing/
CosIng - https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02009R1223-20160812

ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/
NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

Fragrance Websites, if applicable
IFRA (International Fragrance Association) – http://www.ifraorg.org/
RIFM (the Research Institute for Fragrance Materials) should be contacted

Qualifiers

Absorption	Genotoxic	Sensitization
Acute	Irritation	Skin
Allergy	Metabolism	Subchronic
Allergic	Mutagen	Teratogen
Allergenic	Mutagenic	Teratogenic
Cancer	Penetration	Toxic
Carcinogen	Percutaneous	Toxicity
Chronic	Pharmacokinetic	Toxicokinetic
Development	Repeated dose	Toxicology
Developmental	Reproduction	Tumor
Excretion	Reproductive	

DECEMBER 1998 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team/Schroeter Team –December 2, 1998

Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite,
Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite,
and Potassium Metabisulfite

The Belsito and Schroeter Teams issued the following informal data requests:

(1) Concentration of use
(2) Irritation and sensitization
(3) Impurities
(4) Contact urticaria

MARCH 1999 PANEL MEETING – DRAFT REPORT

Full Panel –March 4, 1999

Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite,
Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite,
and Potassium Metabisulfite

Dr. Belsito recalled that the following informal data requests were issued by his Team at the December 2, 1998 Team meeting.

(1) Concentration of use
(2) Irritation and sensitization
(3) Impurities
(4) Contact urticaria

He also said that these four issues have been addressed, and that his Team determined that the seven ingredients being reviewed are safe as used.

Dr. Belsito noted that the patch test data on 1% Sodium Sulfite and 1% Sodium Metabisulfite are negative, by and large, and that the Panel is looking at a use concentration of 0.5% (with wash off at 32%). Additionally, he noted that impurities data were not provided and also acknowledged that Sodium Sulfite, Sodium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are GRAS ingredients. In the absence of impurities data, Dr. Belsito=s Team determined that the impurities that would be acceptable in a cosmetic product could be defined.

Dr. Belsito indicated that his Team's concern about contact urticaria was based on reports indicating that these ingredients cause anaphylactic reactions in asthmatics when ingested. He said that this does not seem to be much of a problem because this may not be an IgE-mediated event, these ingredients don't seem to cause contact urticaria when applied to the skin, and these ingredients are GRAS ingredients.

Dr. Schroeter said that Sodium Bisulfite should not be regarded as safe as used because there is concern regarding the genotoxicity of this ingredient. He noted that data included in the CIR report indicate that Sodium Bisulfite is mutagenic in mammalian systems and that his Team determined that a two-year carcinogenicity study on this ingredient is needed.

Dr. Belsito asked if there is any concern about the toxicity of Sodium and Potassium Metabisulfite.

Dr. Schroeter noted that mutagenicity test results for these two ingredients were negative.

Dr. Belsito wanted to know how bisulfites and metabisulfites differ chemically.

Dr. Andersen recommended that the Panel issue an insufficient data announcement, focusing on the data on bisulfites that are needed (2-year dermal carcinogenicity study).

Dr. McEwen noted that data on some product containing Sodium Bisulfite that was tested for carcinogenicity may be available, because Sodium Bisulfite is used in hair dyes and a number of carcinogenicity studies have been done on hair dyes. He also said that he did not know whether data on hair dye products containing Sodium Bisulfite would be sufficient for addressing the

Panel's concern about the carcinogenicity of this ingredient. Dr. McEwen recalled that the data on the carcinogenicity of hair dyes have already been submitted to CIR, and noted that he would have to confirm the composition of the formulation that was actually tested.

Dr. Klaassen recalled that an inhalation study on a combination of benz[a]pyrene and sulfate, sulfite, or bisulfite was done in Germany approximately 15 or 20 years ago. He noted that his study might be relevant and that he would attempt to locate it, that is, if it is not referenced in the CIR report.

The Panel voted unanimously in favor of issuing an insufficient data announcement with the following data request on Sodium Bisulfite:

(1) A 2-year dermal carcinogenicity study on Sodium Bisulfite according to NTP methods

The Panel did not request data on any of the remaining ingredients, listed as follows, that are included in this review: Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite.

SEPTEMBER 1999 PANEL MEETING – DRAFT TENTATIVE REPORT

Full Panel –September 10, 1999

Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite,
Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite,
and Potassium Metabisulfite

Dr. Schroeter recalled that an Insufficient Data Announcement with a single data request (2-year dermal carcinogenicity study on Sodium Bisulfite according to NTP methods) was issued at the March 3-4, 1999 Panel meeting and that, to date, this study has not been received. He also noted that the following data, which have been incorporated into the CIR report, were received since the March Panel meeting: (1) ingredient use data, (2) human repeat insult patch test (HRIPT) - hair color containing 0.64% sodium sulfite, and (3) HRIPT - topical feminine cream containing 0.5% sodium sulfite.

Dr. Schroeter stated that his Team is still concerned about the potential DNA-damaging property of Sodium Bisulfite on the skin. However, he acknowledged that Sodium Bisulfite is used in hair dyes and, as such, can be described as safe as used. Dr. Schroeter also noted that his Team determined that a 2-year dermal carcinogenicity according to NTP methods is needed to support the safety of other uses of Sodium Bisulfite as well as Ammonium Bisulfite. He then proposed the following conclusion: Sodium Sulfite, Ammonium Sulfite, Potassium Sulfite, Sodium Metabisulfite, and Potassium Metabisulfite are safe as used in cosmetic formulations. Sodium Bisulfite is safe for use in hair dyes, but additional data are needed to support the safety of other uses. The available data are insufficient to support the safety of Ammonium Bisulfite. The additional data needed is a 2-year dermal carcinogenicity study.

Dr. Belsito said that his Team concluded that the ingredients in the Sodium Sulfite group are safe as used. He also said that the following points supporting this conclusion were made during his Team's review (1) Sodium Bisulfite is a GRAS ingredient. (2) Both positive and negative mutagenicity data on Sodium Bisulfite exist, an observation that the Team was unable to understand. (3) All of the other members of the group had negative carcinogenicity studies. (4) It is likely that the Bisulfite, and not Sodium, is acting as a carcinogen. Thus, the question relating to why the other Bisulfites are benign arose. (5) An equilibrium exists, where metabisulfite ↔ bisulfite ↔ sulfite. Thus, it would be difficult to conclude that most of the ingredients in the group are safe and that one is unsafe, considering that this equilibrium will exist for all of the ingredients in cosmetic formulations.

Dr. Schroeter reiterated his Team's concern over the DNA-damaging property of Sodium Bisulfite.

Dr. Belsito said that this property was not associated with the other bisulfites that were studied.

Dr. Shank said that the DNA-damaging effect is pH-related.

Dr. Slaga noted that at pH 9, the species is all sulfite; the species is all bisulfite at < pH 5.

Dr. Belsito suggested that restrictions could be established based on the pH range that is found to be safe.

Dr. Slaga said that a substance with a pH of > 9 on the skin would be harmful.

Dr. Shank said that there is some confusion over the bisulfite ↔ sulfite equilibrium. He noted that a Sodium Sulfite solution at pH 7 or 5 does not deaminate pyrimidines, but that Sodium Bisulfite does. Therefore, the chemical activity of the two is not the same at a given pH. Dr. Shank added that if one tries to deaminate cytosine with Sodium Sulfite instead of Sodium Bisulfite under exactly the same conditions, the reaction does not work. Sodium Sulfite does not deaminate the cytosine in

DNA. He also noted that the standard use of Sodium Bisulfite by DNA chemists is to remove the amino group from cytosine in order to convert it to uracil. Therefore, Sodium Bisulfite should be a mutagen, and this was the case in half of the mutagenicity tests. Sodium Sulfite was not a mutagen. Dr. Shank reiterated that there is a problem with the argument relating to the equilibrium between metabisulfite, bisulfite, and sulfite. The reactivity does not agree with the equilibrium - pH data.

Dr. Klaassen recommended inclusion of the preceding comments by Dr. Shank in the report discussion.

Dr. Schroeter agreed that the effect of pH on the bisulfite ↔ sulfite equilibrium should be included in the report discussion.

Dr. Andersen noted that the additional information on chemistry was provided to the Panel yesterday, and could be added to the report. He also agreed that Dr. Shank's comments on deamination activity should be incorporated.

Dr. Belsito wanted to know why metabisulfite and bisulfite behave differently in terms of DNA effects.

Dr. Shank said that he is not aware of any reports on metabisulfite versus bisulfite in terms of DNA effects.

Dr. Belsito wanted to know if the Schroeter Team concluded that the metabisulfites are safe, considering the absence of data on DNA effects.

Drs. Shank and Schroeter noted that the Panel does not have any data that would support an unsafe conclusion on metabisulfites.

Dr. Belsito wanted to know if it could be said that metabisulfite is converted to bisulfite.

Dr. Shank said that this reaction (metabisulfite ↔ bisulfite) is questionable.

Dr. McEwen noted that data from short-term assays support a concern about the potential mutagenicity of bisulfites. He also noted the lack of activity of the sulfite in the short-term assays and experimental evidence that the sulfite does not deaminate cytosine. With this in mind, Dr. McEwen said that further information would be needed before the use of bisulfites in leave-on products could be approved.

Dr. McEwen requested that Ammonium Bisulfite have an exemption for permanent wave products. He added that the potential for skin contact with Ammonium Bisulfite in a permanent wave product would be very slight, as would be the case for Sodium Bisulfite in hair colorants. Thus, if Sodium Bisulfite is considered safe for use in hair dyes, then Ammonium Bisulfite should be considered safe for use in permanent wave products.

Dr. Belsito expressed concern over any decision to declare metabisulfites safe, given the level of concern about the safety of bisulfites that is evident. He also suggested that a chemist be invited to address any concerns that are related to sulfite vs. bisulfite chemistry (including any reactions between the two), and that someone also be invited to explain why Sodium Bisulfite was positive in some mutagenicity assays and negative in others. Dr. Belsito recommended that the Panel's review of this ingredient group be tabled until presentations to the Panel have been made.

The Panel voted unanimously in favor of tabling the report on the Sodium Sulfite ingredient family, pending presentations to the Panel on sulfite vs. bisulfite chemistry and the mixed mutagenicity test results on Sodium Bisulfite.

Dr. Shank noted that no percutaneous absorption data are included in the CIR report and recommended that the Panel request a dermal absorption study with [35S]-labeled compounds. He stated that this study would be done instead of the 2-year dermal carcinogenicity study on Sodium Bisulfite that was requested in the Insufficient Data Announcement.

Dr. Belsito asked if dermal absorption data would essentially erase any concern about the dermal carcinogenicity of Sodium Bisulfite.

Dr. Shank said that the dermal absorption data would provide an opportunity to demonstrate that the concentration of Sodium Bisulfite applied in a formulation would never reach significantly high levels that would result in the deamination of DNA in the skin. He also said that if the Panel is not satisfied with this approach, then a deamination study could be done, looking for uracil in skin DNA.

Dr. Belsito wanted to know whether it is likely that Sodium Bisulfite will pass through the stratum corneum.

Dr. Shank predicted that the rate of absorption of Sodium Bisulfite (in formulation) through the skin would be so slow that there wouldn't be enough bisulfite ion to deaminate the DNA.

Dr. Bergfeld wanted to know how CIR will proceed at this point, taking into consideration the Panel's recommendations.

Dr. Andersen solicited the Panel's guidance in selecting individuals to address the Panel on the issues relating to sulfite and bisulfite chemistry and the mixed results for Sodium Bisulfite in mutagenicity assays.

Dr. McEwen said that the relationship of using bisulfite as a research tool versus any potential for in vivo harm is the concern that needs to be addressed, and finding a person who could address this concern would probably be difficult. He said that finding a person to address the chemistry of products containing bisulfites should not be a problem.

Dr. Belsito said that if industry could provide a study indicating that Sodium Bisulfite does not penetrate the skin at the maximum concentration for leave-on use of this ingredient and that there is no deamination of DNA in the skin, these data would resolve the issue of carcinogenicity. Thus, after reviewing these data, there would not necessarily be any need for chemistry data. However, in the absence of these data, Dr. Belsito said that it would be helpful to know the relative ratio of the various products that will be formed in a cosmetic formulation over a pH range that one would expect in a cosmetic product.

DECEMBER 1999 PANEL MEETING – DRAFT TENTATIVE REPORT

Full Panel – December 21, 1999

Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite,
Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite,
and Potassium Metabisulfite

Dr. Bergfeld noted that Dr. Charles Warner, FDA, gave a presentation on the equilibrium chemistry of sulfurous acid, sulfur dioxide, bisulfite, sulfite, and metabisulfite at yesterday's Team meetings. This presentation is summarized in the section on Team Meeting Minutes at the end of this document. Dr. Belsito recalled that during the review of this group of ingredients at the September 9-10, 1999 Panel meeting, the Panel focused on the issue of bisulfite genotoxicity and the lack of genotoxic potential associated with sulfites and metabisulfites. Furthermore, Dr. Belsito indicated that after reviewing information indicating the existence of an equilibrium between sulfite, bisulfite, and metabisulfite, the Panel requested assistance in learning more about the chemistry of these reactions. He also noted that Dr. Warner discussed with the Panel how the effects of pH in the presence or absence of water produce an equilibrium between the sulfite, bisulfite, and the metabisulfite.

The Panel also learned from Dr. Warner's presentation that a very rapid bioconversion of sulfites to sulfates occurs in vivo, to a greater extent in rats than in humans. With this in mind, Dr. Belsito said that the Panel recognized the need to review the genotoxicity data on bisulfites again. Upon completion of this review, it was noted that all of the in vivo genotoxicity studies were negative, whereas, the positive genotoxicity studies were in vitro studies. Dr. Belsito said that this was thought to have been an effect of bioconversion, though bioconversion was not studied in either of the genotoxicity tests.

Dr. Belsito said that the Panel does not have skin penetration data on the ingredients being reviewed. However, his Team determined that penetration of these highly charged particles would be poor. Dr. Belsito also noted that these ingredients are used at low concentrations in leave-on products, < 0.5%, but are used at higher concentrations in hair wave sets.

Dr. Belsito called the Panel's attention to studies in the section on Phototoxicity. He noted that these studies would be more properly referred to as cellular toxicity studies indicating a membrane effect of sulfites on erythrocytes that have been exposed to light. It was requested that these studies be moved to the section on Cellular Toxicity. Dr. Belsito emphasized that these studies should not be considered phototoxicity tests that can be used as a model in the future.

The Panel voted unanimously in favor of issuing a Tentative Report with the conclusion that Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are safe as used in cosmetic formulations.

MAY 2000 PANEL MEETING – DRAFT FINAL REPORT

Full Panel – May 19, 2000

Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite,
Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite,
and Potassium Metabisulfite

Dr. Belsito stated that the Panel issued a Tentative Report with a safe as used conclusion at the December 20-21, 1999 Panel meeting, and that no comments on the report were received during the 90-day comment period.

Dr. Belsito also noted that because of the ingredient use product categories indicated in the FDA database (see Cosmetic Use section of report), it appears that the ingredients being reviewed could be used in aerosolized products. However, he added that information provided by CTFA indicates that none of the products within those broad categories are spray products. Dr. Belsito requested that this point of clarification be included in the Cosmetic Use section as well as the report discussion. In the Cosmetic Use section, he favored identifying each product category in Table 3 that includes sprays with an asterisk and inserting a footnote indicating that neither of the products in these categories that were reported to CTFA as being used were sprays.

The Panel concluded that Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are safe as used in cosmetic formulations and voted unanimously in favor of issuing a Final Report on this ingredient group.

SEPTEMBER 2019 PANEL MEETING – INITIAL REVIEW/RE-REVIEW

Belsito Team – September 16, 2019

DR. BELSITO: Yeah. Okay. Sulfites. Let's have some wine, folks.

DR. SNYDER: Sulfites. We're ready for some wine.

DR. KLAASSEN: We can go to the vegetable bar.

DR. BELSITO: Oh, but then we still have more. We have MIPA.

DR. SNYDER: Saving the best for last.

DR. KLAASSEN: We got four more.

DR. BELSITO: I know, but MIPA.

DR. SNYDER: Three re-reviews tomorrow.

DR. BELSITO: Okay. Sulfite. This is the latest we've ever gone. We're going to just go straight to the bar and leave our computers here.

DR. LIEBLER: Leave them on for tomorrow morning.

DR. KLAASSEN: We're in the wrong room.

DR. BELSITO: Yeah, we'll stay here, let the other group argue among themselves.

Okay. So I said reopen, significant increased use, reports of contact sensitization, enhanced asthmatic response to dust mite. And then I had a question for Paul on PDF Page 18, the developmental effects.

DR. SNYDER: This is a re-review, right? This isn't new data, right?

DR. BELSITO: No, there's a lot of new data on contact dermatitis to sulfites in the literature now. Not just the asthmatic responses that you think of, the IgE-mediated that caused them to be labeled on foods.

In fact, the North American Contact Dermatitis Group just added sulfite to our standard panels for 2019-2020. So, we don't have any data. But if you read through it you'll see all these case reports keep coming out on sulfites, and incidences in large groups of 1 to 2 percent of the patch test population, not the general population.

DR. SNYDER: The group effects weren't that very high of a dose, 260 milligrams per kilogram. Yeah.

DR. BELSITO: Okay. I just said most studies were positive, but they're high.

DR. SNYDER: Wait, no. The second study; "The study on protection against. . . .induced testicular toxicity."

DR. BELSITO: And then this probably goes to Tom, but at 0.001 millimolar, it seems that these could cause activation of proto-oncogenes and inactivation of tumor suppressor genes. This is page 21 of the PDF. So I don't know if any of you have comments or we just want to see what Tom says about that?

DR. LIEBLER: Again, I'm always suspicious of these cell culture studies. The concentrations are up to 2 millimolar. They looked at message and some protein by western blotting. I'm dubious as to the significance of the observation. I'll be interested in seeing what Tom's opinion is. For example, all of these mentioned genes were indicated to be increased or increase in expression. I have to look at the papers to see how much --

DR. BELSITO: At all trusted doses.

DR. LIEBLER: At all doses. P53 is a tumor suppressor gene. So, if you wanted to have a pro-carcinogenic effect, you actually drive down tumor suppressor genes. Protooncogenes, abundances can become activated. But the question more is whether or not they actually become enzymatically activated.

DR. BELSITO: I'm just reading this stuff, men, it's not my area of specialty. I just bring it up because my name goes on the final paper.

DR. LIEBLER: Right.

DR. BELSITO: Okay. So, we'll see what Tom says about this.

DR. LIEBLER: Yes.

DR. BELSITO: Okay.

DR. KLAASSEN: I don't think he'll worry about it.

DR. LIEBLER: I think this is a really questionable significance. High concentration, treatments in cell culture.

DR. BELSITO: I certainly thought the neurotoxicity and cytotoxicity were all concentrations. The mast cell degranulation was concentration. Wilbur, on page 22 of the PDF on pulmonary sensitization, the second paragraph in that area, what is "allerfrtulfite?" The second paragraph, the first word in the second line, "something intranasal group."

MR. JOHNSON: I'll correct it. Just one second. Let me see if I can see it.

DR. BELSITO: A-L-L-E-R-T or A-L-L-E-R-F. Something is misspelled there.

DR. LIEBLER: It looks like it's something sulfite, an attempt to say something sulfite.

MR. JOHNSON: That should be sodium sulfite intranasal group. That's what it should be.

DR. BELSITO: Okay. We just need to correct that.

MR. JOHNSON: Mite intranasal plus sodium sulfite intranasal group.

DR. BELSITO: Okay. Then I guess my question is, on this, would this be an issue for aerosolized products that might contain Sodium Sulfite? Because basically what it's saying is that it's increasing the response to dust mite. And a lot of people are allergic to dust mite. Do we know what the concentration is here?

DR. LIEBLER: No.

DR. BELSITO: It doesn't say, at least not here. They don't say in the paper what the concentration of the sodium sulfite was?

MR. JOHNSON: I'll have to check that out, Dr. Belsito.

DR. BELSITO: Okay.

DR. SNYDER: It's interesting. In the old report we talk about the disparate genotox data and giving a clear consistent picture. All the in vivo data were negative and only the in vitro data were positive.

DR. BELSITO: Yeah. I looked at that. I thought in the old report -- I didn't know that the explanation of mutagenic effect was adequate again. I wanted Tom's opinion.

Bottom line is I think this needs to be reopened. It's a significant increased use, reports of contact sensitization, enhanced asthmatic responses to dust mites that need to be clarified, mutagenic effects that need to be clarified.

The bottom line is we can end the discussion on it. I think it needs to be reopened. We have some sense, and industry will have some sense of the type of information we're looking for.

DR. KLAASSEN: We'll reopen.

Marks Team – September 16, 2019

DR. MARKS: Let's see. Next I have are the sulfites. And this is rereview safety assessment of the sulfites. They were first reviewed in 2003 and concluded that these ingredients, ammonium bisulfite, ammonium sulfite, potassium metabisulfite, potassium sulfite, and sodium bisulfite and sodium metabisulfite and sodium sulfite -- oh, no. Tomorrow, I may just say the sulfites as in your memo were found to be safe.

And the number of uses and the concentration have changed since that 2003 report. There's seven ingredients. I didn't see any alerts, particularly it was mentioned in the original paper, a positive patch test. There was little sensitization data. But since 2003, there have been some published series with positives to sodium metabisulfite in the three to five percent range. But there's a marked decrease in use of sodium metabisulfite from 348 to two. The concentration has also been reduced. So I didn't think we needed to reopen. So I move not to reopen, but, Tom and Ron, your comments.

