Safety Assessment of Inorganic Sulfates as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	February 21, 2014
Panel Date:	March 17-18, 2014

The 2014 Cosmetic Ingredient Review Expert Panel 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.; Ronald A Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Bart Heldreth, Ph.D., Chemist.

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: February 21, 2014
Subject: Draft Report on Inorganic Sulfates

A Scientific Literature Review (SLR) on inorganic sulfates was issued on December 2, 2013. Use concentration data received from the Personal Care Products Council (Council) were incorporated prior to announcement of the SLR. Comments from the Council were received during the 60-day comment period.

Included in this package for your review is the Draft Report on Inorganic Sulfates, the CIR report history, Literature search strategy, Ingredient Data profile, 2013 FDA VCRP data, use concentration data from the Council (data1 pdf file), final reports on sodium sulfate and ammonium, potassium, and sodium persulfates (final1 and final2 pdf files), and comments provided by the Council (pcpc1 and pcpc2 pdf files). These comments will be addressed prior to the March 2014 Panel meeting.

It should be noted that the Expert Panel previously issued final reports on the safety of the following inorganic sulfates in cosmetics: sodium sulfate (published in 2000) and ammonium persulfate, potassium persulfate, and sodium persulfate (published in 2001). The Panel concluded that sodium sulfate is safe as used in rinse-off formulations and safe up to 1% in leave-on formulations. Additionally, the Panel concluded that the 3 persulfates are safe as used as oxidizing agents in hair colorants and lighteners designed for brief discontinuous use followed by thorough rinsing from the hair and skin. Concern over sensitization and urticaria potential was the basis for the Panel's conclusion on persulfates, and the Panel's conclusion on sodium sulfate was based on concern over skin irritation potential.

The current safety assessment has been revised to include new sensitization data on ammonium, potassium, and sodium persulfates from the published literature, in light of the limited sensitization data (on copper sulfate) that were identified initially. With this in mind, the Panel needs to determine whether these sensitization data, which justify the Panel's earlier conclusion on persulfates, can be used to evaluate the sensitization potential of all of the inorganic sulfates reviewed in this safety assessment.

Additionally, after reviewing the available data, the Panel needs to determine whether an insufficient data announcement or tentative report with a safe as used, safe with qualifications, or unsafe conclusion should be issued.

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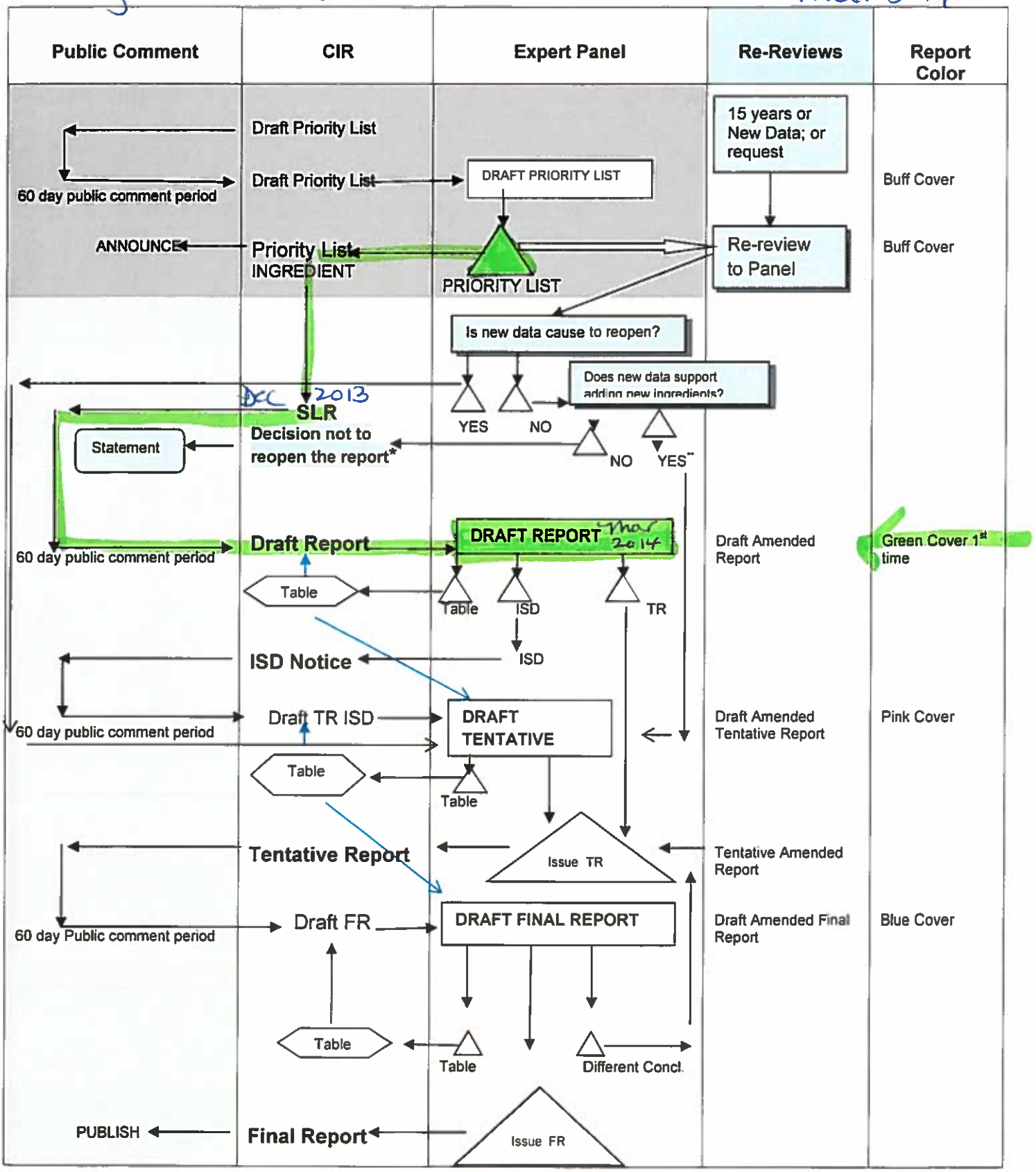
SAFETY ASSESSMENT FLOW CHART

Inorganic Sulfates — Mar 2014



CIR History of:

Inorganic Sulfates

A Scientific Literature Review (SLR) was announced on December 2, 2013. Use concentration data received from the Personal Care Products Council (Council) were incorporated prior to announcement of the SLR. Comments from the Council were received during the 60-day comment period.

Draft Report, Belsito and Marks Teams/Panel: March 17-18, 2013

The draft has been revised to include sensitization data on ammonium, potassium, and sodium persulfate, which may be useful in evaluating the sensitization potential of all of the inorganic sulfates reviewed in this safety assessment.

Inorganic Sulfates
Check List for March, 2014. Analyst – Wilbur Johnson

			Acute toxicity					Repeated dose toxicity				Irritation			Sensitization					
	Skin Penetration	Penetration Enhancement	ADME	Oral	Parenteral	Dermal	Inhale	Oral	Parenteral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
Aluminum Sulfate			X	X	X		X	X	X							X	X	X		
Ammonium Persulfate								X			X					X				
Ammonium Sulfate				X	X		X				X							X	X	
Barium Sulfate			X	X	X						X					X		X		
Calcium Sulfate											X						X		X	
Calcium Sulfate Hydrate														X				X	X	
Copper Sulfate			X	X	X		X	X	X		X	X	X	X	X	X	X	X	X	
Ferric Sulfate							X									X				
Ferrous Ammonium Sulfate			X													X		X	X	
Ferrous Sulfate			X	X	X			X								X	X	X	X	
Hydroxylamine Sulfate			X	X		X		X					X			X		X		
Magnesium Sulfate			X		X				X		X					X	X	X	X	
Manganese Sulfate			X	X	X				X		X						X	X	X	
Potassium Persulfate																X				
Potassium Sulfate																				
Silver Sulfate																		X		
Sodium Bisulfate							X						X							
Sodium Persulfate																				
Sodium Sulfate							X													
Zinc Sulfate			X	X	X			X	X		X			X		X	X	X	X	

Literature Searches on Inorganic Sulfates
(1/2013 and 9/2013)

SciFinder Searches

Search Terms

Aluminum Sulfate
Ammonium Persulfate*
Ammonium Sulfate
Barium Sulfate
Calcium Sulfate
Calcium Sulfate Hydrate
Copper Sulfate
Ferric Sulfate
Ferrous Ammonium Sulfate
Ferrous Sulfate
Hydroxylamine Sulfate
Magnesium Sulfate
Manganese Sulfate
Potassium Persulfate*
Potassium Sulfate
Silver Sulfate
Sodium Bisulfate
Sodium Persulfate*
Sodium Sulfate
Zinc Sulfate

*Searches performed on 12/11/2013

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Table of Contents

INTRODUCTION 1

CHEMISTRY 1

Physical and Chemical Properties 1
Method of Manufacture 1
Composition/Impurities 2

USE 2

Cosmetic 2
Non-Cosmetic 3

TOXICOKINETICS 3

TOXICOLOGY 7

Acute Toxicity 7
Inhalation *7*
Oral *8*
Dermal *10*
Intraperitoneal *10*
Intravenous *11*
Subcutaneous *12*
Intratracheal *12*
Repeated Dose Toxicity 12
Inhalation *13*
Oral *15*
Intraperitoneal *17*
Intravenous *18*
Intratracheal *18*
Cytotoxicity 19
Neurotoxicity 19
Ocular Irritation 20
Skin Irritation 20
Mucous Membrane Irritation 21
Sensitization 21
Animal *21*
Human *21*
In Vitro *24*
Case Reports 24

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY 29

Animal 29
Human 32

GENOTOXICITY 34

In Vitro 34
In Vivo 38
Human 40
Antigenotoxicity 40
Enhancement of Genotoxicity 40

CARCINOGENICITY 41

Animal 41
Human 43
Anticarcinogenicity 43

OTHER EFFECTS **44**

IMMUNOSUPPRESSION 44
EFFECT ON ERYTHROPOIESIS 44
EFFECT ON MELANOGENESIS 44
EFFECT ON CELL PROLIFERATION 44
MODULATION OF HORMONAL EFFECT 45
LIPID PEROXIDATION 45

SUMMARY **45**

INTRODUCTION

The safety of inorganic sulfates as used in cosmetics is reviewed in this safety assessment. These ingredients function mostly as astringents, opacifying agents, skin conditioning agents, and viscosity increasing agents in cosmetic products. Other ingredient functions include cosmetic biocide (zinc sulfate) and skin bleaching agent (calcium sulfate hydrate). Sodium bisulfate functions only as a pH adjuster and ferrous ammonium sulfate functions only as a pesticide in cosmetics. The Cosmetic Ingredient Review (CIR) Expert Panel has evaluated the safety of ammonium persulfate, potassium persulfate, and sodium persulfate in cosmetics, and issued a final report (published in 2001) with a conclusion of safe as used as oxidizing agents in hair colorants and lighteners designed for brief discontinuous use followed by thorough rinsing from the hair and skin.[1] The Expert Panel has also evaluated the safety of sodium sulfate in cosmetics, and issued a final report (published in 2000) with a conclusion of safe as used in rinse-off formulations and safe up to 1% in leave-on formulations.[2]

Concern over sensitization and urticaria potential was the basis for the Panel's conclusion on persulfates, and the Panel's conclusion on sodium sulfate was also based on concern over skin irritation potential. New sensitization data on ammonium, potassium, and sodium persulfates were identified, and are included in this safety assessment. The majority of these data are from human predictive and provocative (mostly hairdressers with dermatitis/asthma) patch testing. These data on persulfates may be useful in evaluating the sensitization potential of all of the inorganic sulfates reviewed in this safety assessment.