DR. SHANK: I agree. Don't reopen.

DR. SLAGA: I agree, too. But we would have a -- we don't have a summary, then, do we?

DR. BERGFELD: Not yet. That's the next one.

DR. SLAGA: Let me just -- in the past report related to this, genotoxicity was mainly looked at in the Ames. And here, they had a list of in vitro, in vivo -- not necessarily mutagenicity. A lot of people call these clastogenic, like tumor promotors can enhance all these type effects. There's a mixed bag of -- there's both positive and negative data related to this response. But if you consider it a clastogenic and we really had true genotoxicity with the Ames before, that's why I say do not reopen. But sometimes it's better to discuss this in some document.

Someone may see all that, that there's both positive and negative related to the micronuclei test and this type of a test, which, this time, that's what they used. And that's the data in it. But I didn't have any concern. Nothing else would say the clastogenic effect would lead to -- it doesn't lead to cancer by itself, but it can enhance cancer in carcinogenic agents.

DR. SHANK: And the concentrations used is small. In cosmetics, it's small. In the genotox studies they were high.

DR. MARKS: So, Tom, you wanted to include that in the discussion of the rereview document?

DR. SLAGA: That could be discussed in the summary.

DR. MARKS: Because I think that's what you're asking.

DR. SLAGA: Yeah. Somewhere, it should be -- it's not a concern. Their genotoxicity in the past report was related to Ames or bacterial test. And then this was related to in vitro/in vivo related to micronuclei test and a couple other test, which a lot of people consider clastogenic.

DR. MARKS: So how would you like -- because you mentioned the --

DR. SLAGA: The problem is if you discuss it too much then people have a concern for it. To me, it doesn't raise the risk at these low doses.

MR. JOHNSON: The low doses don't raise the risk with respect to genotoxicity?

DR. SLAGA: Yeah.

DR. BERGFELD: I think that should go in the discussion that we have.

DR. SLAGA: There's a positive and negative. So you have to take either the weight of evidence, which would be different -- I forget how many different studies there were.

DR. MARKS: So do you want to make a very limited, low --

DR. SLAGA: Yeah. Very limited discussion because we had no concern with clastogenic effects.

DR. MARKS: Pardon? What did you say, Tom?

DR. SLAGA: We had no concern related to the clastogenic -- possible clastogenic effect, C-L-A-S-T-O-G-E-N-I-C.

MR. GERMILLION: Just a clarifying question. It sounds like there's new studies on the genotoxicity and other carcinogenicity that suggests other risks that maybe weren't apparent when this was done initially. But also I see the concentration of use is really down. So is what you're saying the new data on toxicity isn't significant given how low the concentration of use is? Or even if it was at the level that it was back in 2003?

DR. SLAGA: Well, in a nutshell, this data by itself doesn't raise the risk, in my eyes.

DR. MARKS: So I'm going to, Tom, ask you to summarize. But I may just say in the discussion we'll mention that the low doses of these ingredients being used it doesn't raise the risk of genotoxicity. Therefore, we have no concern for a clastogenic effect. Does that sound accurate?

DR. SLAGA: Yeah. That's fine.

DR. MARKS: Okay. And then, Tom, I may ask you to comment. Okay. So move don't reopen tomorrow.

MR. JOHNSON: Just one concern, Dr. Marks. You mentioned something about sodium metabisulfite and sensitization and your lack of concern about the more recent data?

DR. MARKS: Yes. They're low ranges from three to six percent. There have been no epidemics. The North American group have actually tested it, so I didn't feel that raised concern in terms of having significant amount of sensitization.

MR. JOHNSON: Thank you.

DR. MARKS: So, I reaffirmed the original conclusion. Any other comments?

Full Panel –September 17, 2019

DR. MARKS: The panel reviewed these sulfite ingredients in 2003 -- I won't list all those -- and the conclusion was safe. We're at the point now to decide whether to re-review these.

Since that time, there had been in the original paper a positive patch test, but we have a series of positive patch tests to sodium metabisulfite in the three to six percent range. But the uses have decreased dramatically from 348 to 2, and the concentration has also decreased from 0.4 to 0.25. And we also thought at low dosage there wasn't a risk of genotoxicity and no concerns for clastogenic effect.

So, our team felt that we could move to not reopen; and those discussion points would be included in re-review summary.

DR. BERGFELD: Is there a second, or a comment?

DR. BELSITO: Well, I mean, there's been a significant increased use overall in these sulfites. You just picked one ingredient where there was a decrease. And certainly increased reports of contact sensitization, which could pertain as a read across.

We also notice that the presence of sulfites in something that was aerosolized could potentially enhance the asthmatic response to dust mite. And we're a bit concerned about safety in materials that could potentially be inhaled, particularly, for asthmatics who are allergic to dust mite which is a very common aeroallergen. So we thought we needed to reopen this and look at all of the data.

DR. MARKS: That's fine.

DR. SLAGA: I don't have any problem with it.

DR. MARKS: Yeah. Ron, okay?

DR. SHANK: All right.

DR. MARKS: I'll withdraw the motion that I made then and reopen.

DR. BERGFELD: So, it's been moved and seconded to reopen. Any other discussion? Tom?

DR. SLAGA: Yeah actually, the more I thought about it and going through the review, that we had do not reopen, but there was a number of -- listed under genotoxicity -- they had micronuclei test and a few others, which are more clastogenic.

But it would be nice to have all the data, in a re-review you would have the -- the last time it was reviewed we had the Ames test too, some others that were negative. So it would be more weight of evidence. We'd have it all together, so that would be good.

DR. BERGFELD: All right, I'd like to call the question then, all those in favor to re-open, please indicate by raising your hand. Unanimous, thank you. Then the next ingredient is in the mint family, Scutellaria. Dr. Belsito.

Safety Assessment of Sulfites as Used in Cosmetics

Status: Draft Amended Report for Panel Review
Release Date: May 15, 2020
Panel Date: June 8-9, 2020

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst, CIR.

1620 L STREET, NW, SUITE 1200 ◊ WASHINGTON, DC 20036-4702 ◊ PH 202.331.0651 ◊ FAX 202.331.0088 ◊ CIRINFO@CIR-SAFETY.ORG

INTRODUCTION

The Expert Panel for Cosmetic Ingredient Safety (Panel) published a safety assessment of Sulfites in 2003.[1] Based on the available data, the Panel issued the following conclusion: Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite are safe as used in cosmetic formulations. In accordance with Cosmetic Ingredient Review (CIR) Procedures, the Panel evaluates the conclusions of previously-issued reports every 15 years. The Panel determined that the original conclusion should be reconsidered; therefore, a re-review is underway.

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), the functions in cosmetics that are reported for this group of sulfites include: antioxidants, hair-waving/straightening agents, and reducing agents (see Table 1).[2]

The published data in this document were identified by conducting an exhaustive search of the world's literature from yer 1998 forward. A list of the typical search engines and websites used, sources explored, and endpoints that Panel evaluates, is available on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data may be provided by the cosmetics industry, as well as by other interested parties.

CHEMISTRY

Definition and Structure

All 7 sulfite ingredients are inorganic salts that conform to the general structure in Figure 1. Individual structures are presented in Table 1.[2]

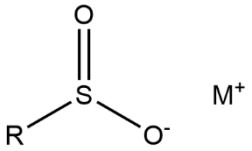


Figure 1. Sulfite ingredients, wherein M is sodium, potassium, or ammonium; and R is OH, OM, or SO_3M

Physical and Chemicals Properties

An ultraviolet (UV) spectral analysis of Sodium Metabisulfite indicates an absorbance peak at 209 nm.[1] Sodium Sulfite has been identified as having a similar absorbance pattern.

The sulfites reviewed in this safety assessment are all water-soluble compounds. Properties of sulfites are presented in Table 2.[1,3,4]

Method of Manufacture

Sodium Sulfite and Sodium Metabisulfite may be manufactured by reacting sulfur dioxide with sodium carbonate (soda ash). This step is followed by purification and drying to form crystals or powder.[5,6]

Composition/Impurities

Food grade specifications for sulfites are listed in Table 3.[7] Specifications for Sodium Metabisulfite and Potassium Metabisulfite listed in the *United States Pharmacopoeia* are presented in Table 4.[8,9]

USE

Cosmetic

The safety of these cosmetic ingredients is evaluated based, in part, on data received from the United States (US) Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.[10] Use data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.[11]

In the 2003 original report and in 2020, Sodium Sulfite is the ingredient with the highest use frequency.[1,10] The use frequency of 911 for this ingredient in the original report increased to a value of 1713 in 2020.[10] A substantial increase in the use frequency from 348 to 916 in 2020 is being reported for Sodium Metabisulfite. Of the ingredients reviewed in the 2003 report, Sodium Metabisulfite had the highest use concentration (14% in rinse-off products).[1] In 2019, this ingredient is being used at substantially lower concentrations of up to 0.6% in rinse-off products.[11] The sulfite with the highest reported use concentration in 2019 is Sodium Sulfite, which is being used at concentrations up to 3% in rinse-off products. This was also the highest use concentration of Sodium Sulfite in the 2003 original report.[1] The sulfite with the highest reported use

concentration in leave-on products in 2019 is Sodium Metabisulfite, which is being used at concentrations up to 0.25% in indoor tanning spray preparations.[11] Frequency and concentration of use data are presented in Table 5.

Sodium Metabisulfite is being used in indoor tanning spray preparations at maximum use concentrations ranging from 0.02% to 0.25%. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm, with propellant sprays yielding a greater fraction of droplets/particles below 10 μm, compared with pump sprays.[12-15] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[12,13]

All of the sulfites reviewed in this safety assessment are included on the European Union's list of substances which cosmetic products must not contain, except subject to the restrictions laid down. The restrictions that are listed include: a maximum concentration (in ready for use preparation) of 0.67% (as free sulfur dioxide) in oxidative hair dye products; a maximum concentration (in ready for use preparation) of 6.7% (as free sulfur dioxide) in hair straightening products; a maximum concentration (in ready for use preparation) of 0.45% (as free sulfur dioxide) in self-tanning products for the face; and; a maximum concentration (in ready for use preparation) of 0.40% (as free sulfur dioxide) in other self-tanning products.[16] Additionally, all of the sulfites reviewed in this safety assessment are included on the European Union's list of preservatives allowed in cosmetic products, whereby the required maximum concentration of each (in ready for use preparation) is 0.2% (as free sulfur dioxide).[16]

Non-Cosmetic

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 kernels during the wet-milling process.[17,18]

In 1974, 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.[19] 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.[20] Review articles 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 sulfur dioxide/kg body weight/day) from a three-generation oral-dose study using rats.[21-23] At the 30th meeting of JECFA in 1987, JECFA retained the ADI of 0 to 0.7 mg/kg body weight allocated to this group of compounds.

According to the US FDA, Ammonium Sulfite, Potassium Sulfite, and Sodium Sulfite are color additives (for food use) that are exempt from certification (21 CFR 73.85). The following 4 sulfites are GRAS for use as preservatives in food for human consumption: Potassium Metabisulfite (21 CFR 182.3637), Sodium Bisulfite (21 CFR 182.3739), Sodium Metabisulfite (21 CFR 182.3766), and Sodium Sulfite (21 CFR 182.3798). Sodium Bisulfite and Sodium Sulfite are also among the constituents of cellophane, which may be safely used for packaging food in accordance with the prescribed conditions (21 CFR 177.1200). Furthermore, Sodium Metabisulfite and Sodium Sulfite are permitted in food for human consumption, and may be safely used in the preparation of steam that will contact food under the conditions that have been established (21 CFR 173.310).

The labeling for any prescription drug product to which sulfites have been added as an inactive ingredient, regardless of the amount added, must bear a warning statement that relates to concerns about allergenicity (21 CFR 201.22).

TOXICOKINETIC STUDIES

Absorption, Distribution, Metabolism, and Excretion (ADME)

Sulfites are generated in the human body by processing of the sulfur-containing amino acids, cysteine and methioneine.[1] Endogenous sulfate is maintained at a low, steady-state concentration by a mitochondrial enzyme, sulfate oxidase, that promotes the oxidation of sulfite to sulfate that is excreted in the urine. Sulfite that enters the body via ingestion, inhalation, or injection is metabolized by sulfite oxidase to sulfate. 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 that it receives, but a finite amount will pass through the organ and enter the systemic circulation.

Sulfites can also be metabolized to thiosulfates (enzymatic reaction of sulfite with disulfide bonds).[1] 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. Review articles note that hepatic sulfite oxidase activity was estimated to be 10 to 20 times greater in rats when compared to humans.

Animal

Oral

Oral dosing studies using dogs and rats demonstrated rapid metabolic clearance.[1] In all species, ≤ 10% of the administered dose was excreted unchanged in the urine.

Human

Total serum sulfite concentrations in 41 women and 35 men have been determined.[1] 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 inter-assay coefficients of variation (CVs) for total serum sulfite at 5.4 µmol/L were 8.1% and 22%, respectively. The mean concentrations (± 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.

TOXICOLOGICAL STUDIES

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.[1] Oral-dose toxicity studies from 1920 to 1972 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.

Acute Toxicity Studies

Oral

Potassium Metabisulfite

Acute oral LD_{50} values of 1040 and 1800 mg/kg have been reported for rats.[1]

Sodium Metabisulfite

The acute oral LD_{50} for Sodium Metabisulfite was 1131 and 1903 mg/kg for female and male rats, respectively.[1] 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.

Inhalation

Ammonium Sulfite

Groups of 8 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.[1] *The aerosol had a mass mean aerodynamic diameter (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 Ammonium Sulfite exceeded 400 mg/m^3.*

Beagle dogs (5 female and 3 male) were exposed nose-only for 1 h to 1 mg/m^3 of aerosolized Ammonium Sulfite mixed with sulfate.[1] *Sulfur dioxide and ammonia gas concentrations were monitored and were less than 0.5 and 5 ppm, respectively. No significant difference was observed between pre-exposure and post-exposure 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.*

Sodium Sulfite

Noting the lack of inhalation studies available for forms of sulfur dioxide other than sulfurous acid, Sodium Sulfite with a MMAD of 0.36 µm was studied.[1] *Guinea pigs were exposed head-only for 1 h to 474, 669, and 972 µg/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 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 µg/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.*

Short-Term Toxicity Studies

Oral

Sodium Metabisulfite

Anemia developed in mice that had been dosed orally (feeding) with ≥ 2% Sodium Metabisulfite for 10 to 56 days.[1] Increased hematopoiesis and splenomegaly were observed with doses of ≥ 4%. Hemorrhagic erosions, inflammation, and necrosis of the stomach were observed in rats fed 4%, 6%, or 8% Sodium Metabisulfite. According to another report, 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).[1] 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. Slightly decreased hemoglobin content and slightly increased relative kidney weight were the only treatment-related adverse effects in the group receiving the NOAEL concentration. No treatment-related effects were found in the groups receiving 1/3 NOAEL and 1/10 NOAEL concentrations.

Groups of 10 male and 10 female rats were administered biscuits containing Sodium Metabisulfite at levels of 0 (control), 10, 35, and 75% (corresponding to 10 - 15, 35 - 45, 150 - 170, and 310 - 340 mg/kg diet, respectively).[24] For each concentration of Sodium Metabisulfite administered, the concentrations of supplements in the diet were: casein + DL-methionine (13% + 0.1%), sugar (6%), minerals (1%), and vitamins (1%). The animals were fed the diets for 28 days. Biscuits made with Sodium Metabisulfite (0.29 kg/100 g of flour) were ground and mixed with supplements of protein, sugar, vitamins, and minerals to obtain a nutritionally adequate rat diet. None of the animals died, and no clinical abnormalities were reported. Growth rate, food consumption and food conversion efficiency were not affected by treatment. No dose-related changes in the following parameters were observed: hematology, clinical chemistry, ocular examination, renal function, urinalysis, organ weights or gross and microscopic examinations. The liver concentrations of vitamins A, B1, C, and E were not significantly changed, except for an increase in vitamin E in males of the highest dose group after 28 days of exposure. Based on these data, the no-observed-adverse-effect level (NOAEL) of Sodium Metabisulfite in baked biscuits was judged to be 310 mg/kg diet or 25 mg/kg body weight/day.

Groups of 10 male and 10 female rats were administered biscuits consisting of Sodium Metabisulfite at levels of 0 (control), 10, 35, and 75% (corresponding to 10 - 15, 35 - 45, 150 - 170, and 310 - 340 mg sulfite/kg diet, respectively).[24] The animals were fed the diets for 85 days. The composition of the diet is the same as described in the short-term study above. None of the animals died, and no clinical abnormalities were reported. Growth rate, food consumption and food conversion efficiency were not affected by treatment. No dose-related changes in the following parameters were observed: hematology, clinical chemistry, ocular examination, renal function, urinalysis, organ weights or gross and microscopic examinations. Clinical chemistry results showed statistically significant differences between treated rats and controls in serum calcium, inorganic phosphate and bilirubin in males, and in urea, uric acid and alkaline phosphatase in females. These differences were not considered to be biologically significant, since they showed no clear dose relationship and all values were within the normal range for rats of this strain and age. Based on these data, the NOAEL of sulfites in baked biscuits was judged to be 310 mg sulfite/kg or 25 mg/kg/day.

Inhalation

Sodium Metabisulfite

The short-term inhalation toxicity of Sodium Metabisulfite was evaluated using Sprague-Dawley rats, divided into the following 4 exposure groups: fresh air (negative control), and groups exposed to Sodium Metabisulfite at target concentrations of 5, 20, and 100 mg/m^3.[25] The animals were exposed 5 days per week (6 h per day) for 10 days. The actual exposure concentrations of Sodium Metabisulfite were determined to be 5.5 ± 2.4, 29.3 ± 7.7, and 110 ± 38.9 mg/m^3 in the low-, medium-, and high-exposure groups, respectively. When compared to rats in the low- and medium-exposure groups, the body weight significantly decreased ($p < 0.05$) in the high-exposure group on the 14th day of exposure. No other clinical symptoms were observed during the exposure. In all exposure groups, short-term exposure resulted in no clear changes in any of the following lung inflammatory responses factors: tumr necrosis factor alpha (TNF-α), interleukin-6 (IL-6), IL-1β, transforming growth factor-beta 1 (TGF-β1), and monocyte chemoattractant protein-1 (MCP-1). At histopathological examination of the liver, multifocal infiltration of mononuclear cells was observed in the control and Sodium Metabisulfite exposure groups. There was no notable difference in the incidence rate and severity of this finding when the 3 exposure groups were compared to controls. Focal bile ductile hyperplasia was noted in 1of 4 rats in the low-dose group. A diffuse type of steatosis was evident in a rat of the medium- and high-dose groups, but it was minimal in severity. In the lungs, no specific abnormal findings were observed, although focal mineralization in the arterial wall was noted in a rat of the high-dose group. According to the histopathological results, dosing with Sodium Metabisulfite did not cause any toxic effects in the liver, lung, or nasal cavity.

Sodium Sulfite

Groups of 6 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).[1] The particle size was ~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 ≥ 5 mg/m^3, and increased wet to dry weight ratios of right apical lobes were observed in rats dosed with ≥ 1 mg/m^3. The investigators concluded that the rats responded with "mild pulmonary edema." Exposure to ≥ 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.

Subchronic Toxicity Studies

Oral

Sodium Bisulfite

Groups of 50 male and 50 female crossbred 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.[1] Seventy percent of males and 68% of females receiving 160 mg/kg/day 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% 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.

Chronic Toxicity Studies

Oral

Sodium Bisulfite

A three-part, 3-year study using the Osbourne-Mendel strain of rats evaluated the chronic toxicity of Sodium Bisulfite.[1] All three parts used a balanced incomplete block design method. Sodium Bisulfite at concentrations of 0.1% (615 ppm as sulfur dioxide) or more added to the diet were toxic to rats. No observed significant effect on growth by Sodium Bisulfite was observed at concentrations less than 1%. A definite trend toward smaller average weights and smaller gains in weight was observed as the concentration was increased from 1% to 2%. Sodium Bisulfite at a concentration of 0.25% (1538 ppm as sulfur dioxide) caused decreased survival time that continued to shorten as the concentrations of sulfite increased. The lowest dose of sulfite that produced histopathological changes was 0.1% (615 ppm as sulfur dioxide). From 0.25% (1538 ppm as sulfur dioxide) 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 to the destruction of vitamins.

The skulls and teeth from the 43 rats of the study described above were utilized for the study of vitamin deficiencies.[1] 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% caused a pronounced lack of pigmentation of the enamel, sudden atypical atrophy of the enamel organ, often accompanied by the following: 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.

A study was designed to evaluate the effects of preservatives alone, in combination, and with added stress factors, in crossbred white and Wistar mice exposed to Sodium Bisulfite over a 17-month period.[1] Survival rates, reproduction, and tumor incidences were evaluated. 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) alone died. These mortality rates were much greater when 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

Hyperplastic and inflammatory changes were found in the nonglandular stomach of rats fed Sodium Metabisulfite at 0.5% to 8% for 10 to 56 days or 0.125% for up to 2 years.[1] 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 study was designed to represent human exposure to sulfites.[1] *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 8 animals (3 enzyme-deficient groups and 3 normal groups) received Sodium Metabisulfite in the drinking water; another 6 groups received the bound form. The 3 sulfite doses (measured as sulfite 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. Hematological parameters were comparable among rats; 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 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.*

Inhalation

Sodium Metabisulfite

Eight male beagle dogs were continuously exposed to a 1 mg/m^3 metabisulfite aerosol for 290 days.[1] *The MMAD of the aerosol was 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 the 3 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.*

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Potassium Metabisulfite

Potassium Metabisulfite was not teratogenic for mice at 125 mg/kg or rats at 155 mg/kg.[1] *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 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.*

For 20 months, 2 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.[1] *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 2 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.

Sodium Bisulfite

Sodium Bisulfite was not teratogenic for mice, rats, hamsters, or rabbits at doses of 150, 110, 120, and 100 mg/kg, respectively.[1]

Sodium Metabisulfite

Sodium Metabisulfite was not teratogenic for mice, rats, hamsters, or rabbits at doses of 160, 110, 120, and 123 mg/kg, respectively.[1] *It was also negative in sulfite oxidase-deficient rats when tested at doses up to 3.5 mmol/kg.*

In a study utilizing sulfite oxidase-deficient virgin female Wistar rats, the endogenous and exogenous toxicity of sulfites was examined.[1] *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 sodium sulfate (12.5 mM) in their drinking water. The three treatment groups, consisting of sulfite oxidase-deficient animals, received either tungsten and sodium sulfate (25 mM), or tungsten and sodium sulfate (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. Toxicity due to decreased feed consumption, reactions with feed constituents of the diet, and irritation of the gut were observed in this study. These effects and anemia were produced by the large concentrations of sulfite in the diet or gut; systemic sulfite does not appear to be related to any toxicity seen in this study.*

Sodium Metabisulfite was added to the drinking water (375 and 750 ppm as sulfur dioxide) of three generations of rats for 2.5 years.[1] *Generation I consisted of three groups: 13 females in each group, with group I having 5 males and group II 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 control groups of generation I. Generation II was derived similarly from generation II. 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.*

In a three-generation study, groups of 40 rats (20 per sex) received 0.125, 0.25, 0.5, 1, or 2% Sodium Metabisulfite administered in a thiamine-rich diet beginning shortly after weaning.[1] *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 F1a 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. 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 rats 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.*

A study similar to the preceding 3-generation study was conducted using groups of 40 guinea pigs (20 of each sex) fed 0.06, 0.16, 0.35, 0.83, and 1.72% Sodium Metabisulfite.[1] *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. 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, hear, 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.*

The effects of Sodium Metabisulfite on testicular function and morphometric values of the epididymis were evaluated using groups of 8 adult male Wistar rats.[26] The experimental groups received Sodium Metabisulfite (in distilled water) by gavage at the following doses for 28 consecutive days: 10, 100, and 260 mg/kg. An equal volume of normal saline was

administered to the control group via gavage. The rats were anaesthetized after 28 days and the left testis (with the head of epididymis) was excised. The epididymal sperm were analyzed for motility, morphology, and the number of sperm. Study results showed that normal morphology, count, and motility of sperm, and testosterone level, were decreased in all of the groups dosed with Sodium Metabisulfite. The serum level of testosterone (ng/ml) in the 260 mg/kg dose group decreased significantly ($p = 0.001$) in comparison with the control group. When compared to the control group, dosing with Sodium Metabisulfite resulted in a statistically significant lower total number of spermatogonia, primary spermatocyte, spermatids, and Leydig cells. The treated groups also showed a significant decrease in the mean diameter of the epididymal tubules and mean height of the epithelial cell when compared to the controls. The data also revealed that normal morphology sperm percentage was reduced significantly ($p < 0.001$) in the 100 mg/kg and 260 mg/kg dose groups. The immotile sperm were significantly increased ($p < 0.001$) in the 260 mg/kg dose group in comparison with the control group. In their conclusion, the authors suggested that Sodium Metabisulfite decreases sperm production and has the potential to affect fertility adversely in male rats.[26]

Three groups of 7 Sprague-Dawley rats were dosed by gavage with 0.7 mg/kg, 7 mg/kg, or 70 mg/kgSodium Metabisulfite daily for a period of 7 weeks.[27] The control group was dosed (by gavage) with distilled water. No change was identified in the parameters of spermatozoa in the 0.7 mg/kg/day group. However, when compared to the control group, a significant ($p < 0.05$) decrease was observed in the number of spermatozoa, percentage of normal morphology spermatozoa, and percentage of motile spermatozoa in the animals of the 2 higher dose groups (7 mg/kg/day and 70 mg/kg/day). The results also revealed a negligible change in the testicle volumes in these 2 groups. The seminiferous tubule volumes were reduced by 25% and 26% in rats exposed to 7 mg/kg/day and 70 mg/kg/day, respectively, when compared to the control group ($p < 0.01$). The results also indicated that the total volume of the seminiferous tubule germinal epithelium decreased by values of 28% and 36% in the rats that received 7 mg/kg/day and 70 mg/kg/day, respectively, when compared to the control group ($p < 0.01$).

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 on GDs 8 to 20.[1] *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 have produced signs of fetal toxicity but not teratogenicity*.

GENOTOXICITY STUDIES

The genotoxicity studies (in non-italicized text) summarized below are presented in Table 6.

In Vitro

The genotoxicity of Sodium Metabisulfite was evaluated in a chromosome aberrations and sister chromatid exchange (SCE) assay involving human lymphocytes.[28] A statistically significant increase in chromosome aberrations and SCE (dose-dependent) was observed at all test concentrations (75 mg/ml, 150 mg/ml, and 300 mg/ml) and treatment periods (24 h and 48 h).[28] Potassium Metabisulfite was evaluated in a chromosomal aberrations assay involving whole blood from human subjects.[29] Cell cultures were treated with 25, 50, 100, or 200 µg/ml concentrations of Potassium Metabisulfite (in twice-distilled water; 24-h and 48-h incubation periods). Structural chromosomal aberrations were observed at all concentrations and exposure periods (24 h and 48 h). Potassium Metabisulfite increased the percentage of numerical chromosomal aberrations only at the highest concentration of 200 µg/ml (48-h treatment). For the analysis of micronuclei in binucleated lymphocytes (micronucleus test), fresh human blood samples were cultured with Potassium Metabisulfite.[29] Cell cultures were treated with 25, 50, 100, or 200 µg/ml concentrations of Potassium Metabisulfite (in twice-distilled water; 24-h and 48-h incubation periods). A dose-dependent effect (without statistical significance) on the induction of structural and numerical chromosomal aberrations for 12-h and 24-h treatment periods was observed.

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 two-fold increase over control was needed to be considered positive.[1]

Sodium Bisulfite

Under in vitro conditions, bisulfite deaminates the nucleoside cytosine to uracil in single-stranded DNA.[1] The reaction proceeds rapidly at pH 5 to 6, with bisulfite solutions of ≥ 1 M (which are not normal physiological conditions). 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. 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. Under acidic conditions, Sodium Bisulfite can induce mutations in Salmonella typhimurium that contain his G46 (base-pair substitution sensitive) and his D6610 mutations, lambda phage, and some Escherichia coli strains. At lower concentrations and neutral pH, Sodium Bisulfite was not mutagenic to S. typhimurium or E. coli.

Sodium Bisulfite induced transformation SCEs, but not chromosomal aberrations in hamster embryo or ovary cells.[1] *Sodium Bisulfite did not induce mutations in two loci in Chines hamster V70 cells. It failed to increase DNA metabolism (which would have indicated DNA repair and mutagenesis), but did reduce the number of functioning replicons. The results suggested that Sodium Bisulfite induced hamster cell transformations through mechanisms other than mutation. Sodium Bisulfite induced SCEs and chromosomal aberrations in human lymphocytes.*

Sodium Metabisulfite

Sodium Metabisulfite was negative in an Ames/microsome assay.[1] *It was negative in the host-mediated assay using mice to test mutagenicity against bacteria and yeast, the cytogenic assay using rats, and a cytogenic assay using sulfite oxidase-deficient hamsters and mice. Results of one dominant lethal assay using rats indicated further testing was needed.*

Sodium Sulfite

Sodium Sulfite was negative in plate and suspension tests using Saccharomyces cerevisiae and *S. typhimurium and did not interfere with mitotic division of oocytes in mice.*[1]

In Vivo

An unscheduled DNA synthesis assay was performed to evaluate the genotoxicity of Sodium Bisulfite, using groups of 4 male rats of the Wistar Hanlbm:WIST (SPF) strain.[30] Sodium Bisulfite (formulated in citrate/sodium hydroxide buffer at pH 5) was administered orally at doses of 625 and 1250 mg/kg (dose volume = 10 ml/kg). No dose level of the test substance revealed unscheduled DNA synthesis induction in the hepatocytes of treated animals. A micronucleus test on Sodium Bisulfite was performed using groups of 12 mice (6 males and 6 females per group) of the NMRI strain.[30] Sodium Bisulfite (formulated in citrate/sodium hydroxide buffer at pH 5) was administered intraperitoneally at doses of 75 mg/kg, 150 mg/kg, and 300 mg/kg (dose volume = 15 ml/kg). The number of micronucleated polychromatic erythrocytes (PCE) was similar to those seen in controls. Therefore, no evidence of mutagenic potential was found. In the in vivo micronucleus test, Sodium Bisulfite and Sodium Sulfite were evaluated for their potential to induce micronuclei in mouse bone marrow PCE.[31] Groups of 10 male and groups of 10 female Kunming mice were used. A mixture of Sodium Bisulfite and Sodium Sulfite (3:1 M/M) in saline was injected intraperitoneally in single doses of 20, 100, 500, and 750 mg/kg. Results indicated that the test material induced micronuclei in a dose-dependent manner. At each dose of the test material, the formation of micronuclei was statistically significantly greater ($p < 0.01$) than the negative (saline) control values. The genotoxic effects of Sodium Metabisulfite on different tissues of the mouse were evaluated using the comet assay (liver and blood cells) and the micronucleus test (blood and bone marrow cells).[32] For all tissues, significant increases in damage index and damage frequency values were observed in the Sodium Metabisulfite-treated groups (1 and 2 g/kg doses), when compared to the control animals. The genotoxicity of Potassium Metabisulfite (in twice-distilled water) was evaluated in a chromosomal aberrations assay using groups of 4 (2 males and 2 females per group) albino rats.[29] The rats were intraperitoneally treated with three different doses (150, 300, and 600 mg/kg, single doses) of Potassium Metabisulfite for 12 h and 24 h before the animals were killed. Potassium Metabisulfite induced structural and numerical chromosomal aberrations at all doses (non-statistically significant dose response) and treatment times when compared to the control.

Sodium Bisulfite

Sodium Bisulfite was negative in all in vivo studies (dominant lethal test in rats and tests on the induction of chromosome aberrrations in mouse germ cells) using mammalian systems.[1]

Comutagenicity

Sodium Bisulfite

The combined effect of Sodium Bisulfite and a nitrogen nucleophile, i.e., semicarbazide, methoxyamine, or hydroxylamine was investigated.[1] *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. The comutagenicity of bisulfite with UV against Chinese hamster V79 cells has also been reported.*

Sodium Sulfite

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

Antimutagenicity

Sodium Bisulfite, Sodium Sulfite, and Potassium Metabisulfite

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 and instant and freshly brewed coffee.[1] *Sodium Bisulfite "effectively" inhibited the mutagenic activity of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), but had no effect on the mutagenicity of N-acetoxy-2-acetylaminofluorene. Sulfites also prevented the induction of lambda prophage, and suppressed the mutagenicities of 1,2-dicarbonyls.*

In mammalian cell systems, Sodium Bisulfite suppressed the mutagenicity of coffee in hamster lung cells and the mutagenicity of B[a]P or x-ray irradiation in C3H/10T-1/2 mouse cells.[1] *Sodium Bisulfite also suppressed the induction of SCEs by coffee in AUXB1 cells. In Chinese hamster ovarian (CHO) cells, it suppressed the induction of SCEs and the proportion of endoreduplicated cells (ERCs) by glyoxal, methylglyoxal, kethoxal, and diacetyl.*

CARCINOGENICITY STUDIES

A review of sulfur dioxide, Sodium Sulfite, Sodium Bisulfite, and Sodium and Potassium Metabisulfites by the International Agency for Research on Cancer (IARC) 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.[1] *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, when 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 sulfate 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.*

In Vitro

Sodium Bisulfite

Sodium Bisulfite caused neoplastic transformation of Syrian hamster fetal cells and was associated with qualitative and quantitative polypeptide changes.[1] *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 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.*

Oral

Potassium Metabisulfite

Groups of 100 ICR/JCL mice (50 per sex) received 1% or 2% Potassium Metabisulfite in the drinking water for 24 months.[1] *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% for the 2% dose group.*

Sodium Bisulfite

A three-part, 3-year study using the Osbourne-Mendel strain of rats evaluated the chronic toxicity of Sodium Bisulfite.[1] *All three parts used a balanced incomplete block design method. In the first part, of the study, rats were fed either one or 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 was further divided into groups that received three different*

concentrations of sulfite: 0.5%, 1%, and 2%. This part of the study was replicated for males and females (3 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 were as follows: weekly prepared at both 1% and 2% Sodium Bisulfite, aged fed at 0.1%, 0.25%, 1%, and 2%, controls at 1% and 2%, and two diets with 0.25% and 1% sodium sulfate. This part of the study had a duration of 1.5 year. 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% of Sodium Bisulfite were utilized, along with a control and 0.25% and 1% sodium sulfide. There was no evidence of carcinogenicity in rats that were fed Sodium Bisulfite at concentrations up to 2%.

Sodium Metabisulfite

An experiment using normal sulfite oxidase activity female rats was conducted. These rats were fed 0%, 1%, 2%, or 6% powdered Sodium Metabisulfite. All diets containing Sodium Metabisulfite were supplemented with 50 ppm thiamine. Powdered Sodium Metabisulfite in the feed was associated with destruction of thiamine. 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.

Parenteral

Sodium Bisulfite

Hamster fetal cells that had been transformed by Sodium Bisulfite produced tumors in nude mice after subcutaneous (SC) inoculation.[1] *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 that has been reported was considered a contributing factor. Sodium Bisulfite was considered a nonclastogenic carcinogen.*

Co-Carcinogenicity

Oral

Potassium Bisulfite

In a two-stage stomach carcinogenesis experiment, male out-bred Wistar rats were given MNNG in the drinking water and sodium chloride in the feed for 8 weeks.[1] *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, when 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.*

Sodium Bisulfite

A co-carcinogenicity study that was performed involved 365 mice of both sexes that were divided into 5 groups and a control group.[1] *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 animals ingested the preservatives, and then were injected intraperitoneally with Ehrlich's ascites carcinoma. The observation period for all 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."*

OTHER RELEVANT STUDIES

Cytotoxicity

Potassium Metabisulfite

Human dermal fibroblasts (1×10^5 cells/ml) were incubated for 7 days with Potassium Metabisulfite, diluted in the culture medium (pH = 7) to three different concentrations: 150, 300, and 600 mg/l.[33] Cell viability was determined by the trypan blue exclusion method and Potassium Metabisulfite cytotoxicity was evaluated using the MTT cell proliferation assay. The highest dose of Potassium Metabisulfite caused a dramatic cell death from 1 day of incubation and, after 3 days of exposure, almost all cells had died. The 2 lower concentrations were also toxic, but to a lesser extent. The MTT assay

demonstrated that Potassium Metabisulfite exposure slowed cell proliferation in a dose-dependent manner. The cells treated with 300 mg/l of Potassium Metabisulfite suffered a sharp slowdown in the proliferation, reaching a halt after the third day. A slowdown of proliferation was also evident for cells treated with 150 mg/l of Potassium Metabisulfite, but in this case, a stunting was not observable.

Sodium Bisulfite

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.[1] In another study, Sodium Bisulfite 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 < 0.8×10^{-3} M.

Sodium Metabisulfite

In the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the treatment of A549 cells with Sodium Metabisulfite induced a dose-dependent decrease in cell viability at concentrations above 100 mg/ml.[25] The half maximal inhibitory concentration (IC_{50}) was determined as 281.5 mg/ml.

Phototoxicity

Sodium Metabisulfite and Sodium Sulfite

A study was performed in which suspensions of human erythrocytes (from 3 donors) were each incubated with Sodium Sulfite and Sodium Metabisulfite (identified as sodium disulfite).[1] 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 long-wavelength UV (UVA) or mid-wavelength UV (UVB) light. Hemolysis was measured as a function of absorbance of 550-nm light. 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 UVA-SUN 5000 (emission: 320 to 460 nm; UVA doses up to 100 J/cm^2) apparatus.

Neurotoxicity

Sodium Metabisulfite

A study was performed to investigate the possible toxic effects of sulfite on pyramidal neurons.[34] Male Wistar albino rats were used in this study. The two groups of rats used were control (5 rats) and test (5 rats). Sulfite in the form of Sodium Metabisulfite (25 mg/kg/day) was given orally via drinking water for 8 weeks. Control rats received standard rat chow and tap water. At the end of the 8th week, all animals were killed. The brain was removed immediately and sections were prepared for microscopy. Neurons were estimated in total and in a known fraction of CA1 and CA2-CA3 subdivisions of the left hippocampus. Toxicity was investigated by counting cell numbers in CA1 and CA2-CA3 subdivisions. Results showed that sulfite treatment caused a significant decrease in the total number of pyramidal neurons in three subdivisions of the hippocampus (CA1 and CA2-CA3) in test animals, when compared to the control group ($p < 0.05$). It was concluded that exogenous administration of sulfite causes loss of pyramidal neurons in CA1 and CA2-CA3 subdivisions of the rat hippocampus.

Activation of Epithelial Lung Cells

Sodium Sulfite

The role of Sodium Sulfite on human epithelial lung cells and its effect on neutrophil adhesion to these epithelial cells was studied.[35] The results of this study indicate that Sodium Sulfite (0.01 M to 10 M) induces tyrosine phosphorylation events and interleukin-8 production in A549 cells. Human neutrophil adhesion to Sodium Sulfite-induced A549 cells was increased when compared to untreated A 549 cells. The authors concluded that Sodium Sulfite can activate human A549 cells. They also concluded that neutrophil adhesion to Sodium Sulfite-induced A549 cells is increased via an ICAM-1, ICAM-3, and VCAM-1 (these are 3 adhesion molecules) independent mechanism.

Effect on Mast Cell Degranulation

Sodium Sulfite

An experiment was performed to determine whether Sodium Sulfite had an effect on mast cell degranulation.[36] Rat basophilic leukemia (RBL-2H3) cells were exposed to varying concentrations of Sodium Sulfite (0.5 mM to 5 mM). Sodium Sulfite induced degranulation of RBL-2H3 cells with a maximum degranulation of 13% observed at 2 mM Sodium Sulfite. Optimal immunoglobulin E (IgE) cross-linking induced degranulation of 14.3%. These data represented the means of 3 experiments. A significant correlation was observed between Sodium Sulfite concentration and percentage degranulation using the Spearman rho correlation method ($p < 0.001$). To evaluate whether sulfite also induces degranulation of human basophils, peripheral blood mononuclear cells were isolated from 2 volunteers and exposed to 1, 2, and 5 mM Sodium

Sulfite. Results for each volunteer are expressed as a percentage of total histamine released by cell lysis. Sodium Sulfite (5mM) induced degranulation of 13% and 10% for volunteers 1 and 2, respectively. Anti–human IgE induced degranulation of 14.8% and 16% for volunteers 1 and 2, respectively. The authors noted that these results confirm that sulfite-induced mediator release is an important trigger in human basophils comparable with mediator release induced by IgE cross-linking.

Effect on Allergic Pulmonary Sensitization Co-Elicitation

Sodium Sulfite

The effects of Sodium Sulfite, and its interaction with a house dust mite (*Dermatophagoides pteronyssinus*), on allergic sensitization and airway inflammation were investigated.[37] BALB/c mice were divided into the following four groups: control (n = 10), mite intranasal (n = 12), Sodium Sulfite intranasal (n = 12), and mite intranasal + Sodium Sulfite intranasal (n = 12). In non-control groups, the mice were sensitized subcutaneously on day 8 and day 15 with mite allergen. Mite allergen was then administered intranasally from day 15 to day 22 in the mite intranasal group and in the mite intranasal + Sodium Sulfite intranasal groups. Sodium Sulfite was administrated to the Sodium Sulfite intranasal group and to the mite intranasal + Sodium Sulfite groups intranasally from day 1 to day 22. Plasma *Dermatophagoides pteronyssinus*-specific IgE, IgG2a, lung histopathology and cytokine levels (interleukin-5 (IL-5) and interferon-gamma (IFN-g)) were analyzed.

When the mite intranasal (or Sodium Sulfite intranasal) group was compared with the mite intranasal + Sodium Sulfite intranasal group, *Dermatophagoides pteronyssinus*-specific IgE levels were significantly higher in the mite intranasal + Sodium Sulfite intranasal group ($p < 0.01$). Also, the *Dermatophagoides pteronyssinus*-specific IgG2a level was significantly lower in mite intranasal + Sodium Sulfite intranasal group than in the mite intranasal or (or Sodium Sulfite intranasal) group ($p < 0.01$). The peribronchiolar, alveolar and total inflammatory scores were increased in the mite intranasal + Sodium Sulfite intranasal group, when compared to the control group ($p < 0.05$, $p < 0.01$, $p < 0.01$, respectively). The authors concluded that Sodium Sulfite may enhance allergic sensitization as well as airway inflammation in mite allergen sensitized BALB/c mice.