CHEMISTRY

The inorganic sulfates (See Figure 1) are salts of sulfuric acid. Except for the ammonium salts, these ingredients are mineral salts readily found in nature (but may also be easily synthesized).[3] While most of these ingredients are readily soluble in water, barium sulfate is virtually insoluble, and almost no absorption occurs even when passed through the human digestive tract (making it a non-toxic, imaging contrast agent).[4]

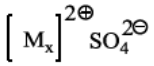

Figure 1. Inorganic Sulfates – wherein "M" is an ammonium or metal cation

The formulas of the inorganic sulfates reviewed in this safety assessment are included in Figure 2.[5] Table 1 contains the definitions and functions of these cosmetic ingredients.

Physical and Chemical Properties

Properties of inorganic sulfates reviewed in this safety assessment are included in Table 2.[6]

Method of Manufacture

The inorganic sulfates are typically manufactured by mining of natural minerals (as many inorganic sulfates occur naturally in hydrated form) or by reaction of available ore or inorganic oxides, hydroxides, or carbonates, with sulfuric acid.[3] These methods produce hydrated inorganic sulfates. To produce the anhydrous salts, an additional step of dehydration (e.g., by heating and reduced pressure) must occur. For example, hydrated magnesium sulfate can be mined as kieserite or epsomite (Epsom salts), or it can be prepared by dissolving magnesium oxide, magnesium hydroxide, or magnesium carbonate in sulfuric acid. Heating of this hydrate reversibly drives off water and produces anhydrous magnesium sulfate (a potent desiccant).

Composition/Impurities

Barium Sulfate

Barytes is the naturally occurring rock form of $BaSO_4$.[7] A study was performed to characterize the mineralogical forms of barium and the trace heavy metal impurities in commercial barytes of different origins using electron probe microanalysis (EPMA), X-ray diffraction (XRD), and inductively coupled plasma mass spectrometry (ICP-MS). Qualitative EPMA results indicated the presence of different minerals in commercial barytes, including barite ($BaSO_4$), barium feldspar, galena (PbS), pyrite (FeS_2), sphalerite (ZnS), quartz (SiO_2), and silicates. Quantitative EPMA confirmed that the barite crystals in the barytes contain some strontium and a little calcium, whereas, trace heavy metals occur in the associated minerals. Analysis of *aqua regia* extracts of barytes samples by ICP-MS has indicated the presence of a large number of elements in the associated minerals. Arsenic, copper, and zinc concentrations correlate closely in all 10 samples.

Chromium has been detected in commercial samples of pharmaceutical grade barium sulfate at concentrations ranging from 0.45 to 1.06 µg/g.[8]

Calcium Sulfate Hydrate

Calcium sulfate hydrate is also known as gypsum. The concentrations of ^{226}Ra (isotope of radium) vary from 0.5 to 35 pCi/g.[9]

USE

Cosmetic

The inorganic sulfates reviewed in this safety assessment function mostly as astringents, opacifying agents, skin conditioning agents, and viscosity increasing agents in cosmetic products. Other ingredient functions include cosmetic biocide (zinc sulfate) and skin bleaching agent (calcium sulfate hydrate). Furthermore, sodium bisulfate functions only as a pH adjuster and ferrous ammonium sulfate functions only as a pesticide in cosmetics. Ingredient functions are listed in Table 1.

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2013, the following inorganic sulfates reviewed in this safety assessment are being used in cosmetic products: aluminum sulfate, ammonium sulfate, barium sulfate, calcium sulfate, copper sulfate, magnesium sulfate, manganese sulfate, potassium sulfate, sodium bisulfate, and zinc sulfate.[10] Results from a survey of ingredient use concentrations provided by the Personal Care Products Council in 2013 indicate that these ingredients are being used at concentrations up to 49% (magnesium sulfate, in bath capsules diluted for bath use). The next highest maximum use concentration reported is 37%, for barium sulfate in lipstick (leave-on product).[11]

Summarized 2013 data on frequency and concentration of use in cosmetics are presented in Table 3. Data on sodium sulfate and ammonium, potassium, and sodium persulfate, are also included in this table for comparison. These are similar ingredients previously reviewed by CIR and the data may be useful for assessing the safety of the inorganic sulfates reviewed in this assessment.

Cosmetic products containing inorganic sulfates may be applied to the skin and hair, or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

The following ingredients are used in cosmetic products that are sprayed (highest maximum use concentration = 15% [barium sulfate]): barium sulfate (up to 15%) and magnesium sulfate (up to 11%). Additionally, the following ingredients are being used in powders (highest maximum use concentration = 15.8% [barium sulfate]): aluminum sulfate (up to 0.2%) and barium sulfate (15.8%). Because these ingredients are used in aerosol/pump hair sprays or powders, they could possibly be inhaled. 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 spray.[12,13,14,15] 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]

Water soluble zinc salts (except for zinc 4-hydroxy-benzenesulfonate and zinc pyrithione) are included on the list of substances that cosmetic products marketed in the European Union must not contain, except when subject to certain restrictions.[16] The restriction for zinc sulfate and other water-soluble zinc salts is 1% (calculated as zinc).

Non-Cosmetic

The following ingredients are FDA-approved direct food additives: aluminum sulfate, ammonium sulfate, calcium sulfate, copper sulfate, ferric sulfate, ferrous sulfate, magnesium sulfate, manganese sulfate, potassium sulfate, sodium sulfate, and zinc sulfate.[17] Additionally, the following ingredients have been approved by FDA as active ingredients in over-the-counter drug products: aluminum sulfate, copper sulfate, ferrous sulfate, magnesium sulfate, and zinc sulfate.[18] Other non-cosmetic uses of inorganic sulfates are summarized below.

Aluminum Sulfate

Throughout the world, aluminum sulfate (alum) is used in municipal water treatment plants to clarify water. Treatment with aluminum salts removes aluminosilicate particles from drinking water.[19]

Barium Sulfate

Barium sulfate has been used as a gastrointestinal contrast agent in roentgenographic procedures.[20]

Barytes (the naturally occurring rock form of $BaSO_4$) has been referred to as the standard densification agent used in drilling fluids worldwide.[7]

Magnesium Sulfate

Magnesium sulfate is an anticonvulsant that is used to prevent or treat seizures in obstetric patients with preeclampsia or eclampsia, and as tocolytic agent in patients with premature labor.[21] It is also used to prevent early mortality in patients with acute myocardial infarction and, in asthmatic patients, as an adjunctive treatment for acute exacerbations of moderate to severe asthma. Magnesium sulfate has been used to inhibit premature labor in women.[22,23]

Silver Sulfate

A silver sulfate antimicrobial dressing (Mepilex® Ag Antimicrobial Soft Silicone Foam Dressing) that provides a rapid, sustained silver release is available in Sweden.[24] This dressing is indicated for low to moderately exuding wounds, such as partial thickness burns, leg ulcers, foot ulcers, and pressure ulcers.

Zinc Sulfate

Zinc salts include the following 3 pesticide active ingredients: zinc chloride, zinc oxide, and zinc sulfate monohydrate (or zinc sulfate).[25] Zinc salts are used as herbicides to control the growth of moss on structures, walkways, patios, and lawns in rainy areas, primarily in the Northwestern United States. Other non-pesticidal uses of zinc salts in the U.S include use in fertilizers, animal feed, dry cell batteries, and as galvanizers.

TOXICOKINETICS

Animal

Aluminum Sulfate and Aluminum

Aluminum kinetics after i.v. bolus administration of aluminum sulfate was studied using groups of 6 male Sprague-Dawley rats (ages not stated).[26] The animals received either 0.1 or 1.0 mg/kg of aluminum sulfate. Blood and plasma aluminum profiles were generally monoexponential. Increasing the administered dose increased the the elimination half-life from 1.20 h to 2.41 h ($p < 0.05$). A corresponding decrease in systemic clearance was observed (from 49.6 to 18.4 ml/kg·h; p

< 0.05). Values for the volume of distribution were 78.3 ± 17.2 and 58.9 ± 8.5 ml/kg for the low and high doses, respectively. At both doses, blood:plasma concentrations ranged from 0.8 to 1.0, indicative of considerable uptake/binding of the element by blood cells.

Groups of 4 male Fischer rats (ages not stated) received 0.1 mg/kg (bolus) of aluminum sulfate via the portal or systemic (ileocolic or femoral vein) route of administration.[27] Both blood and bile were serially sampled over an 8-h period post-dosing. Blood aluminum concentrations decreased in a monoexponential fashion, with half lives of 0.7 h (portal) and 1.08 h (systemic); the difference was statistically significant ($p < 0.05$). The corresponding mean systemic clearances were 48.9 and 35.1 ml/h·kg ($p < 0.05$). Following portal administration, the systemic availability was 0.66, indicating a significant "first-pass" effect. Biliary aluminum recovery (% dose) was negligible both following portal administration (0.83 ± 0.062%) and systemic administration (1.3 ± 0.22%). An immediate decrease in bile flow (~ 40%; $p < 0.05$) was observed only after portal administration of aluminum sulfate; suppression of bile flow persisted throughout the study. Liver recovery of aluminum at 8-h post-dosing was greater following portal administration (65.4 ± 4.1 %) versus systemic administration (39.4 ± 2.52%).

Barium Sulfate

Rats inhaled barium sulfate (40 mg/m^3) for up to 2 months.[28] The rats were killed at 2-week intervals after the beginning of the exposure period, and the barium content of the lungs, lymph nodes, jaw, and femur was determined. Lymph transport was negligible. The barium content of bone increased initially, then gradually decreased during treatment. After 2 weeks of exposure, the barium content of the lungs was high, but decreased rapidly and then increased considerably over the next 4 weeks of treatment.

Copper Sulfate and Zinc Sulfate

A study of the percutaneous absorption and cutaneous bioavailability of zinc and copper from zinc sulfate and copper sulfate formulations topically applied to human skin *in vitro* (Franz diffusion cell) demonstrated increased zinc and copper concentrations in whole skin and epidermis.[29]

Ferrous Ammonium Sulfate

Ferrous ammonium sulfate absorption was studied using male Sprague-Dawley rats (number not stated).[30] Some of the rats in this study were made iron-deficient by feeding them a diet that contained only 4 to 8 mg Fe/kg. The animals were dosed orally with ferrous ammonium sulfate (up to ~ 50,000 µg) for 5 days. The absorption of ferrous ammonium sulfate was greater in iron-deficient animals (up to ~ 3,000 µg iron absorbed) than in normal animals (up to ~ 500 µg iron absorbed).

Ferrous Sulfate

Twenty to 30 pregnant Sprague-Dawley rats (ages not stated) were injected i.v. with 5 µCi $^{59}FeSO_4$ and uptake in maternal, placental, and fetal tissues was evaluated at 8, 12, 15, 17, 19, 20, and 21 days of gestation.[31] Large amounts of ^{59}Fe were transferred from maternal plasma to the fetuses late in gestation. ^{59}Fe was rapidly transported across the placenta, and there was no indication of a large iron pool within it. The authors noted that the uptake of ^{59}Fe by the maternal liver and spleen is diminished during the period of rapid iron accumulation by the fetuses.

Groups of 40 young adult male Sprague-Dawley rats (5 to 6 weeks old) received ferrous sulfate at dietary levels of 35 mg Fe/kg (2.84 mg/kg/day), 70 mg Fe/kg (5.69 mg/kg/day), and 140 mg Fe/kg (11.54 mg/kg/day), respectively.[32] Untreated control animals (10 rats) received a low-iron diet (< 5 mg iron/kg diet). Twenty rats from each test group were killed after 31 days of feeding, and the remaining 20/group were killed after 61 days of feeding. It was concluded that iron, from the diet, accumulated in the liver, spleen, and kidneys in a dose-dependent manner.