Effect on Gene Expression

Sodium Bisulfite and Sodium Sulfite

The effect of Sodium Bisulfite and Sodium Sulfite on the expression of proto-oncogenes and tumor suppressor genes was evaluated using cultured human bronchial epithelial (BEP2D) cells.[38] The mRNA and protein levels were measured by real-time reverse transcriptase polymerase chain reaction (RT-PCR) and western blotting, respectively, following exposure to different test substance concentrations. Sodium Bisulfite and Sodium Sulfite caused mRNA and protein over-expression of c-fos, c-jun, and c-myc oncogenes at all tested doses (0.001 – 2 mM). Over-expression of H-ras (oncogene) and p53 (tumor suppressor gene) genes were observed in cells receiving Sodium Bisulfite or Sodium Sulfite at concentrations of 0.1 – 2 mM, and the under-expression of p16 and Rb tumor suppressor genes was also observed. The authors noted that, in the lung, changes in c-fos, c-jun, c-myc, H-ras, p53, p16, and Rb genes have been observed in preneoplastic as well as cancerous tissue. Furthermore, the authors concluded that these data support the hypothesis that derivatives of sulfur dioxide (i.e., Sodium Bisulfite and Sodium Sulfite) could cause the activation of proto-oncogenes and inactivation of tumor suppressor genes. They also stated that sulfur dioxide derivatives may play a role in the pathogenesis of sulfur dioxide-associated lung cancer.

DERMAL IRRITATION AND SENSITZATION STUDIES

Irritation

Animal

Sodium Bisulfite

Sodium Bisulfite (0.5 ml of a 38% solution) was applied to the clipped backs of 6 albino rabbits.[1] *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 h and 48 h after initial application. Sodium Bisulfite was not corrosive*.

Sodium Metabisulfite

Sodium Metabisulfite (0.5 ml of an undiluted solution) was applied for 4 h to the clipped backs of 6 albino rabbits.[1] *Sodium Metabisulfite did not produce a primary irritation response. Sodium Metabisulfite (solid, 5 g) was applied for 24 h to clipped but intact sites on the trunk of 6 male albino rabbits. Skin irritation was not observed. 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).*

Sensitization

Human

Sodium Sulfite

A hair-coloring agent with 0.64% Sodium Sulfite was used in a repeat insult open patch test involving 100 participants.[1] The panelists received 0.2 ml or 0.2 g of the test material directly onto a designated area of the back. The procedure was repeated until 9 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 h 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 cannot be considered a primary irritant or primary sensitizer.

Samples of 0.5% Sodium Sulfite in a topical feminine cream were patch tested using 100 panelists.[1] 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 9 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 9 exposures and were scored 24 h and 48 h after application. No adverse reactions were observed and, according to investigators, the test material cannot be considered a primary irritant and primary sensitizer.

OCULAR IRRITATION STUDIES

Animal

Sodium Metabisulfite

Sodium Metabisulfite (100 mg) was placed into the right conjunctival sac of each of 2 rabbits.[1] *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 h 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 iritis, and mild conjunctivitis were 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.*

Human

Sodium Metabisulfite

A double-blind study tested the 5 individual components of an eye drop therapy for glaucoma.[1] Five male glaucoma patients applied 2 drops of an eye drop preparation containing 0.075% Sodium Metabisulfite twice daily for 1 week. There was a 1-week treatment-free period between applications of different solutions. The order of administration of the 5 preparations (each comprising 1 of 5 individual components of the eye drop) 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.

CLINICAL STUDIES

Sulfur dioxide is one of the species to which the sulfites reviewed in this safety assessment may convert. In 2019, the United States Environmental Protection Agency (USEPA) completed their review of the primary health-based National Ambient Air Quality Standards (NAAQS) for SO_x, a group of closely related gaseous compounds that includes sulfur dioxide (SO_2) (40 CFR Part 50). The current primary standard is set a a level of 75 parts per billion (ppb), as the 99th percentile of daily maximum 1-h SO_2 concentrations, averaged over 3 years. Based on the EPA's review of key aspects of the currently available health effects evidence, quantitative risk and exposure information, advice from the Clean Air Scientific Advisory Committee (CASAC), and public comments, the EPA is retaining the current standard without revision.

Provocative Studies

Sulfite Sensitivity

Many asthmatics with bisulfite sensitivity have negative allergy skin tests suggesting a nonatopic nature.[1] It has also been reported that IgE, total eosinophil counts, and histamine concentrations were normal during acute reactions, suggesting the lack of an IgE mechanism. Approximately 2% to 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 control normal subjects, but inhalation sensitivity alone is not considered indicative of sulfite sensitivity. In the majority of instances, manifestations include dermatological 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. It has been reported that the results of skin tests, provocative challenge test, and passive transfer tests suggested that some metabisulfite-sensitive reactions can be IgE mediated.

Sodium Metabisulfite

A tertiary-referral clinic population was studied to estimate safe exposure doses for use in epidemiological studies of acute versus allergic reactions.[1] A positive response was defined as a 15% decrease in the amount of air expired in 1 sec 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.

Five male glaucoma patients were patch tested with 0.5% Sodium Metabisulfite (ingredient of eye drops for glaucoma).[1] The patients had elevated intraocular pressure and histories of local sensitivity reactions to dipivalyl epinephrine (the active component of eye drops). None of the patients had positive reactions to 0.5% Sodium Metabisulfite.

Retrospective and Multicenter Studies

The retrospective and multicenter studies (in non-italicized text) summarized below are presented in Table 7.

Potassium Metabisulfite

Two of 100 patients with chronic idiopathic urticaria had a positive urticarial response after each was challenged (single-blind) orally with Potassium Metabisulfite (amount not stated) and 10 other food additives simultaneously.[39] The 2 patients were subjected to a double-blind placebo-controlled challenge after a 2-week period, and a late reaction to this challenge was not observed.

Sodium Metabisulfite

One of 475 patients with contact allergy to cosmetic ingredients had an allergic reaction to Sodium Metabisulfite (concentration not stated).[40] Nine of 405 contact dermatitis patients had positive patch test reactions to 1% Sodium Metabisulfite in petrolatum.[41] In another study, 70 of 996 patients patch tested with 1% Sodium Metabisulfite in petrolatum, as part of the British standard patch test series, had positive reactions.[42] Additionally, in the prospective arm of this study, 380 patients were patch tested with 3 concentrations of Sodium Metabisulfite (0.01%, 0.1%, and 1%). Results were as follows: 14 patients (3.68%) with positive patch test reaction to 1% Sodium Metabisulfite, 7 patients with positive patch test reaction to 0.1% Sodium Metabisulfite, and 3 patients with a positive patch test reaction to 0.01% Sodium Metabisulfite. In a study with a larger patient population, 71 of 1751 contact dermatitis patients patch tested with 1% Sodium Metabisulfite in petrolatum had reactions that were classified as allergic.[43] Of these, 33 were reported as relevant, with an identifiable source at the time of reporting, and 38 were of unknown relevance. Fifty-one of 1518 patients (most of whom had hand eczema) had positive patch test reactions to 2% Sodium Metabisulfite in petrolatum.[44] Another 10 patients had weak irritant reactions when patch tested. The patch test reactions were considered of current relevance in 2 cases; in a third case, a previous relevance was probable. In 14 cases (27%), the relevance was considered questionable and no relevance was found in 24 patients (47%).

Sodium Sulfite and Sodium Metabisulfite

Of the 183 patients patch tested with 1% Sodium Metabisulfite in petrolatum at a dermatology center, 5.5% had positive allergic reactions.[45] In the same patient population patch tested, 3.8% of the patients had positive reactions to 1% Sodium Sulfite in petrolatum. One-hundred twenty-four of 2763 patients had positive patch test reactions to Sodium Metabisulfite (tested with 2% initially and later with 1%).[46] The reactions were considered to be relevant in 80 cases (64.5%), of which 11 were occupational. None of the 39 patients patch tested with 2% Sodium Sulfite had positive reactions.

Sodium Sulfite

Patch tests were performed on 1762 dermatologic patients with Sodium Sulfite (1% in petrolatum).[1] 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."

Sodium Bisulfite, Sodium Metabisulfite, Sodium Sulfite, and Potassium Metabisulfite

The results of patch testing 2894 eczematous patients over a 2-year period were reported.[1] Positive reactions to Sodium Metabisulfite (1% in petrolatum, following a 2-day occlusive exposure) were noted in 50 patients (1.7% incidence).

All 50 patients also reacted to Potassium Metabisulfite (1% in petrolatum) and to Sodium Bisulfite (1% and 5% in petrolatum). Only 2 reacted to Sodium Sulfite (1% in petrolatum). Prick and intradermal tests of 20 patients with a Sodium Metabisulfite solution (10 mg/ml) were negative and oral challenge of 5 patients with 30 mg and 50 mg Sodium Metabisulfite did not provoke a flare-up of dermatitis or patch test. The dermatitis was considered occupational in 7 cases. Five of the remaining 43 cases were considered allergic contact dermatitis resulting from the use of topical preparations.

Case Reports

The case reports (in non-italicized text) summarized below are presented in Table 8.

Ammonium Bisulfite

A patient with a history of rhinitis had a positive reaction (+++/++) reaction to a color bleaching ointment.[47] A positive reaction (++) was also reported when the patient was patch tested with 2% Ammonium Bisulfite (ingredient of ointment) in petrolatum.

Sodium Bisulfite

Positive patch test reactions to 0.1% and 1% Sodium Bisulfite in petrolatum were observed in a myasthenia gravis patient who had been given a high-calorie infusion that contained 0.04% Sodium Bisulfite.[48] Additionally, pruritus (positive reaction) was observed at the 0.04% Sodium Bisulfite infusion patch test site, but a positive reaction was not observed at the 0.002% Sodium Bisulfite infusion patch test site.

Sodium Metabisulfite

Three cases of Sodium Metabisulfite-induced asthma in workers with different jobs in the fishing industry have been reported.[49] It was noted that it appears that high exposures to Sodium Metabisulfite, and thus sulfur dioxide (in excess of 30 ppm), occur in this industry. Also, Sodium Metabisulfite can react with water, releasing toxic sulfur dioxide gas. A patient with severe pruritus was subjected to a series of double-blind, placebo-controlled challenges with Sodium Metabisulfite.[50] Sodium Metabisulfite (10 mg) ingestion caused pruritus of the trunk, upper limbs, and head. A positive (++) patch test reaction to Sodium Metabisulfite (concentration not stated) was observed in an eczema patient who had used a topical antibiotic cream that contained Sodium Metabisulfite (concentration not stated).[51] A positive (+) reaction to Sodium Metabisulfite (concentration not stated) was also observed in a renal transplant patient who had used the same topical antibiotic cream.

A hairdresser with a history of allergic reactions had positive (++) patch test reactions to Sodium Metabisulfite (0.02, 0.064, 0.2, and 0.64%).[52] The patch testing of 5 control patients in this report resulted in an irritant reaction in 1 patient tested with 0.064% Sodium Metabisulfite, and a weak irritant reaction (slight erythema) to 0.02% Sodium Metabisulfite in the same patient. In a separate patch test session 15 additional control patients were patch tested with the following concentrations of Sodium Metabisulfite: 0.02, 0.064, 0.2, and 0.64%. Three of the 15 controls had an irritant reaction to 064% Sodium Metabisulfite and a fourth control patient had a weak irritant reaction to this concentration. One of the 4 also had an irritant reaction to 0.2% Sodium Metabisulfite, and another had a weak irritation reaction at this concentration. A positive (++) patch test reaction to 1% Sodium Metabisulfite in vaseline was observed in an eyelid dermatitis patient who had used eyedrops containing Sodium Metabisulfite.[53] A hand dermatitis patient who came in contact with plastic bags that contained a preservative solution of Sodium Metabisulfite had a positive (+) patch test reaction to 1% Sodium Metabisulfite in petrolatum.[54] A dermatitis patient who had been treated with gentamicin solution and ketoconazole cream (both contained Sodium Metabisulfite) also had a positive patch test reaction to 1% Sodium Metabisulfite in petrolatum.[55] A positive (++) patch test reaction to 2% Sodium Metabisulfite in petrolatum was observed in a patient who had used an antihemorrhoidal cream containing Sodium Metabisulfite.[56] A positive (++) patch test reaction to 2% Sodium Metabisulfite in petrolatum was also observed in a patient who had received an anesthetic injection containing Sodium Metabisulfite (concentration not stated).[57] Prick test results for Sodium Metabisulfite (0.05, 0.1, 1, and 10% in water) were negative in this patient.

Sodium Metabisulfite, Sodium Sulfite, and Potassium Metabisulfite An agricultural worker with dermatitis was tested with sulfites.[58] Patch and prick test results were positive for 1% Potassium Metabisulfite and 1% Sodium Metabisulfite, but not for Sodium Sulfite. Intradermal test results were also positive for 0.1% Potassium Metabisulfite and 0.1% Sodium Metabisulfite, but not for Sodium Sulfite. Another case report relates to an employee in the wine industry (dermatitis patient) was responsible for preparing a solution of Potassium Metabisulfite (10% in water) for different stages of winemaking.[59] The patient was patch tested with Sodium Metabisulfite and Potassium Metabisulfite (each at 1% in petrolatum), and reactions were scored on days 2 and 4. The only positive patch test reactions were to Potassium Metabisulfite (1% in petrolatum) on day 2 (++ reaction) and day 4 (+++ reaction). Results were negative in 10 control subjects patch tested with Potassium Metabisulfite. A patient with monoclonal mast cell activation syndrome after food ingestion was subjected to double-blind, placebo-controlled food challenge.[60] Anaphylaxis was reported at 15 min after oral administration of Potassium Metabisulfite (300 mg).

Sulfur Dioxide

In a case study, a 46-year-old carpenter was exposed to sulfur dioxide gas.[1] The patient complained of eruptions on his forearms that, within 5 days, spread to all of his extremities; also, his eyelids were swollen. The patient was diagnosed with symmetrical exanthema. Two positive exposure tests confirmed that the reactions were due to sulfur dioxide.

Other Clinical Reports

Sodium Bisulfite

Twelve volunteers (6 males, 6 females) were placed on a thiamine-deficient diet for 15 days.[1] 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 6 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.

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.[1] Also, published reports have described isolated cases of seizures associated with i.v. administration of large doses of morphine containing Sodium Bisulfite as a preservative.

SUMMARY

The 7 sulfites reviewed in this safety assessment are all water-soluble, inorganic salts. Sodium Sulfite and Sodium Metabisulfite may be manufactured by reacting sulfur dioxide with sodium carbonate (soda ash). This step is followed by purification and drying to form crystals or powder.

The *Food Chemicals Codex* acceptance criteria for potential impurities in Sodium Bisulfite, Sodium Metabisulfite, Sodium Sulfite, Potassium Metabisulfite, and Potassium Sulfite include not more than 2 mg/kg for lead (all 5 ingredients) and not more than 5 mg/kg for selenium (all except for Sodium Sulfite). According to the *Food Chemicals Codex*, the acceptance criterion for selenium as a potential impurity in Sodium Sulfite is not more than 0.003%.

In the 2003 original report and in 2020, Sodium Sulfite is the ingredient with the highest use frequency. The use frequency of 911 for this ingredient in the original report increased to a value of 1713 in 2020. A substantial increase in the use frequency from 348 to 916 in 2020 is being reported for Sodium Metabisulfite. Of the ingredients reviewed in the 2003 report, Sodium Metabisulfite had the highest use concentration (14% in rinse-off products). In 2019, this ingredient is being used at substantially lower concentrations of up to 0.6% in these products. The sulfite with the highest reported use concentration in 2019 is Sodium Sulfite, which is being used at concentrations up to 3% in rinse-off products. This was also the highest use concentration of Sodium Sulfite in the 2003 original report.

Groups of 10 male and 10 female rats were administered biscuits containing Sodium Metabisulfite at levels of 0 (control), 10, 35, and 75% (corresponding to 10 - 15, 35 - 45, 150 - 170, and 310 - 340 mg/kg diet) for 28 and 85 days.. None of the animals died, and no clinical abnormalities were reported. The NOAEL of sulfites in baked biscuits for both studies was determined to be 310 mg sulfite/kg or 25 mg/kg/day.

The short-term inhalation toxicity of Sodium Metabisulfite exposure was evaluated using the following groups of Sprague-Dawley rats: fresh air (negative control), and groups exposed to Sodium Metabisulfite at target concentrations of 5, 20, and 100 mg/m^3. The actual exposure concentrations of Sodium Metabisulfite were determined to be 5.5 ± 2.4 mg/m^3, 29.3 ± 7.7 mg/m^3, and 110 ± 38.9 mg/m^3 in the low-, medium-, and high-exposure groups, respectively. The animals were exposed 5 days per week (6 h per day) for 10 days. According to the histopathological results, dosing with Sodium Metabisulfite did not cause any toxic effects in the liver, lung, or nasal cavity.

The effects of Sodium Metabisulfite on testicular function and morphometric values of the epididymis were evaluated using groups of 8 adult male Wistar rats. The experimental groups received Sodium Metabisulfite (in distilled water) by gavage at the following doses for 28 consecutive days: 10 mg/kg, 100 mg/kg, and 260 mg/kg. Normal morphology sperm percentage was reduced significantly ($p < 0.001$) in the 100 mg/kg and 260 mg/kg dose groups. The immotile sperm were significantly increased ($p < 0.001$) in the 260 mg/kg dose group in comparison with the control group. The authors suggested that Sodium Metabisulfite decreases sperm production and has the potential to affect fertility adversely in male rats. A study on protection against Sodium Metabisulfite-induced testicular toxicity was performed. Three groups of 7 Sprague-Dawley rats were dosed by gavage with the following doses of Sodium Metabisulfite daily for a period of 7 weeks: 0.7, 7, and 70 mg/kg/day. No change was identified in the parameters of spermatozoa in the 0.7 mg/kg/day group. However, when compared to the control group, a significant ($p < 0.05$) decrease was observed in the number of spermatozoa, percentage of normal morphology spermatozoa and percentage of motile spermatozoa in the animals of the 2 higher dose groups (7 mg/kg/day and 70 mg/kg/day).

The genotoxicity of Sodium Metabisulfite was evaluated in a chromosome aberrations and sister chromatid exchanges assay involving human lymphocytes. A statistically significant increase in chromosome aberrations and sister chromatid

exchanges (dose-dependent) was observed at all test concentrations (75 mg/ml [$p < 0.05$], 150 mg/ml [$p < 0.01$], and 300 mg/ml [$p < 0.01$]) and treatment periods (24 h and 48 h). Potassium Metabisulfite was evaluated in a chromosomal aberrations assay involving whole blood from human subjects. Cell cultures were treated with 25, 50, 100, or 200 µg/ml concentrations of Potassium Metabisulfite (in twice distilled water; 24-h and 48-h incubation periods). Structural chromosomal aberrations were observed at all concentrations and exposure periods (24 h and 48 h). Potassium Metabisulfite increased the percentage of numerical chromosomal aberrations only at the highest concentration of 200 µg/ml (48-h treatment). For the analysis of micronuclei in binucleated lymphocytes (micronucleus test), fresh human blood samples were cultured with Potassium Metabisulfite. Cell cultures were treated with 25, 50, 100, or 200 µg/ml concentrations of Potassium Metabisulfite (in twice distilled water; 24-h and 48-h incubation periods). A dose-dependent effect (without statistical significance) on the induction of structural and numerical chromosomal aberrations for 12-h and 24-h treatment periods was observed.

An unscheduled DNA synthesis assay was performed to evaluate the genotoxicity of Sodium Bisulfite, using groups of 4 male rats of the Wistar Hanlbm:WIST (SPF) strain. Sodium Bisulfite (formulated in citrate/sodium hydroxide buffer at pH = 5) was administered orally at doses of 625 and 1250 mg/kg (dose volume = 10 ml/kg). No dose level of the test substance revealed unscheduled DNA synthesis induction in the hepatocytes of treated animals. A micronucleus test on Sodium Bisulfite was performed using groups of 12 mice (6 males and 6 females per group) of the NMRI strain. Sodium Bisulfite (formulated in citrate/sodium hydroxide buffer at pH 5) was administered intraperitoneally at doses of 75 , 150, and 300 mg/kg (dose volume = 15 ml/kg). The number of micronucleated PCE was similar to those seen in controls. Therefore, no evidence of mutagenic potential was found. In the in vivo micronucleus test, Sodium Bisulfite and Sodium Sulfite were evaluated for their potential to induce micronuclei in mouse bone marrow PCE. Groups of 10 male and groups of 10 female Kunming mice were used. A mixture of Sodium Bisulfite and Sodium Sulfite (3:1 M/M) in saline was injected intraperitoneally in single doses of 20, 100, 500, and 750 mg/kg. Results indicated that the test material induced micronuclei in a dose-dependent manner. At each dose of the test material, the formation of micronuclei was statistically significantly greater ($p < 0.01$) than the negative (saline) control values. The genotoxic effects of Sodium Metabisulfite on different tissues of the mouse were evaluated using the comet assay (liver and blood cells) and the micronucleus test (blood and bone marrow cells). For all tissues, significant increases in damage index and damage frequency values were observed in the Sodium Metabisulfite-treated groups (1 and 2 g/kg doses), when compared to the control animals. The genotoxicity of Potassium Metabisulfite (in twice distilled water) was evaluated in a chromosomal aberrations assay using groups of 4 (2 males and 2 females per group) albino rats. The rats were intraperitoneally treated with three different doses (150, 300, and 600 mg/kg, single doses) of Potassium Metabisulfite for 12 h and 24 h before the animals were killed. Potassium Metabisulfite induced structural and numerical chromosomal aberrations at all doses (non-statistically significant dose response) and treatment times when compared to the control.

Human dermal fibroblasts (1×10^5 cells/ml) were incubated for 7 days with Potassium Metabisulfite, diluted in the culture medium (pH 7) to three different concentrations: 150, 300, and 600 mg/l. Cell viability was determined by the trypan blue exclusion method and Potassium Metabisulfite cytotoxicity was evaluated using the MTT cell proliferation assay. The highest dose of Potassium Metabisulfite caused a dramatic cell death from 1 day of incubation and, after 3 days of exposure, almost all cells had died. The 2 lower concentrations were also toxic, but to a lesser extent. The MTT assay demonstrated that Potassium Metabisulfite exposure slowed cell proliferation in a dose-dependent manner.