Hydroxylamine Sulfate

Ten male Sprague-Dawley rats (ages not stated) were given hydroxylamine sulfate i.v. (40 mg/kg or 16.6 mg hydroxylamine base/kg) and then killed at intervals ranging from 2 minutes to 3 h.[33] At 2 minutes, blood from a pair of animals contained 0.9 and 1.6 µg base/ml. Thus, more than 99% of administered hydroxylamine sulfate was cleared from the blood almost immediately after injection. Similar blood concentrations (between 0.9 and 2.0 µg/ml) were reported for animal pairs killed at 10, 30, and 60 minutes. Plasma concentrations of the test substance were comparable to those found in the blood. When blood and plasma filtrates were hydrolyzed with acid for 2.5 h, the analytical values increased 2- to 5-fold. The

authors noted that it appeared that the blood contained an acid-labile derivative of hydroxylamine, possibly acetohydroxamic acid.

Magnesium Sulfate

In a study involving pregnant female Long-Evans rats, s.c.-injected magnesium sulfate crossed the placenta, entered the fetal blood-brain barrier, and was concentrated in the forebrain.[34]

Manganese Sulfate

Groups of 12 Crl:CD (SD)BR rats (6 weeks old) were exposed (inhalation) to manganese sulfate at concentrations of 0.03, 0.3, or 3 mg Mn/m^3 7 days per week (6 h/day) for a total of 14 exposures.[35] The target nominal particle size (mass mean aerodynamic diameter [MMAD]) was 1.5 to 2 µm, with a geometric standard deviation (δ_g) of < 2. Increased whole-body ^{54}Mn clearance rates were observed in the 3 mg Mn/m^3 exposure group. Elevated manganese concentrations in the lung, olfactory bulb, and femur were observed at ≥ 0.3 mg Mn/m^3. Elevated manganese concentrations in the striatum, testes, liver, and bile were observed at 3 mg Mn/m^3.

The influence of dietary manganese on the pharmacokinetics of inhaled manganese sulfate was studied using Crl:CD (SD)BR rats.[36] Postnatal day (PND) 10 rats were placed on either a low (2 ppm), sufficient (10 ppm) or high (100 ppm) manganese diet. At a dietary concentration of 2 ppm, reduced body weight gain, decreased liver manganese concentrations, and reduced whole-body manganese clearance rates were noted. Beginning on PND 77 ± 2, male littermates were exposed (6 h/day) to 0, 0.03 or 0.3 mg $MnSO_4/m^3$. The target nominal particle size (MMAD) was approximately 1.5 to 2 µm. Rats exposed to 0.03 mg $MnSO_4/m^3$ had elevated lung manganese concentrations when compared to controls. Rats exposed to 0.3 mg $MnSO_4/m^3$ exhibited increased striatal, lung, and bile manganese concentrations. There were no significant interactions between the concentration of $MnSO_4$ inhaled and the dietary manganese level in relation to tissue manganese concentrations. Rats exposed to 0.3 mg $MnSO_4/m^3$ also had increased ^{54}Mn clearance rates and shorter initial phase elimination half-lives when compared to control rats. The authors suggested that these results suggest that marginally manganese-deficient rats exposed to high levels of inhaled manganese compensate by increasing biliary manganese excretion. Therefore, they do not appear to be at increased risk for elevated brain manganese concentrations.

A study was performed to determine manganese body burden in maternal and fetal CD rats following pre-breeding (2 weeks), mating (up to 14 days), and gestational (from gestation day [GD] 0 through 20) exposure to $MnSO_4$ aerosol (0, 0.05, 0.5, or 1 mg Mn/m^3, 7 days/week [6 h/day]). Manganese concentrations in the lung (maternal) were increased at exposure concentrations ≥ 0.05 mg Mn/m^3 and in brain and placenta at ≥ 0.5 mg Mn/m^3. Increased fetal liver manganese concentrations were noted after *in utero* exposure to ≥ 0.5 mg Mn/m^3. Concentrations of manganese in all other fetal tissues examined were not different from air-exposed controls. The authors concluded that the placenta partially sequesters inhaled manganese to limit exposure to the fetus.

Groups of 30 male Sprague-Dawley rats of the CD (Crl:CD[SD] IGS BR) strain (ages not stated) were exposed (inhalation) to manganese sulfate (6 h/day, 5 days/week) at concentrations of 30.1 ± 6.4, 294.8 ± 66.0, or 3,220 ± 578 µg Mn/m^3 for 13 weeks.[37] A fourth group (control) was exposed to 0.3 ± 0.02 µg Mn/m^3. Based on the cascade impactor tests 95% of the particulate MMAD was below 6 µm (respirable fraction). Compared to the control group, manganese concentrations in the 3,000 mg/m^3 group were significantly increased in blood, kidney, lung, testis, and in all brain regions analyzed. Significant differences were also observed in the 300 $µg/m^3$ exposure group. Lung Mn concentrations were significantly increased in all 3 test groups. Increased kidney and testis Mn concentrations were observed at the highest concentration of exposure.

For groups of 4 to 6 male Rhesus monkeys (17 to 22 months old) exposed to $MnSO_4$ at a concentration of ≥ 0.06 mg M/m^3, increased manganese concentrations (compared to air-exposed controls) were observed in the globus pallidus, putamen, olfactory epithelium, olfactory bulb, and cerebellum.[38] The authors noted that high-dose exposure to manganese is associated with Parkinsonism. However, this neurotoxicity endpoint was not evaluated in this study.

Zinc Sulfate

In a study involving 6 male rabbits (ages and strain not stated), zinc sulfate was injected into the jugular vein at a dose of 3.3 µCi of [^{65}Zn]zinc sulfate/kg.[39] Blood samples were obtained at intervals up to 6 h post-injection. The animals were killed at 6.5 h post-injection and the following organs or tissue fragments were removed: liver, kidneys, whole skin with fur, and small intestine. The distribution half-life of zinc sulfate was 0.134 h^{-1}. Urinary elimination did not exceed 1%

of the administered dose. Distribution to the liver accounted for 17% of the total radioactivity, which represented the highest organ-specific concentration reported.

Zinc sulfate was administered intraperitoneally (1.5 mg Zn/ml) to rats once per day for 3 consecutive days.[40] The animals were subsequently killed at intervals, and biochemical and histological examinations of organs were performed. At day 1 after the final dose, the amount of zinc in each tissue was described as high. Most of the zinc was eliminated from the tissues by day 14 after the last injection; however, the level of zinc in the tissues remained significantly high when compared to controls. By 3 weeks, the zinc injected into animals was completely eliminated from the tissues, and zinc tissue levels were described as normal. Additional study results are included in the Repeated Dose Toxicity section of this report.

Human

Ferrous Sulfate

The bioavailability of iron in 5 ferrous sulfate preparations was evaluated using 10 healthy male volunteers.[41] The preparations administered included an oral solution, 2 types of film-coated tablets, and 2 types of enteric-coated tablets. Blood samples were obtained hourly on the day before each treatment and on the day of treatment to assess baseline serum iron concentrations. Spectrophotometry was used to measure serum iron concentrations. To determine relative bioavailability, the mean area under the curve (AUC; mean serum iron concentration [µMol/L0 vs. time [hours]) for each treatment was compared with the mean AUC for the oral solution. The AUC, the maximum concentraioin, and the time to achieve the maximum concentration were compared by analysis of variance. The enteric-coated preparations resulted in AUCs less than 30% of the AUC for the oral solution. The 2 film-coated products resulted in AUCs that were essentially equivalent to that of the oral solution. It was concluded that the bioavailability of iron in the enteric-coated preparations was low, relative to that of the film-coated products and the oral solution.

Magnesium Sulfate

The concentration in serum and the cumulative renal excretions of magnesium were measured in 3 eclamptic and 7 severely eclamptic patients given an initial therapeutic dose of magnesium sulfate i.v. (3 g) and intramuscularly (10 g).[42] The highest single plasma level was 6.0 mEq per liter (7.2 mg/100 ml) at 60 minutes post-treatment in an oliguric eclamptic woman. The average peak level at 60 minutes was 4.5 mEq. per liter. At the end of 4 h, the cumulative renal excretions ranged from 38% to 53% of the injected dose.

Magnesium sulfate (U.S.P., 13.9 g) was administered orally to 7 healthy men (ages not stated) in 4 equal hourly increments.[43] Urinary excretion (corrected for baseline excretion rate) was described as an amount of inorganic sulfate equivalent to 30.2 ± 17.2% of the administered dose during the first 24 h. Excretion during the subsequent 48 h was negligible.

Zinc Sulfate

A study was performed to evaluate the effect of oral zinc sulfate on psoriasis (9 women, 10 men; average age = 32.8 years) and to monitor the absorption, excretion, and cutaneous distribution of therapeutic zinc sulfate in psoriatic patients (2 women, 2 men).[44] Using a double-blind cross-over design, 19 outpatients with psoriasis received 1 zinc sulfate tablet (220 mg) or 1 placebo tablet (220 mg sucrose) three times daily after meals for 2 months. They were then switched to the opposite tablet for an additional 2 months. Pharmacokinetic parameters were evaluated in 4 of these patients. The majority of the zinc sulfate (dietary and therapeutic) ingested was excreted in the feces during the baseline zinc sulfate treatment periods. After therapy was discontinued, rapid excretion of zinc in the urine and feces continued. Upon study completion, 75% of ingested zinc sulfate had been excreted, indicating a lack of prolonged storage of therapeutic zinc. The mean serum zinc concentration of 12 control subjects (0.94 ± 0.11 ppm) exceeded the mean value for the 4 psoriatic patients (0.71 ± 0.29 ppm).

TOXICOLOGY

Acute Toxicity

Inhalation

Animal

Ammonium Sulfate, Copper Sulfate, and Sodium Sulfate

Random-bred guinea pigs (number and ages not stated) were exposed for 1 h to aerosols of the following sulfate salts: ammonium sulfate, ammonium bisulfate, copper sulfate, and sodium sulfate.[45] The particle size range was limited to 0.1 to 0.8 µm (mass median diameter). Except for sodium sulfate, all of the sulfates caused a slight increase in pulmonary flow resistance and a slight decrease in pulmonary compliance. The order of irritant potency was as follows: ammonium sulfate > ammonium bisulfate > cupric sulfate. The degree of response increased with decreasing particle size.

Aluminum Sulfate and Copper Sulfate

Groups of male and female CD_1 mice were exposed for 3 hours to the following concentrations of copper sulfate (as mg SO_4/m^3; [calculated mg metal/m^3 in brackets]): 2.53 [3.3] (23 males, 24 females), 0.93 [1.21] (100 males, 100 females), and 0.43 [0.56] (24 males, 23 females).[46] Similarly, groups of mice (same strain) were exposed to aluminum sulfate: 2.71 [0.51] (56 males, 54 females), 2.31 [0.43] (61 males, 62 females), and 1.84 [0.34] (24 males, 24 females). Groups of untreated mice served as controls. The MMAD of particles was 0.75 µm. Exposure to any of the test concentrations significantly increased the mortality rate (males and females). The magnitude of the response appeared to be directly linearly related to the exposure concentration.