A study was performed to investigate the possible toxic effects of sulfite on pyramidal neurons from male Wistar albino rats. Sulfite in the form of Sodium Metabisulfite (25 mg/kg/day) was given orally via drinking water for 8 weeks. Results showed that sulfite treatment caused a significant decrease in the total number of pyramidal neurons in three subdivisions of the hippocampus (CA1 and CA2-CA3) in test animals, when compared to the control group ($p < 0.05$).

The role of Sodium Sulfite on human epithelial lung cells and its effect on neutrophil adhesion to these epithelial cells was studied. Results indicated that Sodium Sulfite (0.01 M to 10 M) induces tyrosine phosphorylation events and interleukin-8 production in A549 cells (i.e., activation of A549 cells). Human neutrophil adhesion to Sodium Sulfite-induced A549 cells was increased when compared to untreated A549 cells.

An experiment was performed to determine whether Sodium Sulfite had an effect on mast cell degranulation. Rat basophilic leukemia (RBL-2H3) cells were exposed to varying concentrations of Sodium Sulfite (0.5 mM to 5 mM). Sodium Sulfite induced degranulation of RBL-2H3 cells with a maximum degranulation of 13% observed at 2 mM Sodium Sulfite. To evaluate whether sulfite also induces degranulation of human basophils, peripheral blood mononuclear cells were isolated from 2 volunteers and exposed to 1, 2, and 5 mM Sodium Sulfite. Sodium Sulfite (5mM) induced degranulation of 13% and 10% for volunteers 1 and 2, respectively.

The effects of Sodium Sulfite, and its interaction with a house dust mite (*Dermatophagoides pteronyssinus*), on allergic sensitization and airway inflammation were investigated using the following 4 groups of BALB/c mice. control ($n = 10$), mite intranasal ($n = 12$), Sodium Sulfite intranasal ($n = 12$), and mite intranasal + Sodium Sulfite intranasal ($n = 12$). The peribronchiolar, alveolar and total inflammatory scores were increased in the mite intranasal + Sodium Sulfite intranasal group, when compared to the control group ($p < 0.05$, $p < 0.01$, $p < 0.01$, respectively). The authors concluded that Sodium Sulfite may enhance allergic sensitization as well as airway inflammation in mite allergen sensitized BALB/c mice.

The effect of Sodium Bisulfite and Sodium Sulfite on the expression of proto-oncogenes and tumor suppressor genes was evaluated using cultured human bronchial epithelial (BEP2D) cells. Sodium Bisulfite and Sodium Sulfite caused mRNA and protein over-expression of c-fos, c-jun, and c-myc oncogenes at all tested doses (0.001 – 2 mM).

A number of multicenter patient studies on Sodium Metabisulfite have been identified in the published literature. One of 475 patients with contact allergy to cosmetic ingredients had an allergic reaction to Sodium Metabisulfite (concentration not stated). Nine of 405 contact dermatitis patients had positive patch test reactions to 1% Sodium Metabisulfite in petrolatum. In another study, 70 of 996 patients patch tested with 1% Sodium Metabisulfite in petrolatum, as part of the British standard patch test series, had positive reactions. Additionally, in the prospective arm of this study, 380 patients were patch tested with 3 concentrations of Sodium Metabisulfite (0.01, 0.1, and 1%). Results were as follows: 14 patients (3.68%) with positive patch test reaction to 1% Sodium Metabisulfite, 7 patients with positive patch test reaction to 0.1% Sodium Metabisulfite, and 3 patients with a positive patch test reaction to 0.01% Sodium Metabisulfite. In a study with a larger patient population, 71 of 1751 contact dermatitis patients patch tested with 1% Sodium Metabisulfite in petrolatum had reactions that were classified as allergic. Of these, 33 were reported as relevant, with an identifiable source at the time of reporting, and 38 were of unknown relevance. Fifty-one of 1518 patients (most of whom had hand eczema) had positive patch test reactions to 2% Sodium Metabisulfite in petrolatum. Another 10 patients had weak irritant reactions when patch tested. The patch test reactions were considered of current relevance in 2 cases; in a third case, a previous relevance was probable. In 14 cases (27%), the relevance was considered questionable and no relevance was found in 24 patients (47%).

In addition to the multicenter studies on Sodium Metabisulfite, patient populations have also been tested with Sodium Sulfite and Potassium Sulfite. Of the 183 patients patch tested with 1% Sodium Metabisulfite in petrolatum at a dermatology center, 5.5% had positive allergic reactions. In the same patient population patch tested, 3.8% of the patients had positive reactions to 1% Sodium Sulfite in petrolatum. One-hundred twenty-four of 2763 patients had positive patch test reactions to Sodium Metabisulfite (tested with 2% initially and later with 1%).[46] The reactions were considered to be relevant in 80 cases (64.5%), of which 11 were occupational. None of the 39 patients patch tested with 2% Sodium Sulfite had positive reactions. Two of 100 patients with chronic idiopathic urticaria had a positive urticarial response after each was challenged (single-blind) orally with Potassium Metabisulfite (amount not stated) and 10 other food additives simultaneously. The 2 patients were subjected to a double-blind placebo-controlled challenge after a 2-week period, and a late reaction to this challenge was not observed.

A number of case reports on sulfites, mostly relating to Sodium Metabisulfite, have been identified in the published literature. Positive patch test reactions to the following sulfites have been reported: Ammonium Bisulfite (2%), Potassium Metabisulfite (0.1% and 1%), Sodium Bisulfite (0.04%, 0.1% and 1%), and Sodium Metabisulfite (0.02%, 0.064%, 0.2%, and 0.64%). Additionally, patch and prick test results were positive for 1% Potassium Metabisulfite and 1% Sodium Metabisulfite, but not for Sodium Sulfite. Intradermal test results were also positive for 0.1% Potassium Metabisulfite and 0.1% Sodium Metabisulfite, but not for Sodium Sulfite.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

Table 1. Definitions, idealized structures, and functions of the ingredients in this safety assessment.([2,CIR Staff])

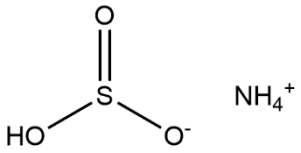
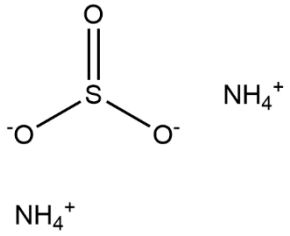
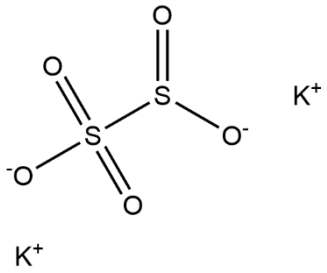
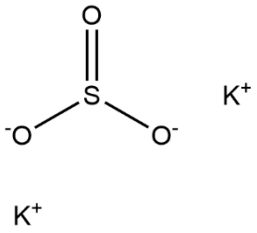
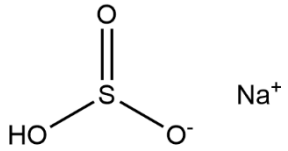
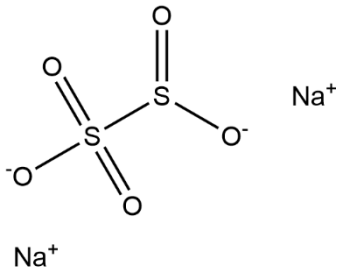
Ingredient CAS No.	Definition & Structures	Function(s)
Ammonium Bisulfite 10192-30-0	Ammonium Bisulfite is an inorganic salt that conforms to the formula: 	Hair-Waving/Straightening Agents; Reducing Agents
Ammonium Sulfite 10196-04-0	Ammonium Sulfite is an inorganic salt that conforms to the formula: 	Hair-Waving/Straightening Agents; Reducing Agents
Potassium Metabisulfite 16731-55-8 4429-42-9	Potassium Metabisulfite is the inorganic salt that conforms to the formula: 	Hair-Waving/Straightening Agents; Reducing Agents
Potassium Sulfite 10117-38-1 23873-77-0	Potassium Sulfite is the inorganic salt that conforms to the formula: 	Antioxidants; Hair-Waving/Straightening Agents; Reducing Agents
Sodium Bisulfite 7631-90-5	Sodium Bisulfite is the inorganic salt that conforms to the formula: 	Antioxidants; Hair-Waving/Straightening Agents; Reducing Agents
Sodium Metabisulfite 7681-57-4 7757-74-6	Sodium Metabisulfite is the inorganic salt that conforms to the formula: 	Antioxidants; Reducing Agents

Table 1. Definitions, idealized structures, and functions of the ingredients in this safety assessment.([2,CIR Staff])

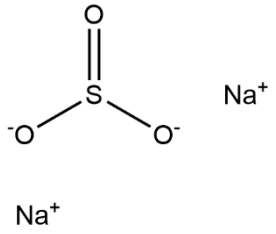
Ingredient CAS No.	Definition & Structures	Function(s)
Sodium Sulfite 7757-83-7	Sodium Sulfite is the inorganic salt that conforms to the formula:  	Antioxidants; Hair-Waving/Straightening Agents; Reducing Agents

Table 2. Physical and Chemical Properties

Property	Value/Results	Reference
Ammonium Bisulfite		
Form	Colorless crystals	1
Formula weight (g/mol)	99.11	3
Solubility	Readily soluble in water	1
log K_{ow}	-5.78 (estimated)	4
Ammonium Sulfite		
Form	Hygroscopic colorless crystals	1
Formula weight (g/mol)	116.14	3
Solubility	Soluble in water; almost soluble in alcohol and acetone	1
log K_{ow}	-4.71 (estimated_	4
Potassium Metabisulfite		
Form	White or colorless free-flowing crystals, crystalline powder, or granules	1
Formula weight (g/mol)	222.33	3
Solubility	Soluble in water; insoluble in alcohol	1
log K_{ow}	-7.18 (estimated)	4
Potassium Sulfite		
Form	White granular powder	1
Formula weight (g/mol)	158.26	3
Solubility	Soluble in water; slightly soluble in alcohol	1
log K_{ow}	-4.53 (estimated)	4
Sodium Bisulfite		
Form	White or yellowish-white crystals or granular powder	1
Formula weight (g/mol)	104.06	3
Solubility	Soluble in water; slightly soluble in alcohol	1
log K_{ow}	-7.51 (estimated)	4
Sodium Metabisulfite		
Form	Colorless crystals or a white to yellowish crystalline powder	1
Formula weight (g/mol)	190.11	3
Solubility	Soluble in water; slightly soluble in alcohol	1
log K_{ow}	-7.35 (estimated)	4
Sodium Sulfite		
Form	White or tan to slightly pink powder	1
Formula weight (g/mol)	126.05	3
Solubility	Soluble in water; sparingly soluble in alcohol	1
log K_{ow}	-7.78 (estimated)	4

Table 3. Food grade specifications.[7]

Requirement	Sodium Sulfite	Potassium Sulfite	Sodium Bisulfite	Sodium Metabisulfite	Potassium Metabisulfite
Assay	95% minimum of Sodium Sulfite	90% minimum, 100.5% maximum of Potassium Sulfite	58.5% minimum, 67.4% maximum of sulfur dioxide	90% minimum, 100.5% maximum of Sodium Metabisulfite	90% minimum of Potassium Metabisulfite
Heavy metals (as Pb)	2 mg/kg maximum	2 mg/kg maximum	2 mg/kg maximum	2 mg/kg maximum	2 mg/kg maximum
Selenium	0.003% maximum	5 mg/kg maximum	5 mg/kg maximum	5 mg/kg maximum	5 mg/kg maximum
Iron			0.005% maximum	10 mg/kg maximum	10 mg/kg maximum
Alkalinity (as potassium carbonate)		0.25% to 0.45%			

Table 4. United States Pharmacopoeia specifications.

Requirement	Sodium Metabisulfite[8,9]	Potassium Metabisulfite[8]
Assay	Sodium Metabisulfite (use American Chemical Society reagent grade) 97% minimum	Potassium Metabisulfite (use suitable grade with content of not less than 98%)
Heavy metals (as Pb)	0.001% maximum	
Iron	0.002% maximum	
Chloride	0.05% maximum	
Thiosulfate	0.05% maximum	
Insoluble Matter	0.005% maximum	

Table 5. Current and historical frequency and concentration of use of Sulfites according to duration and exposure.

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	Ammonium Bisulfite				**Potassium Metabisulfite**			
	2020[10]	**2003[1]**	**2019[11]**	**2003[1]**	**2020[10]**	**2003[1]**	**2019[11]**	**2003[1]**
Totals*	**1**	**NR**	**NR**	**32**	**NR**	**1**	**0.35**	**NR**
Duration of Use								
Leave-On	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Rinse-Off	*1*	*NR*	*NR*	*32*	*NR*	*1*	*0.35*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	1	NR	NR	32	NR	1	0.35	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Potassium Sulfite				**Sodium Bisulfite**			
	2020[10]	**2003[1]**	**2019[11]**	**2003[1]**	**2020[10]**	**2003[1]**	**2019[11]**	**2003[1]**
Totals*	**2**	**1**	**NR**	**NR**	**74**	**58**	**0.0013-0.1**	**0.03-0.7**
Duration of Use								
Leave-On	*2*	*NR*	*NR*	*NR*	*50*	*6*	*0.0013-0.1*	*0.03-0.3*
Rinse-Off	*NR*	*1*	*NR*	*NR*	*24*	*51*	*0.013*	*0.1-0.7*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*1*	*NR*	*NR*
Exposure Type								
Eye Area	NR	NR	NR	NR	8	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	1[a]	NR	NR	NR	11[a];24[c]	1[a]	0.0013[a]	0.03[a];0.05[c]
Incidental Inhalation-Powder	NR	NR	NR	NR	24[c]	NR	0.02[b]	0.05[c]
Dermal Contact	2	NR	NR	NR	69	7	0.02	0.05-0.3
Deodorant (underarm)	1[a]	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	1	NR	NR	5	2	0.0013-0.1	0.03
Hair-Coloring	NR	NR	NR	NR	NR	49	NR	0.7
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	15	1	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Sodium Metabisulfite				**Sodium Sulfite**			
	2020[10]	**2003[1]**	**2019[11]**	**2003[1]**	**2020[10]**	**2003[1]**	**2019[11]**	**2003[1]**
Totals*	**916**	**348**	**0.000005-0.6**	**0.003-14**	**1713**	**911**	**0.000001-3**	**0.01-3**
Duration of Use								
Leave-On	*326*	*28*	*0.0001-0.25*	*0.003-0.4*	*129*	*3*	*0.0000051-0.12*	*0.1-0.4*
Rinse-Off	*590*	*312*	*0.000005-0.6*	*0.1-14*	*1583*	*906*	*0.000001-3*	*0.01-3*
Diluted for (Bath) Use	*NR*	*8*	*NR*	*NR*	*1*	*2*	*NR*	*NR*
Exposure Type								
Eye Area	28	1	0.003-0.03	NR	12	NR	0.03	NR
Incidental Ingestion	NR	NR	0.003	NR	NR	NR	0.0015	NR
Incidental Inhalation-Spray	125[a];115[c]	12[a];2[c]	0.02-0.25	0.003-0.3[a];0.003[c]	45[a];41[c]	NR	0.0000051-0.002[a]	0.1[a]
Incidental Inhalation-Powder	115[c]	2[c]	0.0001;0.001-0.12[b]	0.003[c]	41[c]	NR	0.00001-0.12[b]	NR
Dermal Contact	324	34	0.0001-0.25	0.003-0.4	170	5	0.00001-3	0.1-0.4
Deodorant (underarm)	NR	7[a]	0.04	0.1[a]	6	NR	NR	NR
Hair - Non-Coloring	8	3	0.000005-0.00011	0.1-14	16	12	0.000001-0.35	0.01
Hair-Coloring	537	310	0.29-0.6	NR	1525	893	0.05-1.1	0.5-3
Nail	1	1	NR	NR	1	1	NR	NR
Mucous Membrane	24	8	0.00041-0.1	NR	40	3	0.00005-0.0015	0.2
Baby Products	1	NR	NR	NR	NR	NR	0.00001	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] It is possible these products are powders, but it is not specified whether the reported uses are powders.

[c] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

NR – no reported use

Table 6. Genotoxicity Studies

Ingredient	Strain/cell type	Assay	Dose/Concentration	Results
		In Vitro		
Sodium Metabisulfite	Human lymphocytes	Chromosome aberrations and sister chromatid exchanges assay. Cell cultures incubated for 24 h and 48 h. Negative control (not stated) and positive control (mitomycin C) included	Concentrations of 75 mg/ml, 150 mg/ml, and 300 mg/ml	When compared to negative control, treatment induced as statistically significant increase in chromosome aberrations and sister chromatid exchanges at all concentrations tested (75 mg/ml [$p < 0.05$], 150 mg/ml [$p < 0.01$], and 300 mg/ml [$p < 0.01$]) and treatment periods (24 h and 48 h) in a dose-dependent manner. Potency of Sodium Metabisulfite on induction of chromosome aberrations and sister chromatid exchanges was lower when compared to the positive control. Chromatid breaks, chromosome breaks, and chromatid exchanges were most common chromosomal abnormalities. Treatment also decreased the replication index and mitotic index at concentration of 150 mg/ml and 300 mg/ml (at 24 h and 48 h) in a dose-dependent manner.[28]
Potassium Metabisulfite (in twice distilled water)	Whole blood from human subjects	Chromosomal aberrations assay. Cell cultures treated for 24 h and 48 h. Bromodeoxy-uridine/5-bromo-2'-deoxyuridine (BrdU) control, positive control (ethyl methanesulfonate), and negative control (not stated) used.	Concentrations of 25, 50, 100,and 200 µg/ml	Structural chromosomal aberrations at all concentrations and exposure periods (in dose-dependent manner: $P = 0.01$ at 24 h; $P = 0.05$ at 48 h), when compared to negative and BrdU controls. Treatment increased percentage of numerical chromoso-mal aberrations only at 200 µg/ml (at 48 h). Treatment caused statistically significant increase in total chromosomal aberrations frequencies (compared to control group) in a dose-dependent manner ($P = 0.008$ at 24 h; $P = 0.05$ at 48 h). Results for total chromosomal aberrations comparable to those for positive control. Treatment also decreased mitotic index, when compared to both negative and BrdU controls, in a dose-dependent manner ($P = 0.001$ at 48 h). Results indicate that test substance probably has a genotoxic risk.[29]
Potassium Metabisulfite (in twice distilled water)	Human blood samples	Micronucleus test. Two-thousand binucleated lymphocytes from each donor scored (8,000 binucleated cells scored per concentration). Total of 1000 viable cells scored to determine frequency of cells with nuclei.	Same as in preceding experiment.	Treatment increased percentage of micronuclei, but not in dose-dependent manner. Also increased percentage of micronucleated binuclear cells at the 2 treatment times, when compared to control. Dose-dependent effect on increasing percentage of micronucleated binuclear cells at 48 h. Treatment also decreased nuclear division index at all concentrations and treatment periods. Also, dose-dependent effect on decreasing nuclear division index at 24 h and 48 h ($P = 0.005$ and $P = 0.02$, respectively). Results indicate that test substance probably has a genotoxic risk.[29]

Table 6. Genotoxicity Studies

Ingredient	Strain/cell type	Assay	Dose/Concentration	Results
		In Vivo		
Sodium Bisulfite (formulated in citrate/sodium hydroxide buffer at pH 5)	Primary hepatocytes from groups of 4 male rats of the Wistar Hanlbm:WIST (SPF) strain.	Unscheduled DNA synthesis assay. Animals killed at 2 h and 16 h after oral dosing. Primary hepatocytes (obtained from 3 animals per group) then exposed to methyl-[^{3}HTdR) for 4 h to show its incorporation if unscheduled DNA synthesis occurs. N,N'-Dimethylhydrazine dihydrochloride (40 mg/kg) and 2-acetylaminofluorene (100 mg/kg) served as positive controls.	Doses of 625 mg/kg and 1250 mg/kg (dose volume = 10 ml/kg)	Hepatocytes not substantially affected after treatment. No dose level of test substance caused unscheduled DNA synthesis induction in hepatocytes of treated animals when compared to the vehicle control. The net gain values obtained treatment were consistently negative. There was no substantial shift to higher values in the percentage distribution of nuclear grain counts. It was concluded that Sodium Bisulfite failed to show any evidence of mutagenic potential in this in vivo test for unscheduled DNA synthesis.[30]
Sodium Bisulfite (formulated in citrate/sodium hydroxide buffer at pH 5)	Bone marrow (femur) from groups of 12 mice (6 males and 6 females per group) of the NMRI strain.	Micronucleus test. Mice dosed intraperitoneally (i.p). Bone marrow extracted 24 h and 48 h after dosing. For each animal, at least 2000 polychromatic erythrocytes (PCE) obtained from bone marrow. Cyclophosphamide (40 mg/kg) and vehicle served as positive and negative controls, respectively.	Doses of 75 mg/kg, 150 mg/kg, and 300 mg/kg (dose volume of 15 ml/kg)	Treated mice exhibited normochromatic/polychromatic erythrocyte ratios that were higher when compared to negative controls, demonstrating the bioavailability of Sodium Bisulfite in the bone marrow. The number of micronucleated PCE in treated mice was similar to that observed in controls. It was concluded that Sodium Bisulfite failed to show any evidence of mutagenic potential in this in vivo test for chromosomal alterations.[30]
Mixture of Sodium Bisulfite and Sodium Sulfite (3:1 M/M) in saline	Bone marrow (femur) from groups of 10 male and groups of 10 female Kunming mice	Micronucleus test. Mice injected i.p.with mixture. Negative and positive control groups injected with saline and cyclophosphamide, respectively. Injections of test material and negative control (switched to right side of animal) repeated after 24 h. Animals killed 24 h after last injection. Bone marrow removed from femur and smears prepared.	Single doses of 20 mg/kg, 100 mg/kg, 500 mg/kg, and 750 mg/kg	Mixture induced micronuclei in dose-dependent manner. At each dose of mixture (in groups of male and female mice), formation of micronuclei statistically significantly greater ($p < 0.01$) when compared to negative control values. In male and female controls, background levels of $0.23 \pm 0.05\%$ and $0.22 \pm 0.05\%$ PCE with micronuclei, respectively, were reported. At the highest dose, % PCE values were 0.93 ± 0.06 in male mice and 0.90 ± 0.07in female mice.[31]
Sodium Metabisulfite	Blood and liver cells from male and female mice	Comet assay	Doses of 1 g/kg and 2 g/kg	Statistically significant increase in damage index and damage frequency values at both doses when compared to control animals.[32]
Sodium Metabisulfite	Blood and bone marrow cells from male and female mice	Micronucleus test	Doses of 1 g/kg and 2 g/kg	Statistically significant increase in damage index and damage frequency values at both doses when compared to control animals. Kruskal-Wallis test showed that mean micronucleus frequencies in peripheral blood and bone marrow cells of mice treated with higher dose of test substance showed statistically significant increases when compared to controls. A statistically significant reduction in the ratio of polychromatic to normochromatic erythrocytes also observed. No difference in results between sexes observed. Test results indicate that high oral doses of Sodium Metabisulfite may pose a genotoxic risk.[32]