Exposure to the high concentration of aluminum sulfate significantly increased the mortality rate. Data for the intermediate and low concentrations of aluminum sulfate were quite similar, and the between-group differences were not significant. There was a significant ($p < 0.05$) concentration-response relationship and the overall between-sex difference ($p < 0.05$) in mortality was also significant. Microscopic examination of the trachea after exposure to aluminum sulfate or copper sulfate revealed epithelia similar to those of control mice. Exfoliation of differentiated surface epithelial cells from the tracheal mucosa was a common observation in mice exposed to aluminum sulfate or copper sulfate and in control mice. However, the percentage of desquamation was greater in mice exposed to aluminum sulfate or copper sulfate.[46]

Ammonium Sulfate

The acute inhalation toxicity of ammonium sulfate was evaluated using 10 guinea pigs. Thirteen guinea pigs served as controls. Test animals were exposed to 49.6 mg/m^3 for 1 h.[47] A significant reduction in histamine content of the trachea (µg/g) was observed in the exposed animals. However, none of the animals developed labored breathing, and there were no sifgnificant differences between test and control groups in respiratory frequency, total respiratory system resistance, pseudo-flow rates, or histamine content of the lung.

Copper Sulfate

The metabolism and pulmonary toxicity of intratracheally instilled cupric sulfate were studied using groups of 3 male Wistar rats (10 weeks old).[48] Groups of 3 rats received a single intrathecal instillation of copper sulfate solution (0.4 ml) at a dose of 20 µg copper sulfate/rat, and were killed at 0.5, 3, or 12 h or 1, 2, 3, and 7 days post- instillation. Other groups of 3 rats received copper sulfate solution (0.4 ml) at doses of 2.5, 5.0, 10.0, 30.0, and 50.0 µg copper sulfate/rat. The animals were killed at 2 days post-instillation. Intrathecally-instilled copper sulfate was cleared rapidly from the lung (half-time = 7.5 h). Copper-binding metallothionein (MT) was induced in a dose-dependent manner in the lung tissue after copper sulfate instillation. Lactate dehydrogenase (LDH; an index of cell lysis) and β-glucuronidase (an index of lysosomal secretion) activites peaked at 12 h to 2 days post-instillation, and returned to control levels after 7 days. A dose of 5 µg copper sulfate/rat was sufficient to produce acute inflammatory responses in the rat lung.

Zinc Sulfate

Groups of 48 CD_2F_1 female mice (6 to 8 weeks old) were exposed to zinc sulfate aerosol (≥ 1.2 mg/m^3) or filtered air for 3 h.[49] Microscopic examination indicated that approximately 90% of the airborne particles was ≤ 3 µm in diameter. The estimated MMAD of the evaporated sulfate particles was 0.63 µm. Exposure was followed by respiratory challenge with airborne *Streptococcus pyogenes*, and this exposure sequence resulted in significant excess mortality and reduced survival time in exposed mice. The estimated concentration of zinc sulfate that caused 20% excess mortality (ED_{20}) was 1.45 mg/m^3. The authors noted that the increased susceptibility to respiratory infection appeared to have been, in part, related to the impairment of alveolar macrophage activity by the metallic cation. Throughout the replicate experiments, none of the mice exposed only to zinc sulfate died.

Human

Ammonium Sulfate and Sodium Bisulfate

Sixteen normal subjects (mean age = 27 years) and 17 asthmatic subjects (mean age = 26 years) inhaled ammonium sulfate or sodium bisulfate aerosols.[50] The average MMAD of the particles was ≈ 1.0 µm and the concentrations of exposure were 0.1 and 1.0 mg/m^3. The subjects also inhaled a control NaCl aerosol. By double-blind randomization, all subjects breathed each aerosol for 16 minutes. Additionally, to determine whether sulfate inhalation caused increased reactivity to a known bronchoconstrictor, all of the subjects inhaled carbachol after the 16-minute exposure. When compared to the NaCl control (1 mg/m^3), the sulfates caused significant reduction in specific airway conductance (SG_{aw}) and flow rates in asthmatics. The 2 most sensitive asthmatics demonstrated changes, even at the lower exposure concentration (0.1 mg/m^3). In normal subjects, neither of the 2 sulfates caused significant decreases in SG_{aw} at a concentration of 1 mg/m^3 and the bronchoconstrictor action of cabachol was potentiated by the sulfate aerosols, more or less in relation to their acidity.

Ammonium Sulfate

Six healthy subjects (mean age = 37.2 years) were exposed to a nominal (100 µg/m^3) concentration of ammonium sulfate (MMAD = 0.3 µm) for 2 h.[51] There was little or no evidence of adverse health effects. Neither significant functional changes nor consistent changes in the symptom score were found at low humidity. At high humidity, there was statistically-significant variation in forced expiratory measures, but the changes were small in magnitude.

Ferric Sulfate

The effect of exposure on pulmonary function was evaluated using 20 normal (18 to 55 years old) and 18 asthmatic (20 to 53 years old) volunteers.[52] The subjects were exposed to ferric sulfate aerosol at a nominal concentration of 75 µg/m^3 (equivalent to 20 µg iron /m^3). The concentration and particle size distribution (2 µm mass median aerodynamic diameter) were selected to simulate worst case ambient conditions. A double-blind protocol was followed, whereby each subject was exposed on 2 days (separated by a 3-week period). The subjects were exposed to clean air (sham) on one day and to ferric sulfate aerosol on the other day. The order of exposure was selected randomly. On the average, the 2 groups of subjects did not exhibit significant pre- to post-exposure changes in total respiratory system resistance, forced expiratory flow/volume performance, and single breath nitrogen washout parameters. None of the subjects experienced more than slight changes in symptoms during exposure.

Oral

Aluminum Sulfate

Aluminum sulfate was administered orally (intragastrically) to groups of 10 male and female Sprague-Dawley rats and to groups of 10 Swiss mice.[53] Ages were not stated. Dosing was followed by a 14-day observation period. The Oral LD_{50} for mice and rats was > 9,000 mg/kg.

Ammonium Sulfate

The acute oral toxicity of ammonium sulfate was evaluated using laboratory rats (ages and strain not stated).[54] Ammonium sulfate (37% solution in distilled water) was administered using a stomach tube. The LD_{50} was between 3,000 and 4,000 mg/kg body weight. Additional study details were not included. In another study, the acute oral toxicity of ammonium sulfate (dissolved in water) was evaluated using groups of 6 ddy mice (5 to 6 weeks old; 3 males, 3 females/group).[55] A mean oral LD_{50} value of 3,040 mg/kg body weight (range: 2,670 to 3,440 mg/kg body weight) was reported.

Three Japanese white rabbits (ages not stated) received a total dose of 1,500 mg/kg ammonium sulfate dissolved in saline solution (10 to 15 ml of volume/rabbit) by gastric probe.[56] The 2 control rabbits received 10 to 15 ml of saline. After death, organs were removed and examined microscopically. The following results were observed: mydriasis; slight irregular respiratory rhythms; local convulsions in the face and extremities, which progressed to the entire body; decreased heart rate; and slow and suppressive patterns in the EEG. By 60 to 70 minutes post-ingestion, all rabbits became breathless and experienced cardiac arrest.

Barium Sulfate

Six groups of 16 to 26 young male CBL-Wistar albino rats (ages not stated) received the following 6 total doses of barium sulfate (150% w/v suspension), respectively, by intragastric cannula: 188, 225, 263, 300, 338, and 375 g/kg.[57] For each total dose, 40% was given initially, followed by 35% of the dose 3 h later, and 25% 4 h later. Fifty control rats were dosed with distilled water. Fifty experimental animals died from stomach rupture, and the mean LD_{50} was 307 ± 29 g/kg. Stomach rupture appeared to have been due, in part, to failure of the animal to pass barium sulfate along the gastrointestinal tract. In 90% of the animals, hemorrhagic areas were found in the gastric mucosa, mainly on the anterior and posterior surfaces. The adrenal glands were enlarged, the liver was small, and the stretched abdominal muscle had a watery consistency.

Copper Sulfate

The acute oral toxicity of copper sulfate (in deionized water) was evaluated using a group of 5 Swiss male albino mice and a group of 5 Wistar albino rats.[58] The ages of animals tested and details relating to the test procedure were not stated. Acute oral LD_{50}s of 369 mg/kg (mice) and 794 mg/kg (rats) were reported.

Ferrous Sulfate

An acute oral toxicity study was performed using 40 male albino Swiss-Webster mice, 10 female albino Swiss-Webster mice, 10 female BDF-1 mice, 10 C-57 female mice, and 24 male and female Harlan-Wistar rats.[59] The ages of animals tested were not stated. The mice were dosed intragastrically (i.g.) and then observed for 7 days. An observation period for rats after i.g. dosing was not stated. Mean LD_{50} values of 1,025 mg/kg body weight (male Swiss-Webster mice) and 2,625 mg/kg body weight (Harlan-Wistar rats) were reported. For female mice of the Swiss-Webster, BDF-1, and C-5 strains. Values for % mortality were 70%, 70%, and 90%, respectively.

Ferrous sulfate was administered to male Sprague-Dawley rats (ages not stated) at the following doses in distilled water: 100, 200, 300, 400, or 500 mg iron/kg body weight.[60] Doses ≤ 300 mg/kg were administered to groups of 3 rats. Doses of 400 mg/kg and 500 mg/kg were administered to 6 rats and 55 rats, respectively. Ferrous sulfate was toxic to rats at doses of 250 and 500 mg iron/kg body weight, and the higher dose resulted in 70% mortality within 12 h after dosing. Periportal or midzonal liver cell necrosis was observed in most of the animals. At the lower dose, most of the animals survived and few developed hepatic necrosis. Within 1 h after iron ingestion, the rats developed peripheral vasoconstriction and diarrhea, and had moderate decreases in blood pressure. Electron microscopy revealed parenchymal cells, irreversibly injured by iron, which became swollen prior to necrosis. Also, mitochondrial changes consisting of swelling, contraction, and development of particulate and amorphous densities in the matrix space were observed. The authors suggested that iron caused both systemic toxicity and direct liver cell toxicity.

The following doses of ferrous sulfate were administered by gavage to groups of 8 young male Sprague-Dawley rats (ages not stated): 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 g/kg.[61] The negative control group (0 g/kg ferrous sulfate) also consisted of 8 rats. An LD_{50} of 1.1g Fe/kg was reported. All control animals survived.

The acute oral toxicity of ferrous sulfate (administered by stomach tube) was evaluated using Sprague-Dawley rats (6 males, 6 females; ages not stated).[62] An LD_{50} of 255 mg Fe/kg body weight was reported.

Hydroxylamine Sulfate

An oral LD_{50} of 545 mg/kg (in rats) has been reported for hydroxylamine sulfate.[63]

Manganese Sulfate

The acute oral toxicity of manganese sulfate (in deionized water) was evaluated using a group of 5 Swiss male albino mice and a group of 5 Wistar albino rats.[58] The ages of animals tested and details relating to the test procedure were not stated. Acute oral LD_{50}s of 2,330 mg/kg (mice) and 2,150 mg/kg (rats) were reported.

Zinc Sulfate

The acute oral toxicity of zinc sulfate (in deionized water) was evaluated using a group of 5 Swiss male albino mice and a group of 5 Wistar albino rats.[58] The ages of animals tested and details relating to the test procedure were not stated. Acute oral LD_{50}s of 422 mg/kg (mice) and 1,710 mg/kg (rats) were reported.

Dermal

Hydroxylamine Sulfate

The acute dermal toxicity of hydroxylamine sulfate was evaluated using groups of 10 female New Zealand albino rabbits and groups of 10 Fischer-344 rats (clipped free of hair).[64] The test substance was applied to rabbits topically (24 h under occlusion [plastic cover]) at doses of 0.001, 0.01, 0.1, and 0.5 g/kg. The test substance was also applied topically (24 h under occlusion [gauze]) to rabbits at doses of 0.1, 0.5, and 1.0 g/kg. Rats received topical applications (24 h under occlusion [plastic cover]) of the test substance at doses of 0.01, 0.1, and 0.5 g/kg. The test substance was covered with either a 4" x 4" porous gauze patch opened to cover a 4" x 8" area of skin or a plastic cover. Distilled water served as the negative control and phenhydrazine (PHZ, hemolytic compound) served as the positive control. Hydroxylamine sulfate induced hematotoxic effects consisting of methemoglobin formation, anemia, and reticulocytosis. Both hydroxylamine sulfate and PHZ were more toxic in the rabbit than in the rat, though both chemicals produced similar hematological effects at equivalent doses within the same species. Hydroxylamine sulfate was more toxic when administered under plastic than under gauze. Additionally hydroxylamine sulfate was lethal to the rabbit, but did not induce mortality in rats. Following exposure to 0.5 g/kg and 0.1 g/kg hydroxylamine sulfate (under plastic cover), 90% and 20% of the rabbits died, respectively. There were no mortalities in rabbits exposed to 1.0 g/kg hydroxylamine sulfate (under gauze).

Principal findings at necropsy included a high incidence of enlarged and darkened spleens in animals exposed to hydroxylamine sulfate or PHZ, regardless of the dose or route of exposure. Gross effects on the liver were minimal to absent. At 0.5 g/kg hydroxylamine sulfate, relative spleen weights were statistically greater when compared to negative control rats. No effect on weight of the spleen was evident at lower doses of hydroxylamine sulfate. Liver weight was not affected by treatment.[64]

Intraperitoneal

Aluminum Sulfate

Aluminum sulfate was administered i.p. to 20 male and female Sprague-Dawley rats and to 20 male and female Swiss mice.[53] Ages were not stated. Dosing was followed by a 14-day observation period. The Oral LD_{50}'s for mice and rats were 997 mg/kg and 61 mg/kg, respectively.

Ammonium Sulfate

In a study involving 19 Wistar rats of either sex (ages not stated), a 6% solution of ammonium sulfate was injected i.p. at a dose of 1.0 ml/100 g body weight.[65] The animals were killed at various intervals (range: 2.5 to 90 minutes post-dosing) after injection. Control rats (9 animals) were injected i.v. with colloidal carbon. The lungs of rats killed 15 minutes or longer after injection were heavier when compared to control rats; 5 of 7 rats killed at 15 minutes had abundant frothy edema in the major airways. When rats killed within 10 minutes post-injection were compared to controls, histological findings were very similar and included carbon "plugging" of capillaries, arterioles, and venules; endothelial bleb formation (no carbon in blebs) was the most common lesion. Additionally, when these 2 groups were compared, the same was true for gross examinations, whereby all organs showed blue-gray discoloration by carbon.

Copper Sulfate

The acute i.p. toxicity of copper sulfate (in deionized water) was evaluated using a group of 5 Swiss male albino mice and a group of 5 Wistar albino rats.[58] The ages of animals tested and details relating to the test procedure were not stated. Acute i.p. LD_{50}s of 31.6 mg/kg (mice) and 20 mg/kg (rats) were reported.

Ferrous Sulfate

An acute toxicity study was performed using 105 male albino Swiss-Webster mice (ages not stated).[59] The mice were dosed i.p. and then observed for 7 days. A mean LD_{50} value of 137 mg/kg body weight was reported.

Manganese Sulfate

The acute i.p. toxicity of manganese sulfate (in deionized water) was evaluated using a group of 5 Swiss male albino mice and a group of 5 Wistar albino rats.[58] The ages of animals tested and details relating to the test procedure were not stated. Acute oral LD_{50}s of 147 mg/kg (mice) and 92.6 mg/kg (rats) were reported.

Zinc Sulfate

The acute i.p. toxicity of magnesium sulfate (in deionized water) was evaluated using a group of 5 Swiss male albino mice and a group of 5 Wistar albino rats.[58] The ages of animals tested and details relating to the test procedure were not stated. Acute oral LD_{50}s of 108 mg/kg (mice) and 196 mg/kg (rats) were reported.

Intravenous

Copper Sulfate

The acute i.v. toxicity of copper sulfate (in deionized water) was evaluated using a group of 5 Swiss male albino mice and a group of 5 Wistar albino rats.[58] The ages of animals tested and details relating to the test procedure were not stated. Acute oral LD_{50}s of 23.3 mg/kg (mice) and 48.9 mg/kg (rats) were reported.

Ferrous Sulfate

An acute toxicity study was performed using 55 male albino Swiss-Webster mice and 16 Mongrel dogs.[59] The mice were dosed intravenously (i.v.) and then observed for 7 days. An observation period for dogs after i.v. dosing was not stated. Mean LD_{50} values of 112 mg/kg body weight (mice) and 79 mg/kg body weight (dogs) were reported.

Magnesium Sulfate

Magnesium sulfate was administered i.v. to groups of Crj:CD(SD) rats (males and females, 6 weeks old) at doses of 90, 130, 200, 300, and 450 mg/kg.[66] Deaths occurred at doses $\geq$ 200 mg/kg, and the LD_{50} values were 206 mg/kg and 174 mg/kg for males and females, respectively. Tonic convulsions, abnormal gait, and tachypnea were observed in surviving animals dosed with $\geq$130 mg/kg. These signs were transient and the animals had returned to normal by 15 minutes post-dosing. There were no treatment-related changes in body weight or gross pathology in any dose group. In a second experiment, magnesium sulfate was infused into groups of female beagle dogs (6 months old) at doses of 75, 300, and 1,200 mg/kg (12.5, 50, and 200 mg/kg/h) for 6 h. Deaths were not observed in any of the dose groups. The following signs were observed in the 1,200 mg/kg dose group: vomiting, decreased spontaneous movement, staggering gait, prone position, and flush of the conjunctiva and ear auricles. These signs were transient and the animals had returned to normal by 1 h post-dosing. There were no treatment-related changes in body weight, food consumption, or gross pathology.

Manganese Sulfate

The acute i.v. toxicity of manganese sulfate (in deionized water) was evaluated using a group of 5 Swiss male albino mice and a group of 5 Wistar albino rats.[58] The ages of animals tested and details relating to the test procedure were not stated. An LD_0 of 31.6 was reported for mice dosed with the test material. An acute oral LD_{50} of 44.1 mg/kg was reported for rats.

Zinc Sulfate

The acute i.v. toxicity of zinc sulfate (in deionized water) was evaluated using a group of 5 Swiss male albino mice and a group of 5 Wistar albino rats.[58] The ages of animals tested and details relating to the test procedure were not stated. Acute i.v. LD_{50}s of 23.3 mg/kg (mice) and 69.9 mg/kg (rats) were reported.

Subcutaneous

Zinc Sulfate

The following doses of zinc sulfate were injected s.c. into groups of 7 male ddy mice (6 weeks old): 0.1, 0.5, 1.0, 5.0, 12.5, 25, or 50 mg Zn/kg.[67] The aim of the study was to determine whether dosing with zinc induced pancreatic injury in mice. The control group was injected with saline. Acute cell damage, such as fibrosis and necrosis, was observed in pancreatic exocrine cells, but not in cells of the islets of Langerhans. Histochemical analysis of a pancreas at 24 h post-injection revealed the following: zymogen degranulation, vacuolation, fibrosis, and necrosis. Though there were abundant neutrophils, changes in the islets of Langerhans were not observed.

Barium Sulfate

The effect of intrauterine, s.c. injection of sterile barium sulfate into rabbit fetuses was evaluated. Healthy pregnant rats (number not stated) of gestational periods ranging from 21 to 26 days were used.[68] Two fetuses were selected at random. The dorsum of the fetus was delivered and a sterile aqueous suspension of micro-opaque barium sulfate was injected into the subcutaneous tissue of each dorsolateral surface. The hysterotomy wounds were then closed. The pregnancy was allowed to progress and fetuses were removed at varying postoperative intervals for morphological studies. Similar s.c. injections were performed in newborn rabbits. The rabbits were killed at regular intervals and the morphology of the wounds was studied. Subpannicular injection of sterile barium sulfate in newborn rabbits produced an acute inflammatory response that was observed clearly at 24 h and well-established by 48 h. The process of repair had begun by day 4, whereby the appearance of proliferating capillaries and fibroblasts was observed. Both vascular and cellular components of the acute inflammatory response were more prominent in rabbit fetuses, and appeared earlier (well-developed within 24 h) when compared to newborn rabbits. The process of repair also began earlier; the proliferation of capillaries and fibroblasts was prominent by 48 h. By day 4, the lesion was compact, less cellular, and relatively avascular.

Intratracheal

Barium Sulfate

Following endotracheal administration of barium sulfate (Barosperse™) into 220 Sprague-Dawley rats and 3 dogs, radiographic and histologic studies were performed.[69] Barosperse™ (0.25 ml), under fluoroscopic control, was injected endotracheally into rats, and a 1.75 ml/kg dose of Barosperse™ was administered endotracheally to dogs. The 0.25 ml dose was selected based on the results of a pilot study in which all 30 rats died after intratracheal administration of 0.5 ml Barosperse™. After dosing with Barosperse™, all of the rats and 2 dogs were radiographed for a total of 9 months. Barosperse™ was virtually cleared from the trachea and stem bronchi in 30 minutes. Barosperse™ cleared more slowly from the lungs of 3 dogs, when compared to these data on rats. Diffuse, but patchy, acinar filling resulted in a slow decrease in barium aggregates from the lungs of rats and dogs over a 9-month period. New infiltrates were found in 15% of the rats on serial follow-up. Two of the 3 dogs dosed with Barosperse™ died during the first 24 h; both dogs had diffuse alveolarization of the contrast agent.

Barium in the alveoli and a mild acute inflammatory response were observed in rats at microscopic examination. A few hours after instillation, macrophages were observed in the alveoli and subsequently became evident in thickened septa. Focal alveolar wall granulomata were also observed. After 3 months, focal areas of actue and chronic inflammatory cells with focal fibrosis persisted, and areas of atelectasis and emphysema were also observed. At 6 months, aggregates of macrophages containing barium were the main finding. At 9 months, nodules of phagocytic cells in bronchioles and perivascular structures persisted. At 9 months after Barosperse™ instillation into the lungs, the same histological findings were observed in 3 dogs.[69]

Repeated Dose Toxicity

Animal

Inhalation

Ammonium Persulfate

The repeated dose toxicity of ammonium persulfate was evaluated in Sprague-Dawley rats (20/sex/group).[70] Whole-body exposures were performed 5 days/week (6 h/day) for 13 weeks. Respirable dust aerosol concentrations during group exposure were 5.0, 10.3, or 25 mg/m^3. The control group was exposed to air only. At the end of exposure, 10 rats/sex/group were killed and necropsied. Five rats/sex/group were retained for 6- and 13-week recovery periods. There were no exposure-related deaths during the study. Both males and females in the 25 mg/m^3 exposure group and rats in the 10.3 mg/m^3 exposure group experienced rales and increased respiration. However, the frequency of these signs decreased to zero during the first few weeks of the recovery period. When compared to control animals, body weights of males and females in the 25 mg/m^3 exposure group were depressed significantly during most of the exposure period, but were similar to control values by the end of the recovery period. Also, in the 25 mg/m^3 exposure group, lung weights were elevated after 13 weeks of exposure, but values were similar to controls at 6 weeks post-exposure. Microscopic examination revealed irritation of the trachea and bronchi/bronchioles in the 25 mg/m^3 exposure group, and these lesions had recovered by 6 weeks post-exposure. The authors concluded that the no-observed-adverse-effect level (NOAEL) for ammonium persulfate was 10.3 mg/m^3 and that the no-observed-effect level (NOEL) for ammonium persulfate was 5.0 mg/m^3.