Table 6. Genotoxicity Studies

Ingredient	Strain/cell type	Assay	Dose/Concentration	Results
Potassium Metabisulfite (in twice distilled water)	Bone marrow from groups of 4 (2 male and 2 females per group) albino rats	Chromosomal aberrations assay. Rats injected i.p. with different doses for 12 h and 24 h before animals were killed and bone marrow smears prepared. Urethane served as the positive control and an untreated group served as the negative control. One-hundred metaphases per animal (400 metaphases per group) examined	Doses of 150 mg/kg, 300 mg/kg, and 600 mg/kg	Potassium Metabisulfite induced structural and numerical chromosomal aberrations and a percentage of abnormal cells at all doses and treatment times, when compared to the control. These effects induced by the test substance were greater when compared to the urethane positive control for 12-h treatments. The highest test substance dose of 600 mg/kg had the same effect on induction of structural and numerical chromosomal aberrations as did the urethane positive control. Furthermore, the same was true regarding the percentage of abnormal cells at the 24-h treatment period. A dose-dependent effect (without statistical significance) on the induction of structural and numerical chromosomal aberrations for 12-h and 24-h treatment periods was observed. Potassium Metabisulfite also increased the percentage of abnormal cells in a dose-dependent manner ($P = 0.04$) at the 24-h treatment. It was also capable of inducing structural chromosomal aberrations (especially the chromatid-type abnormalities) instead of numerical chromosomal aberrations. Potassium Metabisulfite decreased the mitotic index when compared to both negative and positive control groups for 12-h treatments. It also decreased the mitotic index at the highest dose for the 48-h treatment, when compared to the control. The authors noted that these test results indicate that Potassium Metabisulfite probably has a genotoxic risk.[29]

Table 7. Retrospective and Multicenter Studies

Ingredient	Number of Subjects	Protocol	Results
Sodium Metabisulfite (concentration not stated)	475 patients with contact allergy to cosmetic ingredients	Retrospective European survey of allergic reactions to cosmetics, during a 4-month period (January – April of 1996)	One allergic reaction to Sodium Metabisulfite reported.[40]
Sodium Metabisulfite (1% in petrolatum) and Sodium Sulfite (1% in Petrolatum)	183 patients	Patch test	Positive allergic reactions in 5.5% of patients tested. 60% of patients with positive reactions to Sodium Metabisulfite (1%) also had positive reaction to Sodium Sulfite (1%). Only 1 patient with negative reaction to Sodium Metabisulfite (1%) had a positive reaction to Sodium Sulfite (1%).[45]
Sodium Metabisulfite (2% initially and later 1% in petrolatum) and Sodium Sulfite (2% in petrolatum)	2763 patients patch tested with Sodium Metabisulfite. 39 patients patch tested with Sodium Sulfite	Patch tested between 1990 and 2010.	One-hundred and twenty-four (4.5%) of the patients had positive patch test reactions to Sodium Metabisulfite. The most frequent localizations of the lesions were on the face (40.3%) and hands (24.2%). Six patients also reported systemic symptoms. Thirteen cases (10.5%) were occupational, 10 of whom presented with hand eczema. Sodium Metabisulfite was the single allergen found in 76 cases (61.3%). The reactions were considered to be relevant in 80 cases (64.5%), of which 11 were occupational. There were no reactions to 2% Sodium Sulfite in petrolatum.[46]
Sodium Metabisulfite (0.01%, 0.1%, or 1% in petrolatum)	380 contact allergy patients (this group and group immediately below in same study)	Patch tested, between February of 2009 and June of 3013, with Sodium Metabisulfite as part of the British standard series.	14 patients with positive reactions to 1% Sodium Metabisulfite. 7 patients with positive reactions to 0.1% Sodium Metabisulfite. 3 patients with positive reactions to 0.01% Sodium Metabisulfite.[42]
Sodium Metabisulfite (1% in petrolatum)	996 contact allergy patients	Patch tested, between February of 2009 and June of 3013, with Sodium Metabisulfite as part of the British standard series	70 patients (7% [39 females and 31 males) had positive reactions. Reactions considered to be of current relevance in 24 cases (34%), of possible relevance in 14 (20%), and of unknown relevance in 32 cases (46%). None of the patients reported systemic symptoms.[42]
Sodium Metabisulfite (1% in petrolatum)	1751 contact dermatitis patients	Retrospective case note review of positive patch test reactions at contact dermatitis investigation unit in United Kingdom. Patch tests read on days 2 and 4	71 (4.1%) positive patch test reactions to 1% Sodium Metabisulfite in petrolatum, interpreted as allergic. Of these, 33 reported as relevant (designated as group A) and 38 were of unknown relevance (designated as group B). The hands were most common primary site of dermatitis in both groups (23% for group A; 25% for group B).[43]
Sodium Metabisulfite (2% in petrolatum)	1518 consecutive patients (839 [55.3%] females; 679 [44.8%] males). Hand eczema was most common diagnosis	Retrospective study (at Department of occupational and Environmental Dermatology in Sweden) to investigate prevalence of positive patch test reactions to Sodium Metabisulfite in patients, and to evaluate the clinical relevance of positive patch test reactions. Patch testing from November of 1998 to April of 2007.	Fifty-one patients (3.4%) had positive patch test reactions to Sodium Metabisulfite; 43 (84.3%) and 8 (15.7%) were males and females, respectively. Sixteen cases (31.3%) had +/- reactions and 35 patients (68.6%) had strong patch test reactions (++ or +++) to Sodium Metabisulfite. The patch test reactions were considered of current relevance in 2 cases. In a third case, a previous relevance was probable. In 14 cases (27%), the relevance was considered questionable, and no relevance was found in 24 patients (47%). In 10 cases, the relevance could not be evaluated because of incomplete patient records. Among the patients in this study with positive patch rest reactions and known relevance, the first one was a male photographer with hand eczema. This patient had contact allergy to photodeveloping chemicals containing Sodium Metabisulfite as well as to Sodium Metabisulfite. The second patient (male) was a textile dyer with hand eczema who cleaned his hands with a Sodium Metabisulfite-containing cleanser. The third patient (female) was a dentist diagnosed with hand eczema. This patient had handled photograph developing chemicals containing Sodium Metabisulfite; therefore, past relevance was probable.[44]

Table 7. Retrospective and Multicenter Studies

Ingredient	Number of Subjects	Protocol	Results
Sodium Sulfite (1% in petrolatum)	183 patients	Patch test	Positive allergic reactions to Sodium Sulfite in 3.8% of patients tested.[45]
Potassium Metabisulfite (amount not stated)	100 patients with chronic idiopathic urticaria (history of more than 6 weeks); 43 reported possible history of food or drug additive sensitivity.	Oral challenge (single-blind) with Potassium Metabisulfite and 10 other food additives simultaneously. Skin symptoms recorded hourly after administration of food additive capsules for 4 h. Patients instructed to continue to check skin at 6 h and 8h after ingestion at home. If positive results observed, double-blind placebo-controlled challenge initiated after 2-week period.	Only 2 patients had positive urticarial response after single-blind challenge. No late reaction to double-blind placebo-controlled challenge.[39]

Table 8. Case Reports

Ingredient	Patients	Protocol	Results
Sodium Bisulfite (0.002%, 0.04%, 0.1%, and 1%)	Female patient previously diagnosed with myasthenia gravis	Administered high-calorie infusion containing 0.04% Sodium Bisulfite. Subsequently patch tested (48-h closed patch test, Finn chamber) with the following: 0.1% Sodium Bisulfite (in petrolatum), 1% Sodium Bisulfite (in petrolatum), high-calorie infusion containing Sodium Bisulfite (0.002%), and high calorie infusion containing Sodium Bisulfite (0.04%). Reactions scored at 48 h and 72 h post-application according to International Contact Dermatitis Research Group (ICDRG) recommendations.	At 3 days after start of infusion, small red pruritic papules developed over most of body. Eruption gradually disappeared after infusion stopped. Positive patch test reactions to 0.1% and 1% Sodium Bisulfite. Pruritus observed after patch testing with 0.04% Sodium Bisulfite infusion. No positive patch test reaction to infusion containing 0.002% Sodium Bisulfite. Authors noted that results suggest that sulfite intake could also cause a type IV allergic reaction, leading to systemic eruption (systemic type IV allergic reaction).[48]
Sodium Metabisulfite (concentration not stated)	Patient with eczema (observed over 8-month period). Condition worsened after use of topical antibiotic cream containing Sodium Metabisulfite	Patch test. Reactions evaluated on days 2 and 4	Positive reaction (++) to cream and to Sodium Metabisulfite on days 2 and 4.[51]
Sodium Metabisulfite (concentration not stated)	Renal transplant patient with otitis externa	Patch test. Reactions evaluated on days 2 and 4	Positive reaction (+) to cream and to Sodium Metabisulfite on days 2 and 4.[51]
Sodium Metabisulfite	3 fishing industry workers	Author noted that it appears that high exposures to Sodium Metabisulfite and sulfur dioxide gas (in excess of 30 ppm) occur within fishing industry. Sodium Metabisulfite can react with water, releasing toxic sulfur dioxide gas.	3 fishing industry workers subjected to Sodium Metabisulfite inhalation exposure diagnosed as follows: irritant-induced asthma (case 1), occupational asthma (case 2), and vocal cord dysfunction with underlying asthma (case 3).[49]

Table 8. Case Reports

Ingredient	Patients	Protocol	Results
Sodium Metabisulfite (0.02%, 0.064%, 0.2%, and 0.64% aqueous	1 female hairdresser and 20 controls	Patch tests.	Hairdresser had positive (++) patch test reactions to all test concentrations. For the initial 5 control subjects patch tested, an irritant reaction to 0.064% was observed in 1 of 5, and a weak irritant reaction (slight erythema) to 0.02% was observed in same subject. In next patch test session, morphology of patient's reactions to Sodium Metabisulfite was different, appearing more irritant than allergic in nature. Also, the reaction to 0.02% was negative. Fifteen additional control subjects were also evaluated in the second patch test session. Three of the 15 controls had an irritant reaction to 0.64%, and a fourth control had a weak reaction to this concentration. Of these 4 controls with reactions, 1 also had an irritant reaction to 0.2% and another had a weak reaction to this concentration.[52]
Sodium Metabisulfite (0.02%, 0.064%, 0.2%, and 0.64% aqueous	1 female hairdresser and 20 controls	Patch tests.	A comment on the preceding study has been published, focusing on the strong positive patch tests to Sodium Metabisulfite in serial dilutions down to 0.02%, and subsequently down to 0.01%. The observation that the authors of the publication correctly tested the patient and controls with serial dilutions, and have shown gradually diminishing strengths of patch test reaction (a pattern of allergy rather than irritancy) was made. The commenters also noted their belief that these findings, taken in combination with the clinical presentation, point more to a diagnosis of contact allergy rather than irritancy. Furthermore, they stated that the reasons for the authors' interpretation of the reactions as irritant reactions were first, the morphology of the reactions, and, second, the results from the controls. They also expressed agreement with the authors' statement that it is frequently impossible to distinguish morphologically irritant from allergic reactions.[61]
Sodium Metabisulfite (1% in petrolatum)	Female patient who used eyedrops containing Sodium Metabisulfite and developed eyelid dermatitis	Patch test. Reactions scored on days 2, 3, and 4	Positive reaction (++) at days 2, 3, and 4.[53]
Sodium Metabisulfite (1% in petrolatum)	Restaurant employee with hand dermatitis who came in contact with plastic bags that contained a preservative solution of Sodium Metabisulfite.	Patch test. Reactions scored on days 2 and 4	Positive (1+) patch test reaction on days 2 and 4.[54]
Sodium Metabisulfite (1% in petrolatum)	Male patient with dermatitis after 3 separate orthopedic surgeries. Pruritic vesicles developed along the surgical incision sites (lasted for 2 to 3 weeks)	Patch test.	Clinically relevant positive reactions to gentamycin injection solution and ketaconazole cream (both contained Sodium Metabisulfite as a preservative) observed. Positive patch test reaction to Sodium Metabisulfite (1% in petrolatum).
Sodium Metabisulfite (1% in petrolatum) and Sodium Sulfite (5% in white soft paraffin)	Female patient with dermatitis of the lips and face after application of a cosmetic day and night creams	Patch tests on constituents of the creams. Reactions scored on days 2 and 4.	Positive reactions to Sodium Metabisulfite (1% in petrolatum and Sodium Sulfite (5% in soft paraffin) on days 2 and 4.[62]

Table 8. Case Reports

Ingredient	Patients	Protocol	Results
Sodium Metabisulfite (1% in petrolatum), Sodium Sulfite (1% in petrolatum), and Potassium Metabisulfite (1% in petrolatum	Non-atopic male agricultural worker with the following symptoms over a 5-year period: itchy erythema, swelling and scaling of face (eyelids included, and more severe on forehead and malar areas). Itchy erythematopapular scaly dermatitis also present on extensor part of forearms. Worker had added Potassium Metabisulfite to wine to prevent yeast and bacteria proliferation and wine oxidation.	Patch tests. Reactions scored on days 2 and 4	Positive (++) reactions to Sodium Metabisulfite (1% in petrolatum) and Potassium Metabisulfite (1% in petrolatum) on days 2 and 4. No reaction to Sodium Sulfite (1% in petrolatum).[58]
Sodium Metabisulfite (1% in sterile saline solution), Sodium Sulfite (1% in sterile saline solution), Potassium Metabisulfite (1% in sterile saline solution)	Same patient (agricultural worker)	Prick tests. Reactions scored on day 0 and day 2	Positive (++) reactions to Sodium Metabisulfite (1% in sterile saline) and Potassium Metabisulfite (1% in sterile saline) on day 2, but not on day 0. No reaction to Sodium Sulfite (1% in sterile saline).[58]
Sodium Metabisulfite (0.1% in sterile saline solution), Sodium Sulfite (0.1% in sterile saline solution), Potassium Metabisulfite (0.1% in sterile saline solution)	Same patient (agricultural worker)	Intradermal tests. Reactions scored on day 0 and day 2.	Positive (++) reactions to Sodium Metabisulfite (1% in sterile saline) and Potassium Metabisulfite (1% in sterile saline) on day 2, but not on day 0. No reaction to Sodium Sulfite (1% in sterile saline).[58]
Sodium Metabisulfite (2% in petrolatum)	Male patient with 1-month history of pruritic, erythematous plaques after use of antihemorrhoidal cream containing Sodium Metabisulfite	Patch test. Reactions evaluated on days 2 and 4	Positive reaction (++) to cream and to Sodium Metabisulfite on days 2 and 4.[56]
Sodium Metabisulfite (in petrolatum) and Sodium Metabisulfite (0.05%, 0.1%, 1%, and 10% aqueous)	Patient who received local anesthetic injection containing Sodium Metabisulfite additive (concentration unknown). Burning sensation around injection site, followed by itching, swelling, and erythema (i.e., type IV hypersensitivity reaction)	Patch and prick tests	Positive (++) patch test reaction to Sodium Metabisulfite (in petrolatum). Negative prick test reactions to 0.05%, 0.1%, 1%, and 10% aqueous Sodium Metabisulfite. Author concluded that type IV hypersensitivity reaction to local anesthetic could be attributed to Sodium Metabisulfite additive.[57]
Sodium Metabisulfite (10 mg)	Male patient with 6-month history of severe pruritus on trunk, upper limbs, and head (with no visible sign of rash). No personal or family history of atopic diseases	Patient ingested Sodium Metabisulfite (10 mg). Series of double-blind, placebo-controlled challenges with Sodium Metabisulfite performed. Last follow-up visit occurred after resuming a full diet, except for nitrates and sulfites.	Approximately 4 h after ingestion of Sodium Metabisulfite (10 mg), patient reported onset of pruritus of the trunk, upper limbs, and head. Signs lasted for 3 days. When dosing was repeated, pruritus at approximately 4 h after challenge. At last follow-up visit, patient was well. No reaction to the placebo reported. Authors noted that results indicate that sulfites may be associated with chronic pruritus.[50]

Table 8. Case Reports

Ingredient	Patients	Protocol	Results
Sodium Metabisulfite (1% in petrolatum) and Potassium Metabisulfite (1% in petrolatum). [Potassium Metabisulfite was used at different stages of winemaking (when the grapes enter the wine cellar, at the end of alcoholic fermentation, and when cleaning the wooden casks.]	Female employee in winemaking industry who was responsible for preparing a Potassium Metabisulfite solution (10% aqueous), sometimes without wearing rubber gloves. No previous history of eczema or problems with the intake of beverages and foods containing sulfites. After several weeks of work, she developed an itchy vesicular eczematous reaction on both hands and forearms. Dermatitis had relapses coinciding with use of the dilution.	Patch testing of Sodium Metabisulfite and Potassium Metabisulfite on upper back of patient. Reactions scored on days 2 and 4 according to International Contact Dermatitis Research Group (ICDRG) standards. 10 control subjects patch tested with Potassium Metabisulfite only	Cessation of work resulted in resolution of dermatitis observed. Positive reaction to 1% Potassium Metabisulfite in petrolatum on days 2 (++ reaction) and 4 (+++ reaction), in patient. No reaction to Sodium Metabisulfite (1% in petrolatum) in patient. Negative reactions to Potassium Metabisulfite (1% in petrolatum) in 10 control subjects. Authors noted that relevance of positive patch test reaction to Potassium Metabisulfite was clearly a case of occupational exposure.[59]
Potassium Metabisulfite (300 mg)	Male patient who developed severe hypotension after food ingestion. Patient had 4-year history of multiple recurrent episodes of severe hypotension and syncope, following flushing, dizziness, tachycardia, and palpitations. Reactions occurred within 30 min of food ingestion. Diagnosis of monoclonal mast cell activation syndrome established	Double-blind, placebo-controlled food challenge. Oral administration of Potassium Metabisulfite (300 mg, equivalent to less than a maximum level of sulfites in wines)	Patient reacted to Potassium Metabisulfite with anaphylaxis. At 15 min after oral administration of Potassium Metabisulfite (300 mg), patient developed flush, nausea, and dizziness, followed by tachycardia and decrease in blood pressure to an unmeasurable value.[60]
Ammonium Bisulfite (2% in petrolatum)	Female patient with history of allergic rhinitis experienced itching on face and itching and erythema on forehead and cheeks after application (by hairdresser) of a henna dye and protective ointments	Patch tests. Reactions scored at 48 h and was later reading	Positive reaction, at 48 h (+++) and late reading (++), to color bleaching ointment containing Ammonium Bisulfite. Positive reaction, at 48 h (++) and late reading (++), to Ammonium Bisulfite (2% in petrolatum).[47]

REFERENCES

1. Andersen F. Final report on the safety assessment of sodium sulfite, potassium sulfite, ammonium sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite and potassium metabisulfite. *Int J Toxicol* 2003;22(2):63-88.

2. Nikitakis J, Kowcz A. International Cosmetic Ingredient Dictionary and Handbook Online Version (wINCI). http://webdictionary.personalcarecouncil.org/jsp/Home.jsp 2019. Accessed 10/30/2019.

3. National Library of Medicine (NlM). 2020. PubChem database. . https://pubchem.ncbi.nlm.nih.gov/compound/90659508 Accessed 1-15-2020.

4. United States Environmental Protection Agency. 2019. Estimation Programs Interface Suite for Microsoft Windows v 4.11. United States Environmental Protection Agency, Washington, DC, USA.

5. Solvay Chemicals, Inc. 2013. Sodium Sulfite. https://www.solvay.us/en/binaries/PSS-Sodium-Sulfite-164364.pdf Accessed 1-27-2020.

6. Solvay Chemicals, Inc. 2012. Sodium Metabisulfite. https://www.solvay.us/en/binaries/PSS-Sodium-Metabisulfite-164360.pdf.

7. United States Pharmacopeial Convention. 2016. Food Chemicals Codex. Tenth *ed*. Rockville, MD: The United States Pharmacopeial Convention.

8. Committee of Revision of the United States Pharmacopeial Convention. 2010. The United States Pharmacopoeia. 33rd ed. Rockville, MD: United States Pharmacopeial Convention.

9. Spectrum Chemical Manufacturing Corporation. 2020. Sodium Metabisulfite, Anhydrous, Granular, Reagent, ACS https://www.spectrumchemical.com/OA_HTML/chemical-products_Sodium-Metabisulfite-Anhydrous-Granular-Reagent-ACS_S1334.jsp Accessed 2-20-2020.

10. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). Voluntary Cosmetic Registration Program - Frequency of use of Cosmetic Ingredients. College Park, MD. 2020. (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 6, 2020; received January 13, 2020.

11. Personal Care Products Council. 2019. Council Concentration of Use by FDA Product Category: Sulfites (Unpublished data submitted by the Personal Care Products Council on January 9, 2019).

12. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: principles on inhalation risk assessment. *Toxicol Lett* 2011;205(2):97-104.

13. Bremmer HJ, Prud'homme de Lodder LCH, van Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. Bilthoven, Netherlands 2006. RIVM 320104001/2006. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Accessed 8/24/2011. Pages1-77.

14. Rothe H. 2011. Special aspects of cosmetic spray evaluation. Unpublished information presented to the 26 September Expert Panel. Washington D.C.

15. Johnsen MA. The Influence of Particle Size. *Spray Technology and Marketing* 2004;14(11):24-27.

16. López-Olmos K, Hernández MP, Contreras-Garduño JA, et al. 2012. Roles of endonuclease V, uracil-DNA glycosylase, and mismatch repair in Bacillus subtilis DNA base-deamination-induced mutagenesis. *J Bacteriol.* Vol 194. 243-252. [Journal of bacteriology]. (J Bacteriol.)

17. Fisher AA. The sulfites. Part III. Facts about sulfites [news]. *Cutis* 1997;60:73-74.

18. Green LF. Sulphur dioxide and food preservation - a review *Food Chem* 1976;1:103-124.

19. World Health Organization. 1974. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meeting Report Series No. 53. WHO Technical Report Series No. 539 and corrigendum.

20. Food and Agriculture Organization of the United Nations/World Health Organization. 1994. Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). United States: International Life Sciences Institute

21. Walker R. Sulphiting agents in foods: Some risk/benefit considerations *Food Addit Contam* 1985;2:5-24.

22. Til HP, Feron VJ, DeGroot AP. Toxiciy of sulfite I: Long-term feeding and multigeneration studies in rats. *Food Cosmet Toxicol* 1972;10:291-310.

23. Til HP, Feron VJ. Toxicology of sulphiting agents. I: Animal studies. *Food Addit Contam* 1992;9:587-595.

24. Ribera D, Jonker D, Narbonne JF, O'Brien J, Antignac E. Absence of adverse effects of sodium metabisulphite in manufactured biscuits: results of subacute (28-days) and subchronic (85-days) feeding studies in rats. *Food Addit Contam* 2001;18(2):103-114.

25. Yoo J, Lim YM, Kim H, et al. Potentiation of Sodium Metabisulfite Toxicity by Propylene Glycol in Both in Vitro and in Vivo Systems. *Front Pharmacol* 2018;9(161).

26. Shekarforoush S, Ebrahimi Z, Hoseini M. Sodium metabisulfite-induced changes on testes, spermatogenesis and epididymal morphometric values in adult rats. *Int J Reprod Biomed (Yazd)* 2015;13(12):765-770.

27. Mahmoudi R, Honarmand Z, Karbalay-Doust S, Jafari-Barmak M, Nikseresht M, Noorafshan A. Using curcumin to prevent structural impairments of testicles in rats induced by sodium metabisulfite. *EXCLI Journal* 2017;16:583-592.

28. Rencüzogullari E, Ila HB, Kayraldiz A, Topakta. Chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes treated with sodium metabisulfite, a food preservative. *Mutat Res* 2001;490(2):107-112.

29. Yavuz-Kocaman A, Rencuzogullari E, Ila HB, Topaktas M. The genotoxic effect of potassium metabisulfite using chromosome aberration, sister chromatid exchange, micronucleus tests in human lymphocytes and chromosome aberration test in bone marrow cells of rats. *Environ Mol Mutagen* 2008;49(4):276-282.

30. The Scientific Committee on Cosmetic Products and non-Food Products (SCCNFP). Opinion concerning inorganic sulfites and bisulfites. https://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out_200.pdf.2003. Accessed 8-8-2019.

31. Meng Z, Sang N, Zhang B. Effects of derivatives of sulfur dioxide on micronuclei formation in mouse bone marrow cells in vivo. *Bull Environ Contam Toxicol* 2002;69(2):257-264.

32. Carvalho IM, Melo Cavalcante AA, Dantas AF, et al. Genotoxicity of sodium metabisulfite in mouse tissues evaluated by the comet assay and the micronucleus test. *Mutat Res* 2011;720(1-2):58-61.

33. Corte L, Roscini L, Zadra C, et al. Effect of pH on potassium metabisulfite biocidic activity against yeast and human cell cultures. *Food Chemistry* 2012;134:1327-1336.

34. Akdogan I, Kocamaz E, Kucukatay V, Yonguc NG, Ozdemir MB, Murk W. Hippocampal neuron number loss in rats exposed to ingested sulfite. *Toxicol Ind Health* 2011;27(9):771-778.

35. Pelletier M, Lavastre V, Girard D. Activation of human epithelial lung a549 cells by the pollutant sodium sulfite: enhancement of neutrophil adhesion. *Toxicol Sci* 2002;69(1):210-216.

36. Collaco C, Hochman D, Goldblum R, Brooks E. Effect of sodium sulfite on mast cell degranulation and oxidant stress. *Ann Allergy Asthma* 2006;96:550-556.

37. Lin HK, Tsai JJ, Wen MC, Tsai MC, Chen CJ, Fu LS. Sodium sulfite aggravated allergic sensitization and airway inflammation in mite allergen sensitized BALB/c mice. *Hum Exp Toxicol* 2011;30(10):1682-1689.

38. Qin G, Meng Z. Effects of sulfur dioxide derivatives on expression of oncogenes and tumor suppressor genes in human bronchial epithelial cells. *Food Chem Toxicol* 2009;47(4):734-744.

39. Rajan JP, Simon RA, Bosso JV. Prevalence of sensitivity to food and drug additives in patients with chronic idiopathic urticaria. *J Allergy Clin Immunol Pract* 2014;2(2):168-171.

40. Goossens A, Beck M, Hanake E, et al. Adverse cutaneous reactions to cosmetic allergens. *Contact Dermatitis* 1999;40:112.

41. Krecisz B, Chomiczewska-Skóra D, Kiec-Swierczynska M. [Preservatives as important etiologic factors of allergic contact dermatitis]. *Med Pr* 2015;66(3):327-332.

42. Ralph N, Verma S, Merry S, Lally A, Kirby B, Collins P. What is the relevance of contact allergy to sodium metabisulfite and which concentration of the allergen should we use? *Dermatitis* 2015;26(4):162-165.

43. Madan V, Walker SL, Beck MH. Sodium metabisulfite allergy is common but is it relevant? *Contact Dermatitis* 2007;57(3):173-176.

44. Kaaman AC, Boman A, Wrangsjö K, Matura M. Contact allergy to sodium metabisulfite: an occupational problem. *Contact Dermatitis* 2010;63(2):110-112.

45. Oliphant T, Mitra A, Wilkinson M. Contact allergy to sodium sulfite and its relationship to sodium metabisulfite. *Contact Dermatitis* 2012;66(3):128-130.

46. García-Gavín J, Parente J, Goossens A. Allergic contact dermatitis caused by sodium metabisulfite: a challenging allergen: a case series and literature review. *Contact Dermatitis* 2012;67(5):260-269.

47. Nassif A. Ammonium bisulfite contact dermatitis: face eczema due to a bleaching ointment used during hair-dying. *Contact Dermatitis* 2006;55(2):124.

48. Honda T, Kitoh A, Miyachi Y, Kabashima K. Drug eruption following high-calorie infusion: a possible systemic type IV allergic reaction to sulphites. *Acta Derm Venereol* 2015;95(7):854-855.

49. Steiner M, Scaife A, Semple S, Hulks G, Ayres JG. Sodium metabisulphite induced airways disease in the fishing and fish-processing industry. *Occup Med (Lond)* 2008;58(8):545-550.

50. Asero R. Food additive-induced chronic pruritus: further evidence. *Clin Exp Dermatol* 2005;30(6):719-720.

51. Tucker SC, Yell JA, Beck MH. Allergic contact dermatitis from sodium metabisulfite in Trimovate cream. *Contact Dermatitis* 1999;40(3):164.

52. Aalto-Korte K, Suuronen K, Alanko K. Sodium metabisulfite - a contact allergen? *Contact Dermatitis* 2009;60(2):115-117.

53. Seitz CS, Bröcker EB, Trautmann A. Eyelid dermatitis due to sodium metabisulfite. *Contact Dermatitis* 2006;55(4):249-250.

54. Sasseville D, El-Helou T. Occupational allergic contact dermatitis from sodium metabisulfite. *Contact Dermatitis* 2009;61(4):244-245.

55. Boyd AH, Warshaw EM. Sulfites: No Longer a Zebra? *Dermatitis* 2017;28(6):364-366.

56. Sánchez-Pérez J, Abajo P, Córdoba S, García-Díez A. Allergic contact dermatitis from sodium metabisulfite in an antihemorrhoidal cream. *Contact Dermatitis* 2000;42(3):176-177.

57. Riemersma WA, Schuttelaar ML, Coenraads PJ. Type IV hypersensitivity to sodium metabisulfite in local anaesthetic. *Contact Dermatitis* 2004;51(3):148.

58. Stingeni L, Bianchi L, Lisi P. Occupational airborne allergic contact dermatitis from potassium metabisulfite. *Contact Dermatitis* 2009;60(1):52-53.

59. García Ortiz JC, Vega Gutiérrez JM, Pérez Velesar MJ, Medina AA. Occupational allergic contact dermatitis from potassium metabisulfite. *Dermatitis* 2014;25(3):150-151.

60. Cifuentes L, Ring J, Brockow K. Clonal mast cell activation syndrome with anaphylaxis to sulfites. *Int Arch Allergy Immunol* 2013;162(1):94-96.

61. Madan V, Beck MH. Sodium metabisulfite--a contact allergen? *Contact Dermatitis* 2009;61(1):58.

62. Malik M, Hegarty M, Bourke J. Sodium metabisulfite - a marker for cosmetic allergy? *Contact Dermatitis* 2007;56:241-242.

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

Received 24 March 2003; accepted 1 July 2003.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Bindu Nair and Amy R. Elmore, former Scientific Analyst/Writers. Address correspondence to F. Alan Andersen, Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

International Journal of Toxicology, 22(Suppl 2):63–88, 2003

ISSN: 1091-5818 print / 1092-874X online
DOI: 10 1080/10915810390239478

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

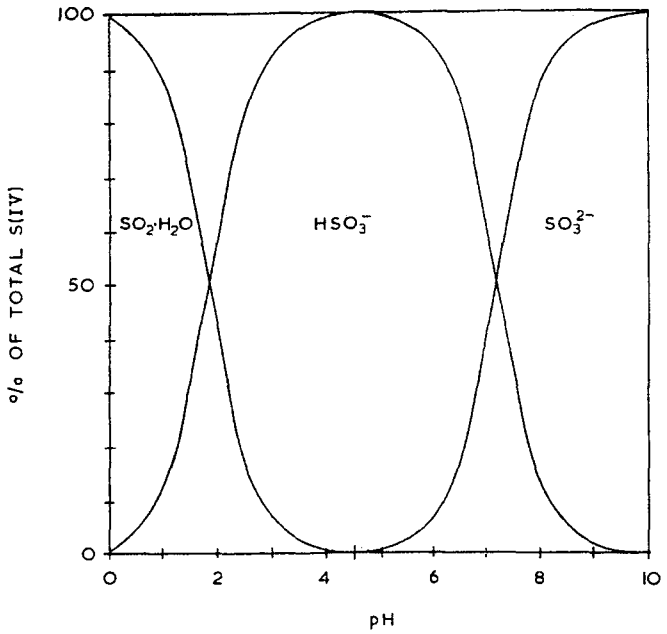


FIGURE 1
Distribution of the species $SO_2 \cdot H_2O$, HSO_3^-, and SO_3^{2-} as a function of pH in dilute solution (Wedzicha 1984).

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 ≥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 ≥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 ≥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 ~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

(Continued on next page)

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

(Continued on next page)

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

(Continued on next page)

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 ~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 ≤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 (~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 ≥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.

REFERENCES

Abe, S., and M. Sasaki 1977. Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals *J Natl Cancer Inst.* 58:1635–1641

American Conference of Governmental Industrial Hygienists (ACGIH). 1987. *Threshold limit values and biological exposure indices for 1986–1997.* 3, 29, 30 Cincinnati, OH: ACGIH

Atkinson, D A., T. C Sim, and J A Grant. 1993. Sodium metabisulfite and SO_2 release: An underrecognized hazard among shrimp fishermen. *Ann Allergy* 71:563–566.

Beckman, L., and I. Nordenson. 1986. Interaction between some common genotoxic agents. *Hum Hered* 36:397–401

Bhattacharjee, D , T K. Shetty, and K Sundaram 1980 Effects on the spermatogonia of mice following treatment with sodium bisulfite. *J Environ Pathol Toxicol* 3:189–193.

Borek, C., A Ong, and H. Mason. 1985 Sodium bisulfite protects against radiogenic and chemically induced transformation in hamster embryo and mouse C3H/10T$^1/_2$ cells. *Toxicol Ind Health* 1:69–74.

Bower, D 1999. Personal communication of information on hair spray particle sizes provided at the September 9, 1999 CIR Expert Panel Meeting [2]

Budavari, S., ed. 1989. *The Merck index. An encyclopedia of chemicals, drugs, and biologicals*, 11th ed Rahway, NJ: Merck & Co.

Calabrese, E., C. Sacco, G. Moore, and S DiNardi 1981. Sulfite oxidase deficiency: A high risk factor in SO_2, sulfite, and bisulfite toxicity? *Med Hypotheses* 7:133–145.

Chen, L. C., H. F Lam, D. Ainsworth, J. Guty, and M O Amdur. 1987 Functional changes in the lungs of guinea pigs exposed to sodium sulfite aerosols. *Toxicol Appl Pharmacol* 89:1–8

Clauzan, R., J. Causeret, and D Hugot 1965 Potassium-metabisulphite: Long term study of toxicity in the rat *Ann Biol. Biochem Biophys* 5:267–281

Combe Incorporated. 1996 100 human subject insult open patch test skin irritation/sensitization evaluation. Unpublished data submitted by CTFA. (9 pages.)[2]

Combe Incorporated. 1998. 100 human subject insult semi-occlusive patch test skin irritation/sensitization evaluation. Unpublished data submitted by CTFA. (10 pages.)[2]

Committee of Revision of the United States Pharmacopeial Convention. 1995 *The National Formulary*, 18th ed. Rockville, MD: United States Pharmacopeial Convention

Corder, E. H., and C. E. Buckely, 3rd 1995. Aspirin, salicylate, sulfite and tartrazine induced bronchoconstriction. Safe doses and case definition in epidemiological studies. *J Clin Epidemiol* 48:1269–1275

Cosmetics Directive of the European Union 1995. Updated version—incorporating all amendments until August 1, 1995 Dir 76/768/EEC, Annex VI, part I http://europa eu.int/comm/index_en htm

Cosmetics, Toiletry and Fragrance Association (CTFA). 1999a Ingredient use data Unpublished data submitted by CTFA (1 page)[2]

CTFA 1999b Ingredient use data Unpublished data submitted by CTFA (2 pages.)[2]

Covino, B. G. 1988 Toxicity of local anesthetic agents *Acta Anaesthesiol Belg.* 39(3 suppl 2):159–164

De Giovanni-Donelly, R 1985 The mutagenicity of sodium bisulfite on base-substitution strains of *Salmonella typhimurium. Teratog Carcinog Mutagen.* 5:195–204

Dinerman, A. A , and A D. Ignat'ev. 1966. Effect of certain food preservatives on the development of certain malignant processes in mice. *Hyg Sanitation* 31:396–401.

DiPaolo, J. A , A. J. DeMarinis, and J Doniger. 1981. Transformation of Syrian hamster embryo cells by sodium bisulfite. *Cancer Lett* 12:203–208.

Doniger, J., R. O'Neill, and J. A. DiPaolo. 1982. Neoplastic transformation of Syrian hamster embryo cells by bisulfite is accompanied with a decrease in the number of functioning replicons. *Carcinogenesis* 3:27–32

Dulak, L., G. Chiang, and A. F. Gunnison. 1984. A sulfite oxidase-deficient rat model: Reproductive toxicology of sulfite in the female *Food Chem Toxicol* 22:599–607.

Eastman Kodak Co. 1980. *Basic toxicity of sodium metabisulfite with cover letter dated 02/15/94.* National Technical Information Service (NTIS) Report No. OTS0556695.

Eberlein-König, B , T. Bergner, S. Diemer, and B. Przybilla. 1993. Evaluation of phototoxic properties of some food additives: Sulfites exhibit prominent phototoxicity. *Acta Dermato-Venereol* 73:362–364.

Ema, M., T Itami, and S. Kanoh. 1985. Effect of potassium metabisulfite on pregnant rats and their offspring. 2. studies on the fetal toxicity of food additives. *Shokuhin Eiseigaku Zasshi (J. Food Hyg Soc Japan)* 26:454–459.

Environmental Protection Agency (EPA). 1994. National Ambient Air Quality Standards 40 CFR 50.4 and 50.5 http://www.epa.gov/airs/criteria.html

Fazio, T., and C. R Warner. 1989. A review of sulphites in food: Analytical methodology and reported findings. *Food Add and Contam* 7:433–454.

Federation of American Societies for Experimental Biology (FASEB). 1976. *Evaluation of the health aspects of sulfiting agents as food ingredients* NTIS report No. PB 265-508.

FASEB. 1985. *The reexamination of the GRAS status of sulfiting agents.* NTIS report No PB 85-164044.

Feron, V. J., and P. Wensvoort. 1972. Gastric lesions in rats after the feeding of sulfite *Pathol Eur.* 7:103–111

Fisher, A. A. 1997. The sulfites: Part III. Facts about sulfites [news]. *Cutis* 60:73–74.

Fitzhugh, O. G., L. F. Knudsen, and A. A Nelson. 1946. Chronic toxicity of sulfites. *J Pharmacol Exp Ther.* 86:37–48

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). 1994. *Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).* United States: International Life Sciences Institute.

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. *FDA database.* Washington, DC: FDA.

FDA. 1986 New sulfite regulations. *FDA Drug Bull.* 16:17–18.

FDA 1992 Modification in Voluntary Filing of Cosmetic Product Ingredient and Cosmetic Raw Composition Statements. Final rule. *Fed Register* 57:3128–3130.

FDA 1998 Cosmetic product formulation data. *FDA database.* Washington, DC: FDA

Food and Drug Research Labs. 1972a. *Teratologic evaluation: FDA 71-20 (sodium bisulfite).* NTIS Report No. PB-221-788.

Food and Drug Research Labs. 1972b. *Teratologic evaluation FDA 71-22 (sodium metabisulfite) in rabbits.* NTIS Report No. PB-221-795.

Food and Drug Research Labs. 1974a. *Teratologic evaluation FDA 71-20 (sodium bisulfite) in rabbits.* NTIS Report No PB-267-195.

Food and Drug Research Labs 1974b *Teratologic evaluation FDA 71-22 (sodium metabisulfite) in rabbits.* NTIS Report No. PB-267-194

Food and Drug Research Labs 1975. *Teratologic evaluation of potassium metabisulfite.* NTIS Report No. PB-245-529.

Franklin Institute Research Laboratories. 1972. *GRAS (generally recognized as safe) food ingredients—sulfiting agents.* NTIS Report No. PB-221-217

[2]Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036-4702, USA

Generoso, W. M., S. W Huff, and K. T. Cain. 1978 Tests on induction of chromosome aberrations in mouse germ cells with sodium bisulfite. *Mutat Res.* 56:363–365.

Grant, J., ed. 1972. *Hackh's chemical dictionary*, 4th ed. New York, NY: McGraw-Hill.

Green, L. F. 1976. Sulphur dioxide and food preservation—a review *Food Chem* 1:103–124.

Gregory, R. E., S. Grossman, and V. R. Sheidler 1992. Grand mal seizures associated with high-dose intravenous morphine infusions: Incidence and possible etiology. *Pain* 51:255–258.

Gunnison, A. F 1981. Sulphite toxicity: A critical review of in vitro and in vivo data. *Food Cosmet Toxicol.* 19:667–682

Gunnison, A. F., C. A. Bresnahan, and E. D. Palmes 1977 Comparative sulfite metabolism in the rat, rabbit, and rhesus monkey. *Toxicol Appl Pharmacol* 42:99–109.

Gunnison, A. F., L. Dulak, J. Chiang, J. Zaccardi, and T J Farruggella 1981. A sulphite-oxidase-deficient rat model: Subchronic toxicity *Food Cosmet Toxicol.* 19:221–232.

Gunnison, A. F., and D. W. Jacobsen. 1987. Sulfite hypersensitivity. A critical review *CRC Crit Rev Toxicol.* 17:185–214.