Ammonium Sulfate

Groups of 10 adult male rats were exposed to ~300 mg/m^3 ammonium sulfate 8 h per day for 1, 3, 7, and 14 days.[71] The particle size of the ammonium sulfate aerosol ranged from 1 to 2 µm in diameter. None of the animals died, and there were no detectable toxicological effects.

The following groups of young adult Sprague-Dawley rats were subjected to various durations of exposure to ammonium sulfate (0.5 mg/m^3): 4 months (15 test + 15 air-exposed controls), 8 months (15 test + 13 air-exposed controls), and 8 months (14 test + 13 air-exposed controls).[72] The animals were exposed 5 days per week (5 h/day). The overall mean values and standard deviations of the 190 daily average concentrations were 0.496 ± 0.027 mg/m^3. The 40-week mean particle sized distribution was 0.44 ± 0.04 µm (MMAD). No significant pathological changes were observed in the nasal cavities of animals in any group. The examination of lungs by light microscopy revealed hemisiderosis, greatest in animals exposed for 8 months (27% incidence). This incidence was significantly greater when compared to controls ($P < 0.05$). Significant interstitial fibrosis ($P < 0.05$) was also observed. When compared to air-exposed rats at 4 months, exposure to ammonium sulfate induced bronchiolar cell hyperplasia ($P < 0.01$). Counts of nonciliated epithelial cells (NEC) per standard area of terminal respiratory bronchioles were significantly higher at 4 months ($P < 0.01$) or 8 months ($P < 0.05$). There were no significant treatment-related effects on vital capacity, total lung capacity, time constant, or CO diffusion capacity.

Barium Sulfate

Groups of male Wistar rats were exposed (whole-body) 5 days per week (7h/day) to barium sulfate dust.[73] At each of 6 time points (not specified), 12 rats were drawn, 6 for bronchoalveolar lavage (BAL) and 6 for dust burden measurements. Animals destined for bronchoalveolar lavage studies were killed 18 h after completion of the final day of exposure for that time point. Considering that dust deposited higher in the respiratory tract would have time to clear, animals used for lung and dust burden analyses were killed 66 h after the end of exposure. Three age-matched sham-exposed animals were used as controls at each time point for each test condition in the lavage studies.

The results of BAL fluid analyses are as follows: The time course of neutrophil recruitment during exposure to barium sulfate resembled that of lymph node burden. Barium sulfate dust produced a low degree of inflammation at the last 3 time points of the higher (75 mg/m^3) exposure concentration. The mean numbers of alveolar macrophages did not change significantly when compared to the background level in control animals. Animals exposed to barium sulfate dust had significantly higher numbers of lymphocytes in BAL fluid when compared to controls.[73]

Histological sections from animals killed at timepoint 6 indicated that inhalationof barium sulfate elicited accumulation of pulmonary macrophages around the dust deposition sites. The highest concentrations of macrophages with phagocytosed dust were at the bifurcations of the terminal airways and bronchioles. In some cases, there was an accumulation of inflammatory cells, including fibroblasts in the interstitium. Some macrophages with their dust burdens had become interstitialized as well, with the lesions becoming microgranulomas. In most cases where centriacinar macrophage aggregations were found, the walls of surrounding alveoli appeared thickened, mainly due to the rounding of epithelial cells, indicative of Type II cell hyperplasia. Barium sulfate did not show significant fibrogenic activity in this study.[73]

Three groups of 30 male Sprague-Dawley rats of the CD (Crl:CD[SD] IGS BR) strain were exposed (inhalation) to manganese sulfate (6 h/day, 5 days/week) at concentrations of 30.1 ± 6.4, 294.8 ± 66.0, and 3,220 ± 578 µg Mn/m^3 for 13 weeks.[37] A fourth group (control) was exposed to 0.3 ± 0.02 µg Mn/m^3. Based on the cascade impactor test results, 95% of the particulate MMAD was below 6 µm (respirable fraction).

Calcium Sulfate

Male F344 rats (n = 36 rats) were exposed, nose-only, to 100 mg/m^3 calcium sulfate 5 days per week (6 h/day) for 3 weeks. A group of 36 rats served as the control group.[74] In both groups, 18 animals were killed immediately after the end of exposure and the remaining animals were killed 3 weeks later. The lungs were removed and prepared for microscopic examination or lavaged (bronchoalveolar lavage [BAL]). Based on the number of alveolar macrophages, the authors stated that there was no inflammatory response. There were no exposure-related changes in protein concentration or γ-GT activity in in the lavage fluid supernatant of animals killed immediately after the end of exposure. Following 3 weeks of recovery, non-protein sulfhydryl (NPSH) concentrations were increased in the lavage supernatant, which may have resulted from a response to protect the lungs from oxidant damage.

Copper Sulfate

The respiratory toxicity of copper sulfate was studied using 2 groups of 3 to 4 male Wistar rats (ages not stated). In the test group, copper sulfate (330 g/l spray [3 dosages]; spray volume = 0.5l) was sprayed for daily periods of 1 h in a self-contained chamber for up to 10 days.[75] The remaining group served as the untreated control. Compared to the control group, higher levels of copper were measured in all tissues examined in the exposed rats; although, only liver and plasma levels were statistically significantly elevated. Liver copper increased dramatically to levels up to 280 ppm. Levels of copper in the lung were described as slightly increased. The distribution of copper in the soluble fractions of the liver and kidney indicated copper accumulation in a low-molecular-weight protein, which was probably metallothionein. The lungs were not evaluated for toxic effects in this study.

Magnesium Sulfate

Male Wistar rats were exposed (inhalation exposure) to 2 types of magnesium sulfate whiskers 5 days per week (6 h/day) for 4 weeks or for 1 year.[76] Magnesium sulfate whisker is a manmade mineral fiber that is synthesized from magnesium sulfate and magnesium hydroxide by hydrothermal reaction at 100˚C to 300˚C. In the 4-week study, short whisker (mean diameter = 1.5 µm) was tested at a mean concentration of 2.3 mg/m^3 and long whisker (mean diameter = 1.8 µm) was tested at a mean concentration of 4.0 mg/m^3. Few whiskers were detected in rat lungs, even at day 1 post-exposure. This finding suggested that magnesium sulfate whiskers are dissolved and eliminated rapidly from the lungs. Histopathological examination indicated that the lung tumor incidence was not significantly different from that of control rats.

Manganese Sulfate

A study was performed to characterize the nasal toxicity of manganese sulfate ($MnSO_4 \cdot H_2O$) in young adult male rats (groups of 8) after exposure to the test substance at a target concentration of 0.01, 0.1, or 0.5 mg Mn/m^3.[77] The overall average concentrations for the manganese sulfate atmospheres were 0.01 ± 0.0001, 0.098 ± 0.009, and 0.478 ± 0.042 mg Mn/m^3. The corresponding geometric MMADs were 1.85 µm, 1.92 µm, and 2.03 µm, respectively. Control rats were exposed to air for same period of time. All animals were exposed 5 days per week (6 h/day) for up to 13 weeks (at least 65 exposure days). Nasal pathology was assessed immediately after the end of exposure and 45 days later. Elevated end-of-exposure olfactory bulb, striatum, and cerebellum manganese concentrations were noted in all groups exposed to the test substance. Exposure to the highest concentration was associated with reversible inflammation within the nasal respiratory epithelium, but the olfactory epithelium was unaffected by manganese inhalation.

Young male rhesus monkeys were exposed to manganese sulfate 5 days/week (6 h/day) for up to 65 days.[78] One cohort of monkeys (n = 4 to 6 animals/exposure concentration) was exposed to air or manganese sulfate at 0.06, 0.3, or 15 mg Mn/m^3 for 65 days. Another 8 animals were exposed to manganese sulfate at 1.5 mg Mn/m^3 for 65 days, and then held for 45 or 90 days before evaluation. A second cohort (n = 4 monkeys per time point) was exposed to manganese sulfate at 1.5 mg Mn/m^3 and then evaluated after 15 or 33 days of exposure. The highest exposure concentration was associated with increased lung manganese concentrations and small airway inflammatory changes in the absence of clinical signs. Exposure concentrations of ≤ 0.3 mg Mn/m^3 were not associated with pulmonary pathology.

Three groups of 30 male Sprague-Dawley rats of the CD (Crl:CD[SD] IGS BR) strain (ages not stated) were exposed (inhalation) to manganese sulfate (6 h/day, 5 days/week) at 30.1 ± 6.4, 294.8 ± 66.0, or 3,220 ± 578 µg Mn/m^3, respectively, for 13 weeks.[37] A fourth group (control) was exposed to air containing a background concentration of 0.3 ± 0.02 µg Mn/m^3. Based on the cascade impactor test results, 95% of the particulate MMAD was below 6 µm (respirable fraction). None of the animals died. After 13 weeks, there were no significant differences ($P < 0.05$) in body weight between the control and 3 test groups. Serum biochemical analyses yielded significant differences in the following between controls and all exposure groups: glucose, creatinine, alanine aminotransferase (ALT), alkaline phosphatase (ALP), sodium, chlorine, and urea. Additional study results are included in the section on Neurotoxicity.

Zinc Sulfate

Groups of 12 healthy male WKY rats (12 weeks old) were exposed, nose-only, to filtered air or aerosolized zinc sulfate at concentrations of 10, 30, or 100 µg/m^3.[79] The doses were administered 3 days per week (5 h/day) for 16 weeks. Necropsy was performed at 48 h after the last exposure, to ensure that any adverse effects observed were due to chronic exposure, rather than the final exposure. There were no significant changes in the following in bronchoalveolar lavage fluid of treated animals: neutrophil or macrophage count, total lavageable cells, or enzyme activity levels (lactate dehydrogenase, *n*-acetyl β-*D*-glucosaminidase, and γ-glutamyl transferase). Changes in the heart, suggestive of a mitochondria-specific effect, included a decrease in cytosolic glutathione peroxidase activity, an increase in mitochondrial ferritin levels, and decreased succinate dehydrogenase activity. There was no evidence of cardiac pathology; however, cardiac gene array analysis indicated small changes in genes involved in cell signaling.

Human

Ammonium Sulfate

Male subjects (21 to 34 years old) and female subjects (between 21 and 33 years old) were exposed to ammonium sulfate aerosol 3 days per week (4 h/day) for 3 consecutive weeks.[80] The mean exposure concentration of ammonium sulfate aerosol (mass median diameter = 0.97 ± 0.05 µm) was 1.0 ± 0.05 ppm. Each subject acted as his or her control. There were no significant changes in pulmonary function or bronchial reactivity to methacholine either during the exposure period or at 24 h post-exposure.

Oral

Animal

Aluminum Sulfate

Groups of 8 male Sprague-Dawley rats received aluminum sulfate (2.5% in drinking water) daily for 3 months or 6 months.[81] Treated animals consumed 17 ± 6 ml of water supplemented with 2.5% aluminum sulfate, corresponding to 33 mg of aluminum per rat per day. Control rats received tap water *ad libitum*. There was no evidence of overt necrosis of the liver or kidney at the end of 3 or 6 months. At 6 months, focal nuclear pyknosis, brush-border detachment, fibrosis, and occasional hepatic fat degeneration were detected. Additionally, the mitotic index was not statistically significantly different in the kidney or liver exposed to aluminum sulfate, when compared to the control groups.

Eight Wistar rats (4 weeks old) received 30 mM aluminum sulfate (in sodium citrate) *ad libitum* for 18 months.[82] Eight control rats had free access to tap water during the same period. After 18 months, rats exposed to aluminum sulfate had a significant decrease in the red blood cell count, blood hemoglobin concentration, and hematocrit, when compared to control rats. Serum iron levels were also significantly lower, but total iron binding capacity and erythrocyte osmotic fragility remained unchanged after treatment.

Ammonium Sulfate

Groups of 20 F344 rats (10 males, 10 females per group) were fed a diet containing the following concentrations of ammonium sulfate for 13 weeks: 0.38%, 0.75%, 1.5%, and 3.0%.[83] The control group was fed diet without the test material. There was no evidence of test material-induced toxicity in relation to the following: body weights, organ weights, and hematological, serum biochemical, or histopathological examinations.

The chronic toxicity of ammonium sulfate was studied using groups of 10 F344/DuCrj rats (5 weeks old; 5 males, 5 females/group).[84] The animals were fed ammonium sulfate at dietary concentrations of 0.1%, 0.6%, and 3.0%, for 52 weeks.

Control animals were fed a diet that did not contain ammonium sulfate. A significant increase in kidney and/or liver weights in males and females was observed in the 3.0% dietary group. However, for all dietary groups, there were no effects on survival, body weights, or hematological, serum biochemical, or histopathological parameters. It was concluded that the no-observed-adverse-effect-level of ammonium sulfate was a dietary concentration of 0.6%, which is equivalent to 256 and 284 mg/kg body weight per day in males and females, respectively.

Copper Sulfate

Four experiments were conducted to investigate the effects of oral copper sulfate exposure in rats and mice. In the 1st experiment, groups of 10 Fischer 344/N rats (5 males, 5 females/group; 6 weeks old) received copper sulfate at concentrations of 300 ppm, 1,000 ppm, 3,000 ppm, and 10,000 ppm in drinking water (2-week exposure) or at concentrations of 1,000 ppm, 2,000 ppm, 4,000 ppm, 8,000 ppm and 16,000 ppm in dosed feed for 15 days.[85] In the 2nd experiment, groups of 10 B6C3F_1 mice (5 males, 5 females/group; 6 weeks old) received copper sulfate at 300 ppm, 1,000 ppm, 3,000 ppm, and 10,000 ppm in drinking water or at 1,000 ppm, 2,000 ppm, 4,000 ppm, 8,000 ppm and 16,000 ppm in dosed feed for 15 days.

In the 3rd experiment, groups of 20 Fischer 344/N rats (10 males, 10 females/group; 6 weeks old) received copper sulfate at the following concentrations in dosed feed for 92 days: 500 ppm, 1,000 ppm, 2,000 ppm, 4,000 ppm, and 8,000 ppm. An additional group of 20 rats (10 males, 10 females/group) was involved in special studies (intermediate time points for clinical pathology determinations). In the 4th experiment, groups of 20 B6C3F_1 mice (10 males, 10 females/group) received copper sulfate at the following concentrations in feed for 92 days: 1,000 ppm, 2,000 ppm, 4,000 ppm, 8,000 ppm, and 16,000 ppm. The control groups of these experiments received feed and drinking water without the test material.[85]

The results of these 4 experiments can be summarized as follows: When administered in drinking water, copper sulfate at 3,000 to 30,000 ppm was lethal to rats and mice within 2 weeks. In feeding studies, copper sulfate at 4,000 to 16,000 ppm caused a significant reduction in body weight gain in mice and rats in both 15-day and 92-day studies. Hyperplasia and hyperkeratosis of the limiting ridge of the forestomach were also observed in rats and mice in these studies. Rats in the feed studies exhibited a dose-related increase in inflammation of the liver and changes in clinical chemistry parameters that were indicative of hepatocellular damage and cholestasis. Histologic changes in the kidneys of rats consisted of a dose-related increase in the number and size of eosinophilic protein droplets in the epithelial cytoplasm and in the lumina of the proximal convoluted tubules. Urinalysis results suggested renal tubular epithelial damage. Iron staining of spleens from treated animals indicated a marked depletion of iron stores in both male and female rats, but not in mice. Hematologic and clinical chemistry alterations in rats in the 92-day study, along with histologic changes in bone in the 15-day feed study indicated microcytic anemia. Copper sulfate produced no adverse effects on any of the reproductive parameters measured in rats or mice of either sex.[85]

Groups of young Sprague-Dawley rats (up to 10 per group; ages not stated) were fed a diet containing 1,200 ppm copper sulfate for 16 weeks.[86] Control rats of the same strain were fed normal diet (copper content < 10 ppm). Copper loading with 1,200 ppm copper (as copper sulfate) altered the growth curves of young rats and induced early hepatic morphological changes, which were consistent with these ultrastructural findings of increased lysosomal number and diversity, appearance of heterochromatin and pycnotic nuclei, and varied mitochondrial alterations in the liver. There was no evidence of major histological changes in the liver.

Ferrous Sulfate

Five mongrel dogs (ages not stated) were given ferrous sulfate in gelatin capsules twice daily (0.5 g/day) for approximately 1 month.[59] The animals were then killed for histopathologic examination. Neither gross nor microscopic lesions/signs suggestive of iron toxicity were observed. No significant differences in stained iron content were apparent in the spleen, liver, and bone marrow. The total red blood cell count and hemoglobin levels were within the normal range.

Three groups of 40 young adult male Sprague-Dawley rats (5 to 6 weeks old) received ferrous sulfate at dietary levels of 35 mg Fe/kg (2.84 mg/kg), 70 mg Fe/kg (5.69 mg/kg), or 140 mg Fe/kg (11.54 mg/kg).[32] Control animals (10 rats) received a low-iron diet (< 5 mg iron/kg diet). Twenty rats from each test group were killed after 31 days of feeding, and the remaining 20/group were killed after 61 days of feeding. After 31 days of feeding, the mean corpuscular hemoglobin was statistically significantly higher in the high dose group when compared to the low dose group. There were no statistically significant differences in red blood cell or coagulation values after 61 days of feeding, and no statistically significant differences in white blood cell counts after 31 or 61 days of feeding. Statistically significant differences in mean body weight were not observed among the groups throughout the study. Neither macroscopic nor microscopic examination revealed any treatment-related changes.

A group of 12 (6 males, 6 females) Sprague-Dawley rats received ferrous sulfate (25 mg/kg/day) in drinking water for 4 weeks.[62] Another group of 12 Sprague-Dawley rats (6 males, 6 females; ages not stated) received ferrous sulfate (25 mg/kg/day) in drinking water for 4 months. The control group received regular tap water. Both groups of animals dosed with ferrous sulfate had gastric mucosal erosions and mucosal irritation ranging from simple mucosal edema and congestion to submucosal hemorrhages in the lower GI tract (colon and rectum). Changes in the stomach, small and large intestines, and rectum were also observed at microscopic examination. Lesions of the stomach, small intestines, colon, or rectum were not observed in control groups.

Hydroxylamine Sulfate

Long-term administration of hydroxylamine sulfate to mice (20 mM [2.62 mg/ml] in drinking water) for 52 weeks caused anemia and splenomegaly.[87] Bone formation (osseous metaplasia) was observed in the spleen of approximately 50% of the animals. The red blood cell count was between 55% and 73% of the control value at specific times after exposure and the white blood cell count was greatly elevated.

Zinc Sulfate

Groups of mice of the ICR strain (groups of 8 [4 males, 4 females/group]) and rats of the Wistar strain (groups of 8 [4 males, 4 females/group]) were fed a diet containing $ZnSO_4$ at 300, 3,000, and 30,000 ppm in the diet for 13 weeks.[88] The control group was fed diet only. In mice, there were no test substance-related toxic signs, though 5 mice (4 males, 1 female) were found dead or killed *in extremis* during the study. Histological findings indicated impairment of the urinary tract and regressive changes in the exocrine pancreas. Both male and female rats in the 30,000 ppm group discarded diet from the food jar by picking it out with their forelimbs, the only sign observed in rats. There were no remarkable signs in male or female rats fed at a level of 3,000 ppm or less in the diet. Two female rats (1 control and 1 at 3,000 ppm) were killed *in extremis* due to suppurative pyelitis during the study. Moribund animals of either sex were not found in the 30,000 ppm group.

Animals in the 30,000 ppm group (rats and mice) experienced retarded growth and low feed intake, and had abnormal values in a few hematological parameters and regressive changes of the exocrine pancreas. Additionally, mice had decreased water intake and significant deviations in biochemical parameters; toxic lesions appeared in the stomach, intestine, and spleen of both sexes and in the kidneys of female mice. The maximum no-effect-level of $ZnSO_4$ was 3,000 ppm, determined to be equivalent to the following milligram dose: male mice (458 mg/kg/day), female mice (479 mg/kg/day), male rats (234 mg/kg/day), and female rats (243 mg/kg/day).[88]

Human

Ferrous Sulfate

The relative frequency of adverse side effects attributable to oral treatment with iron was studied using 278 healthy 1-year-old infants. Iron sulfate and placebo dose groups consisted of 124 and 154 infants, respectively.[89] Laboratory tests of iron status were performed on venous blood, and infants with a hemoglobin of greater than 10.5 g/dL were randomly chosen to receive 1.2 mL of ferrous sulfate drops ($\approx$ 3 mg $FeSO_4$/kg/day) or an equal volume of placebo for 3 months. Repeat blood testing was then performed. Test results indicated no significant difference ($P > 0.50$) in the frequency of vomiting, diarrhea, or fussiness in iron-treated infants (6%), when compared to placebo-treated infants (9%). It was concluded that once daily, moderate-dose $FeSO_4$ therapy administered to fasting 1-year-old infants resulted in no more gastrointestinal side effects than placebo therapy.

Intraperitoneal

Aluminum Sulfate

Seven adult male albino Wistar rats were each injected i.p. with aluminum sulfate (1 mg/200 g body weight) 3 times per week over a period of 2 weeks.[90] The animals were killed and kidney tissues prepared for routine histology and electron microscopy. The control rats were dosed i.p. with saline. Aluminum sulfate at this dose caused severe damage to the kidneys. The glomeruli and proximal tubuli had swellings, adherence, hemorrhage, an increase in mesengial matrix, and marked interstitial fibrosis.

Manganese Sulfate

Ten male albino rats were injected i.p. with 6 mg/kg manganese sulfate ($MnSO_4 \cdot 4\ H_2O$), in physiological saline, daily for 30 days.[91] Another group of 10 rats served as controls. Gross abnormalities were not observed in treated or control animals. Normal hepatic architecture was reported for control animals. Liver sections from treated rats had mild congestion of central veins and adjacent sinusoids. Mild proliferation of the bile duct was also observed. Manganese sulfate did not produce evidence of hepatocellular damage.

Six male albino rats (control group) were injected i.p. with manganese sulfate in saline (6 mg Mn/kg) daily for 25 days.[92] At 48 h after the last injection, the animals were injected i.p. with saline (0.11 m mole/kg) daily for 8 days. Animals in the control group were injected with saline throughout the experiment. There was no evidence of liver or brain abnormalities at gross examination of all animals injected with manganese sulfate for 25 days, followed by dosing with saline. At microscopic examination, pathomorphological alterations of the liver, but not the brain, were observed. Dilatation and congestion of central veins and adjacent sinusoids were observed in liver sections; Kupffer cells showed hyperplasia.