Gunnison, A. F., and E. D. Palmes 1976. A model for the metabolism of sulfite in mammals. *Toxicol Appl Pharmacol.* 38:111–126

Haskell Labs. 1973. *Department of Transportation skin corrosion test on rabbit skin of sodium bisulfite solution (38%) with cover letter dated 03/15/94.* NTIS Report No. OTS0556762.

Haskell Labs. 1974a. *Skin irritation test on rabbits of sodium metabisulfite with cover letter dated 03/15/94.* NTIS Report No. OTS0556760.

Haskell Labs. 1974b. *Eye irritation test on rabbits of sodium metabisulfite with cover letter dated 03/15/94.* NTIS Report No. OTS0556759.

Haskell Labs. 1975. *Acute oral test of sodium metabisulfite in CHR-CD rats with cover letter dated 03/15/94.* NTIS Report No. OTS0556761

Hayatsu, H. 1977 Co-operative mutagenic actions of bisulfite and nitrogen nucleophiles *J Mol Biol* 115:19–31.

Hayatsu, H., and A. Miura. 1970. The mutagenic action of sodium bisulfite. *Biochem Biophys. Res Commun.* 39:156–160.

Hayatsu, H., Y. Wataya, K. Kai, and S. Iida. 1970 Reaction of sodium bisulfite with uracil, cytosine, and their derivatives. *Biochemistry* 9:2858–2865

Hazleton Labs. 1973. *Skin irritation test with sodium metabisulfite in rabbits with cover letter dated 4/13/94.* NTIS report No. OTS0572413.

Hötzel, D., E Muskat, I Bitsch, W Aign, J -D. Althoff, and H. D. Cremer. 1969. Thiamin-mangel and Unbedenklichkeit von sulfit für den Menschen *Int Z Vitaminforsch.* 39:372–383.

Hui, J. Y., J T Beery, N. A. Higley, and S L Taylor 1989 Comparative subchronic toxicity of sulfite and acetaldehyde hydroxysulfonate in rats *Food Chem Toxicol.* 27:349–360.

International Agency for Research on Cancer (IARC). 1992. Sulphur dioxide and some sulfites, bisulfites and metabisulfites *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* 54:131–188

Irving, J. T., J J. Pindborg, O. G. Fitzhugh, J P Weinmann, and I Schour. 1952. Symptoms of vitamin A and E deficiencies in the incisor of the rat fed sodium sulfite *J D Res* 31:815–824.

Itami, T., M. Ema, H Kawasaki, and S Kanoh. 1989. Evaluation of teratogenic potential of sodium sulfite in rats. *Drug Chem Toxicol* 12:123–135

Jagiello, G M., J. S. Lin, and M. B Ducayen. 1975. Sulfur dioxide and its metabolite: Effects on mammalian egg chromosomes. *Environ Res* 9:84

Jamieson, D M., M. F Guill, B. B. Wray, and J. R. May 1985 Metabisulfite sensitivity: case report and literature review *Ann. Allergy.* 54:115–121

Ji, J. A., S R. Savon, and D. W Jacobsen. 1995. Determination of total serum sulfite by HPLC with flourescence detection. *Clin Chem* 41:897–903

Jonker, D , R. A. Woutersen, P. J. Van Bladeren, H. P Til, and V. J. Feron 1990 4-Week oral toxicity study of a combination of eight chemicals in rats: Comparison with the toxicity of the individual compounds. *Food Chem Toxicol* 28:623–632.

Karg, E., F. Erbe, G A. Ferron, B. Haider, J. Heyder, W.G. Kreyling, J. Peter, T. Tuch, and W. Witte 1988. Facilities for chronic exposure of dogs to sulfite aerosols. *J. Aerosol Sci* 19:971–973.

Kim, S. B , I S Kim, D. M. Yeum, and Y. H. Park. 1991. Mutagenicity of maillard reaction products from D-glucose-amino acid mixtures and possible roles of active oxygens in the mutagenicity *Mutat Res* 254:65–69.

Koepke, J W , H. Staudenmayer, and J C. Selner. 1985. Inhaled metabisulfite sensitivity. *Ann. Allergy* 54:213–215.

Kunz, B. A., and B W Glickman 1983 Absence of bisulfite mutagenesis in the LacI gene of *Escherichia coli Mutat Res.* 119:267–271.

Last, J. A., P. K. Dasgupta, and J. R. Etchison. 1980. Inhalation toxicology of sodium sulfite aerosols in rats *Toxicol Appl. Pharmacol.* 55:299–234

Lavoie, J. C., C. Lachance, and P. Chessex 1994. Antiperoxide activity of sodium metabisulfite A double-edged sword *Biochem Pharmacol* 47:871–876.

Lester, M. R. 1995 Sulfite sensitivity: Significance in human health. *J Am. Coll. Nutr.* 14:229–232.

Lewis, R. J., ed. 1993. *Hawley's condensed chemical dictionary*, 12th ed. New York, NY: Van Nostrand Reinhold

Litton Bionetics. 1972 *Mutagenic evaluation of compound FDA 71-20 sodium bisulfite.* NTIS Report No PB-245-456

Litton Bionetics. 1975. *Mutagenic evaluation of compound FDA 73-53 sodium sulfite.* NTIS Report No. PB 245-488.

Lockett, M. F , and I L Natoff. 1960. A study on the toxicity of sulphite *J Pharm. Pharmacol* 12:488–496.

MacRae, W. D., and H. F. Stich 1979. Induction of sister chromatid exchanges in Chinese hamster cells by the reducing agents bisulfite and ascorbic acid. *Toxicology* 13:167–174.

Mallon, R. G., and T. G. Rossman 1981. Bisulfite (sulfur dioxide) is a comutagen in *E coli* and in Chinese hamster cells. *Mutat. Res* 88:125–133

Meisel, S B., and P. K. Welford. 1992. Seizures associated with high-dose intravenous morphine containing sodium bisulfite preservative. *Ann Pharmacother.* 26:1515–1517.

Meng, Z , and L. Zhang. 1990 Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of workers exposed to sulphur dioxide. *Mutat Res* 241:15–20.

Meng, Z., and L. Zhang 1992. Cytogenic damage induced in human lymphocytes by sodium bisulfite. *Mutat Res* 298:63–69.

Merlo, D J., and D V. Thompson. 1987 In vitro sodium bisulfite mutagenesis of restriction endonuclease recognition sites. *Anal Biochem* 163:79–87.

Ministry of Health, Labor and Welfare (MHLW). 2001 Unofficial translation of MHLW Ordinance No. 331, including attached Tables. Ministry of Health, Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2, 1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100-8045, Japan.

Mitsuhashi, H., N. Yoshihisa, T. Takaharu, K Ueki, A Maezawa, et al. 1998 Sulfite is released by human neutrophils in response to stimulation with lipopolysaccharide. *J Leuk Biol* 64:595–599.

Mukai, F., I. Hawryluk, and R. Shapiro. 1970. The mutagenic specificity of sodium bisulfite *Biochem Biophys Res Commun.* 39:983–988.

Münzner, R 1980. Investigations of the mutagenic effect of bisulfite *Lebensmittel-Wiss-Technol* 13:219–220

Nakasato, F , M. Nakayasu, Y. Fujita, M. Nagao, M. Terada, and T Sugimura. 1984. Mutagenicity of instant coffee on cultured Chinese hamster lung cells *Mutat Res* 141:109–112.

National Academy of Sciences (NAS). 1981. *Food chemicals codex.* 3rd ed. Washington, DC: National Academy Press

Nicklas, R. A. 1989. Sulfites: A review with emphasis on biochemistry and clinical application *Allergy Proc* 10:349–356

Pagano, D A , and E. Zeiger 1987 Conditions affecting the mutagenicity of sodium bisulfite in *Salmonella typhimurium Mutat Res* 179:159–166.

Pal, B. B., and S P Bhunya 1992. Genotoxic effect of a preservative, sodium metabisulphite as revealed by mammalian in vivo bioassays. *Cytologia (Tokyo)* 57:455–461

Pepe, R. C , J. A Wenninger, and G. N. McEwen, Jr., eds. 2002 *International cosmetic ingredient dictionary and handbook*, 9th ed. Washington, DC: CTFA.

Petersen, C S., and T. Menné 1992 Consecutive patch testing with sodium sulfite in eczema patients. *Contact Dermatitis* 27:344–345

Petersen, P. E., R B Evans, M. A Johnstone, and W R. Henderson, Jr 1990 Evaluation of ocular hypersensitivity to dipivalyl epinephrine by component eye-drop testing. *J Allergy Clin Immunol* 85:954–958

Pirila, V., H. Kajanne, and O P. Salo 1963 Inhalation of sulfur dioxide as a cause of skin reactions resembling drug eruption *J Am Acad Dermatol* 10:1077–1081

Popescu, N. C., and J A DiPaolo. 1988 Chromosome alterations in Syrian hamster cells transformed in vitro by sodium bisulfite, a nonclastogenic carcinogen. *Cancer Res.* 48(24 pt 1):7246–7251

Reed, G. A., and B. C. Jones. 1996. Enhancement of benzo[a]pyrene diol epoxide mutagenicity by sulfite in a mammalian test system. *Carcinogenesis* 17:1063–1068

Reed, G A., M. J. Ryan, and K S Adams 1990 Sulfite enhancement of diol epoxide mutagenicity: The role of altered glutathione metabolism *Carcinogenesis* 11:1635–1639.

Registry of Toxic Effects of Chemical Substances (RTECS). 1998. *RTECS database*. Bethesda, MD: National Library of Medicine

Renner, H W., and J Wever. 1983. Attempts to induce cytogenetic effects with sulphite in sulphite oxidase-deficient Chinese hamsters and mice. *Food Chem Toxicol.* 21:123–127

Rosin, M. P, and H F Stich 1979 Assessment of the use of the *Salmonella* mutagenesis assay to determine the influence of antioxidants on carcinogen-induced mutagenesis *Int J Cancer* 23:722–727

Rothenberg, S. J., A. R Dahl, E. B. Barr, and R K. Wolff. 1986 Generation, behavior, and toxicity of ammonium sulfite aerosols. *J Air Pollution Control Assoc* 36:55–59

Seravalli, E., and E. Lear 1987. Toxicity of chloroprocaine and sodium bisulfite on human neuroblastoma cells *Anesth Analg.* 66:954–958

Seravalli, E P., E Lear, and J. E Cottree. 1984. Cell membrane fusion by chloroprocaine. *Anesth Analg.* 63:985–990.

Shapiro, R 1983. Genetics effects of bisulfite: Implications for environmental protection. *Basic Life Sci.* 23:35–60.

Shortle, D., and D Botstein. 1983. Directed mutagenesis with sodium bisulfite *Methods Enzymol* 100:457–468

Shtenberg, A J , and A D. Ignat'ev 1970 Toxicological evaluation of some combinations of food preservatives *Food Cosmet Toxicol* 8:369–380.

Simon, R. A 1986. Sulfite sensitivity. *Ann Allergy* 56:281–288.

SRI International. 1978a. *Microbial mutagenesis testing of substances compound report F76-003, sodium bisulfite*. NTIS Report No PB 89-193676

SRI International 1978b. *Microbial mutagenesis testing of substances compound report F76-004, sodium meta-bisulfite*. NTIS Report No PB 89-193684.

SRI International. 1979 *Study on the mutagenic effects of sodium meta-bisulfite (76-73) by the dominant lethal test in rats* NTIS Report No PB-299-836

Stanford Research Institute 1972. *Study of the mutagenic effects of sodium meta-bisulfite (71-22-)*. NTIS Report No. PB 221-825.

Suwa, Y., M. Nagao, A. Kosugi, and T Sugimura 1982 Sulfite suppresses the mutagenic property of coffee. *Mutat. Res.* 102:383–391.

Takahashi, M., and R Hasegawa. 1985. Enhancing effects of dietary salt on both initiation and promotion stages of rat gastric carcinogenesis. *Princess Takamatsu Symp.* 16:169–182.

Takahashi, M., R Hasegawa, F. Furukawa, K. Toyoda, H Sato, and Y Hayashi. 1986. Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. *Jpn. J Cancer Res.* 77:118–124.

Takenaka, S., P Heilmann, L. Ruprecht, U. Heinzmann, A. B. Murray, G Fürst, A Heini, and J. Heyder 1990. Long-term exposure of dogs to a sulphite aerosol: IV Effects on extrapulmonary airway morphology *J Aerosol Sci* 21(suppl 1):S483–S486.

Tanaka, T., M Fujii, H Mori, and I. Hirono. 1979. Carcinogenicity test of potassium metabisulfite in mice. *Ecotoxicol. Environ Safety* 3:451–453.

Taylor, S. L , N. A Higley, and R. K. Bush. 1986. Sulfites in foods: Uses, analytical methods, residues, fate, exposure assessment, metabolism, toxicity, and hypersensitivity *Adv Food Res.* 30:1–76.

Til, H. P., and V. J. Feron. 1992. Toxicology of sulphiting agents I: Animal studies. *Food Addit. Contam.* 9:587–595.

Til, H. P., V. J. Feron, and A. P. De Groot. 1972a. Toxicity of sulfite 1. long-term feeding and multigeneration studies in rats. *Food Cosmet Toxicol* 10:291–310.

Til, H. P., V. J. Feron, A. P. DeGroot, and P. van der Wal. 1972b. The toxicity of sulphite. II Short and long term feeding studies in pigs *Food Cosmet Toxicol* 10:463–473.

Tsutsui, T., and J C. Barrett. 1990. Sodium bisulfite induces morphological transformation of cultured Syrian hamster embryo cells but lacks the ability to induce detectable gene mutations, chromosome mutations or DNA damage. *Carcinogenesis (eynsham)* 11:1869–1874.

Tucker, J D., R. T Taylor, M. L. Christensen, C L. Strout, and M. L Hanna. 1989a. Cytogenetic response to coffee in Chinese hamster ovary AUXB1 cells and human peripheral lymphocytes *Mutagenesis.* 4:343–348.

Tucker, J D , R. T. Taylor, M. L. Christensen, C. L. Strout, M. L. Hanna, and A. V Carrano. 1989b. Cytogenetic response to 1,2-dicarbonyls and hydrogen peroxide in Chinese hamster ovary AUXB1 cells and human peripheral lymphocytes *Mutat Res* 224:269–279.

Vena, G. A., C Foti, and G. Angelini. 1994 Sulfite contact allergy. *Contact Dermatitis* 31:172–175.

Walker, R 1985. Sulphiting agents in foods: Some risk/benefit considerations. *Food Addit Contam* 2:5–24

Wedzicha, B L. 1984. *Chemistry of sulfur dioxide in foods* New York: Elsevier Applied Publishers

Wirth, P J , J Doniger, S. S. Thorgeirsson, and J. A. Dipaolo 1986. Altered polypeptide expression associated with neoplastic transformation of Syrian hamster cells by bisulfite. *Cancer Res* 46:390–399

Wright, W., Y. G Zhang, C. M. Salome, and A. J Woolcock. 1990. Effect of inhaled preservatives on asthmatic subjects. I. Sodium metabisulfite. *Am Rev. Respir. Dis* 141:1400–1404

Yang, W. H , E. C Purchase, and R. N. Rivington. 1986. Positive skin tests and Prausnitz-Küstner reactions in metabisulfite-sensitive subjects *J Allergy Clin Immunol.* 78(3 pt 1):443-449. [Published erratum 1987. *J Allergy Clin Immunol.* 79:15.]

2020 FDA VCRP Data

Ammonium Bisulfite

05C - Hair Straighteners	1
Total	**1**

Ammonium Sulfite - No FDA Uses

Potassium Metabisulfite - No FDA Uses

Potassium Sulfite

10B - Deodorants (underarm)	1
12I - Skin Fresheners	1
Total	**2**

Sodium Bisulfite

3D - Eye Lotion	6
3G - Other Eye Makeup Preparations	2
5A - Hair Conditioner	1
5F - Shampoos (non-coloring)	3
5G- Tonics, Dressings, and Other Hair Grooming Aids	1
7I - Other Makeup Preparations	2
10A - Bath Soaps and Detergents	8
10E - Other Personal Cleanliness Products	7
12A - Cleansing	3
12C - Face and Neck (exc shave)	23
12D - Body and Hand (exc shave)	1
12F - Moisturizing	6
12G - Night	3
12H - Paste Masks (mud packs)	2
12I - Skin Fresheners	1
12J - Other Skin Care Preps	5
Total	**74**

Sodium Metabisulfite

1C - Other Baby Products	1
3D - Eye Lotion	12
3G - Other Eye Makeup Preparations	16
5C - Hair Straighteners	1
5F - Shampoos (non-coloring)	3
5G - Tonics, Dressings, and Other Hair Grooming Aids	3
5I - Other Hair Preparations	1
6A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	395
6B - Hair Tints	142
7A - Blushers (all types)	1
7C - Foundations	3

7F - Makeup Bases	1
7I - Other Makeup Preparations	4
8G - Other Manicuring Preparations	1
10A - Bath Soaps and Detergents	15
10E - Other Personal Cleanliness Products	9
11G - Other Shaving Preparation Products	1
12A - Cleansing	19
12C - Face and Neck (exc shave)	94
12D - Body and Hand (exc shave)	21
12F - Moisturizing	58
12G - Night	13
12H - Paste Masks (mud packs)	5
12I - Skin Fresheners	5
12J - Other Skin Care Preps	46
13A - Suntan Gels, Creams, and Liquids	8
13B - Indoor Tanning Preparations	36
13C - Other Suntan Preparations	2
Total	**916**

Sodium Sulfite

2A - Bath Oils, Tablets, and Salts	1
3D - Eye Lotion	8
3F - Mascara	1
3G - Other Eye Makeup Preparations	3
5A - Hair Conditioner	2
5C - Hair Straighteners	2
5F - Shampoos (non-coloring)	4
5G - Tonics, Dressings, and Other Hair Grooming Aids	4
5I - Other Hair Preparations	4
6A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	1489
6B - Hair Tints	30
6F - Hair Lighteners with Color	2
6G - Hair Bleaches	1
6H - Other Hair Coloring Preparation	3
7C - Foundations	2
7I - Other Makeup Preparations	1
8C - Nail Creams and Lotions	1
10A - Bath Soaps and Detergents	17
10B - Deodorants (underarm)	6
10E - Other Personal Cleanliness Products	23
11A - Aftershave Lotion	8
11E - Shaving Cream	1
12A - Cleansing	7
12C - Face and Neck (exc shave)	24
12D - Body and Hand (exc shave)	17
12F - Moisturizing	31

12G - Night	7
12H - Paste Masks (mud packs)	2
12I - Skin Fresheners	1
12J - Other Skin Care Preps	9
13B - Indoor Tanning Preparations	2
Total	**1713**

1998 FDA VCRP Data

Ammonium Bisulfite - No FDA Uses

Ammonium Sulfite - No FDA Uses

Potassium Metabisulfite

05D - Permanent Waves	1
Total	**1**

Potassium Sulfite

05D - Permanent Waves	1
Total	**1**

Sodium Bisulfite

02D - Other Bath Preparations	1
05A - Hair Conditioners	1
05F - Shampoos (non-coloring)	1
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	49
12D - Body and Hand (exc shave)	1
12F - Moisturizing	1
12J - Other Skin Care Preps	4
Total	**58**

Sodium Metabisulfite

02D - Other Bath Preparations	8
03D - Eye Lotion	1
05D - Permanent Waves	1
05F - Shampoos (non-coloring)	2
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	309
06E - Hair Color Sprays (aerosol)	1
08A - Basecoats and Undercoats	1
10B - Deodorants (underarm)	7
12D - Body and Hand (exc shave)	2
12F - Moisturizing	1

12J - Other Skin Care Preps	4
13B - Indoor Tanning Preparations	11
Total	**348**

Sodium Sulfite

02A - Bath Oils, Tablets, and Salts	1
02D - Other Bath Preparations	1
05A - Hair Conditioners	1
05D - Permanent Waves	2
05F - Shampoos (non-coloring)	9
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	872
06B - Hair Tints	19
06F - Hair Lighteners with Color	1
06H - Other Hair Coloring Preparations	1
08A - Basecoats and Undercoats	1
10A - Bath Soaps and Detergents	1
12J - Other Skin Care Preps	2
Total	**911**

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: September 11, 2019

SUBJECT: Re-review of the Safety Assessment of Sulfites (draft prepared for the September 16-17, 2019 CIR Expert Panel meeting)

The Personal Care Products Council respectfully submits the following comments on the Re-review of the Safety Assessment of Sulfites.

Key Issue

It would have been helpful if the EU Annex III limitations for the sulfites and the 2003 SCCNFP opinion would have been mentioned in the summary.

Additional Considerations

Short-Term, Inhalation, Sodium Metabisulfite - What were the "lung inflammatory responses factors" that were measured? Were any other organs examined in this study (reference 2)?

DART, Animal, Oral, Sodium Metabisulfite - Please correct: "The epididymis was analyzed for motility..." It is more likely that the "epididymal sperm were analyzed for motility..."

Although the paragraph starts with: "A study on protection against Sodium Metasilicate-induced testicular toxicity was performed.", the paragraph never describes an experiment concerning protection. What was studied and did it reduce the testicular effects of Sodium Metasilicate?

Genotoxicity, In Vivo, Sodium Bisulfite and Sodium Sulfite - In addition to stating the saline and background levels of PCE with micronuclei, it would be helpful to also state the PCE with micronuclei in the cells from treated mice.

Effect on Allergic Pulmonary Sensitization Co-Elicitation - Please correct: "allerfrtulfite" (or explain what this means)

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org