Zinc Sulfate

Zinc sulfate was administered intraperitoneally (1.5 mg Zn/ml) to rats once per day for 3 consecutive days.[40] Remarkable swelling of the liver and slight growth of argentophile fiber, mostly around Glisson's capsules, were observed 14 days after the last injection. The appearance of fibrinogen and an increase in fibronectin in the liver were observed using a fluorescent antibody method. Remarkable enlargement of sinusoids, abnormal growth of microvilli, and a growth of fibers around the endothelial cells were observed using electron microscopy. Also, at 14 days, the amount of hydroxyproline in hepatic tissues was determined in each fraction, and was significantly increased ($p < 0.05$) in the supernatant fraction.

Intravenous

Copper Sulfate

Four male Clun Forest, Suffolk cross sheep were given copper sulfate (20 mg $CuSO_4\ 5H_2O$, dissolved in 40 ml of sterile saline) as a slow i.v. infusion daily.[93] Three control sheep were injected daily with sterile saline (40 ml). Animals dosed with copper sulfate had liver, kidney, and brain damage, similar to that seen in chronic copper poisoning. All of the animals survived for 30 days. Reticulocytes were produced after 4 days and production, sometimes in high numbers, continued throughout the course of the experiment.

Magnesium Sulfate

In a 2-week study, groups of female beagle dogs received the following i.v. doses of magnesium sulfate: 12.56, 50, 100, and 200 mg/kg/h.[94] The test substance was administered as 24-h i.v. infusions. Dosing was followed by a 2-week observation period. One animal in the 200 mg/kg/h dose group died at 32 h, and another animal was euthanized because of morbidity at the same time. Treatment-related changes in the 100 mg/kg/h dose group were as follows: decreased food consumption and body weight gain, anemia, mild prolongation of conduction time in the electrocardiogram, and tubular basophilia in the kidneys. Additionally, decreased calcium level was reported for animals that received doses $\geq$ 50 mg/kg/h, and was considered toxicologically insignificant. These treatment-related changes were not observed at the end of dosing. The NOAEL was considered to be 50 mg/kg/h (for 24 h) in this study.

Magnesium sulfate was administered (24-h i.v. infusion) to groups of 3 female beagle dogs at doses of 12.5, 50, and 100 mg/kg/h for 4 weeks.[95] None of the animals died. The following treatment-related changes were reported (highest dose group): decreased feed consumption and body weight gain, anemic changes, increased urine volume, decreased serum calcium level, increased inorganic phosphorus level, slight prolongation of conduction time in the electrocardiogram, and tubular basophilia in the kidneys. The NOAEL was considered to be $\leq$ 50 mg/kg/h (for 24 h) in this study.

Intratracheal

Zinc Sulfate

Male Sprague-Dawley rats (4 animals) received a single intratracheal injection of zinc sulfate in distilled water (1 mM; volume = 0.1 ml).[96] The four vehicle control rats were dosed with distilled water. At gross or histological examination, lung abnormalities were not observed in test or control animals on days 1, 7, 14, and 28 after dosing.

Cytotoxicity

Ferric Sulfate

Balb/c 3T3 fibroblasts were incubated for 24 h with several dilutions (~0.001 to 30 µg/ml) of ferric sulfate.[97] The number of cells corresponding to each concentration of the test substance was calculated relative to the control (untreated cells), considered as 100%. The LC_{50} was estimated to be = 7.74 mg/mL. Cell viability was evaluated using the MTT assay and the neutral red uptake (NRU) assay. Ferric sulfate was classified as cytotoxic.

Hydroxylamine Sulfate

Femoral bone marrow smears were prepared (from male Sprague-Dawley rats [groups of 4 or 5]) 4 h after i.p. injection of hydroxylamine sulfate; 1,000 nucleated cells were counted.[33] A series of hydroxylamine sulfate doses, increasing successively by a factor of 2, was administered. A dose of 80 mg/kg was the highest administered. Isotonic saline was injected i.v. into 36 control rats, and the animals were killed 4 h later. Hydroxylamine sulfate was inactive in this cytotoxicity assay at doses up to 80 mg/kg. Of the 36 controls, karyorrhectic cells were absent in 14.

In the MTT assay, rabbit skin cultures were exposed to hydroxylamine sulfate at concentrations of 0.5% and 5.0%.[98] A decrease in MTT conversion was induced only at a concentration of 5%. In the same study, the histomorphology of rabbit skin in organ cultures exposed to hydroxylamine sulfate (0.5% and 5.0%) was examined and compared with uncultured skin from the same animal. Hydroxylamine sulfate induced histopathological changes, i.e., severe epidermal damage at a concentration of 5.0% only. Cell proliferation was assessed by the incorporation of bromodeoxyuridine (BrdU) into DNA of cells during the S-phase. The topical exposure of rabbit skin cultures to hydroxylamine sulfate (0.5% and 5.0%), caused a decrease in epidermal cell proliferation in a dose-dependent manner. A statistically significant decrease ($P < 0.05$) in epidermal cell proliferation was noted only at the higher concentration.

Neurotoxicity

Aluminum

A 2013 review article on the subject of aluminum and Alzheimer's disease is available.[99] Alzheimer's disease (AD) is characterized by senile plaques and neurofibrillary tangles (NFT) comprised of amyloid-β protein (Aβ) deposits and hyper-phosphorylated tau protein (p-tau), respectively. Studies conducted in the 1970's have demonstrated increased levels of aluminum in the AD brain and neurofibrillary changes in the brain following aluminum injection, which has led to the suggestion that aluminum may be one of the major causes of AD. Later studies contradicted this hypothesis, in that they revealed that aluminum-induced neurofibrillary changes were different from NFTs in AD. Furthermore, studies have also revealed that the intake of high-dose aluminum-containing antacid drugs did not induce AD. Other *in vitro* and *in vivo* studies have found that aluminum was neurotoxic, and possibly promoting aggregation of Aβ and p-tau. Despite the multitude of studies that have been performed, currently, there is no direct evidence that specifically links aluminum with AD pathogenesis. Therefore, the authors of this review stated that more advanced cohort studies are necessary to better understand the absolute risk of aluminum for AD.

Aluminum Sulfate

Groups of 5 male C57BL/6 mice were treated with 2.5% aluminum sulfate in tap water for 2, 4, 6, and 12 months, respectively.[100] Twenty control mice received tap water. At the end of treatment, the animals were anesthetized with sodium pentobarbitone and the brain was dissected out and prepared for immunohistochemistry. In this study, chronic exposure to aluminum sulfate in drinking water for 12 months resulted in a pattern of deposition that was similar to that observed in humans with congophilic amyloid angiopathy and a reduction in the neuronal expression of GRP78.

Manganese Sulfate

Three groups of 30 male Sprague-Dawley rats of the CD (Crl:CD[SD] IGS BR) strain were exposed (inhalation) to manganese sulfate (6 h/day, 5 days/week) at concentrations of 30.1 ± 6.4, 294.8 ± 66.0, or $3{,}220 \pm 578$ µg Mn/m^3 for 13 weeks.[37] A fourth group (control) was exposed to air containing 0.3 ± 0.02 µg Mn/m^3. Based on the cascade impactor test results, 95% of the particulate MMAD was below 6 µm (respirable fraction). At the 2 highest concentrations, neuronal cell counts in the globus pallidus were significantly different. Locomotor activity at all exposure concentrations and resting time (at middle and high concentrations) for the 2 night resting periods were significantly increased. The total ambulatory count was decreased significantly at all concentrations of exposure.

Four cynomolgus macaque monkeys were trained to perform variable delayed response (VDR), visual discrimination (VD), and object retrieval tasks.[101] Three of these animals were then exposed to manganese sulfate (10 to 15 mg/kg/week) for 272 ± 17 days.the fourth served ax the control. By the end of the exposure period, the treated animals developed subtle deficits in spatial working memory and had modest decreases in spontaneous activity and manual dexterity. Additionally, stereotypic or compulsive-like behaviors, such as compulsive grooming, had increased in frequency by the end of the exposure period. Blood manganese concentrations measured at the end of the exposure period ranged from 29.4 to 73.7 µg/l (mean = 55.7 ± 10.8). Baseline blood manganese values ranged from 5.1 to 14.2 µg/l (mean = 9.2 ± 2.7).

Groups of 4 juvenile rhesus monkeys (between 20 and 24 months old) were exposed to airborne-manganese sulfate. An aerosol concentration of 4.62 $MnSO_4/m^3$, corresponding to 1.5 mg Mn/m^3, was generated in this study.[102] The animals were exposed 5 days per week (6 h/day). Two groups were exposed for 15 or 33 days. Other animals (recovery groups) were exposed for 65 days and then held for 45 days (4 animals) or 90 days (4 animals). Six control animals were exposed to filtered air for 65 days. Compared to controls, test substance exposure caused significant reduction of MT mRNa in the caudate. Putamen MT mRNA levels were unaffected by exposure. Exposure-related decreases in GLT-1 and GLAST levels in the globus pallidus were noted. A significant decrease in GSH levels in the caudate and increased GSH levels in the putamen after 15 and 33 days of exposure were also reported. TH protein levels were significantly reduced in the globus pallidus of monkeys exposed for 33 days, but mRNA levels were significantly increased in the same region. The authors noted that most alterations in these biomarkers of neurotoxicity were reversible upon cessation of manganese exposure.

Ocular Irritation

Copper Sulfate

The ocular effects of intravitreally-injected copper sulfate solutions were studied using New Zealand white rabbits.[103] These injections caused uveitis, which was characterized by prolonged ocular hypotony, increased protein concentrations, and decreased ascorbic acid concentrations in both the vitreous and aqueous humors, and an apparent decrease in the transport function of the anterior uvea. Both the extent and duration of these effects were dose-dependent. Reversible inflammation resulted from injection of the lower doses of copper sulfate (3 µg or 6 µg per eye). The highest dose of copper sulfate injected (30 µg per eye) produced the following signs of ocular chalcosis: hemorrhage, vitreous liquefaction, prolonged hypotony, and local iridial ischemia.

Skin Irritation

Copper Sulfate

The skin irritation potential of copper sulfate was evaluated using groups of 2 to 5 Sprague-Dawley rats (2 to 3 months old).[104] Test concentrations ranging from 0.05% to 25% in water were evaluated. The test material was applied to a defined area (dimensions not stated) of shaved skin for 1 minute using a fine brush. The test material was classified as a non-irritant over the range of test concentrations.

Hydroxylamine Sulfate

The acute dermal toxicity of hydroxylamine sulfate was evaluated using groups of 10 female New Zealand albino rabbits and groups of 10 Fischer-344 rats (on skin clipped free of hair).[64] The test substance was applied to rabbits topically (24 h under occlusion [plastic cover]) at doses of 0.001, 0.01, 0.1, and 0.5 g/kg. The test substance was also applied topically (24 h under occlusion [gauze]) to rabbits at doses of 0.1, 0.5, and 1.0 g/kg. Rats received topical applications (24 h under occlusion [plastic cover]) of the test substance at doses of 0.01, 0.1, and 0.5 g/kg. Distilled water served as the negative control and phenhydrazine (PHZ, hemolytic compound) served as the positive control. In this study, gross signs of toxicity with hydroxylamine sulfate of PHZ included skin irritation with some necrosis at the test site. Necrosis appeared more severe when both chemicals were applied under plastic than when gauze was used. Sloughing of the skin was observed after application of PHZ or hydroxylamine sulfate at the higher dose levels. Edema (at 24 h) was observed in some of the rabbits exposed to hydroxylamine sulfate, but the occurrence was random and not dose-related.

Sodium Bisulfate

Sodium bisulfate (1.5 g in 0.2 ml distilled water) was applied to the skin (trunk and lateral areas clipped free of hair) of 6 male albino rabbits.[105] Applications were made, under 1" x 1" gauze squares, to intact and abraded skin. The trunk was then wrapped with rubber sheeting. The patches were removed after 24 h and reactions were scored. Reactions ranging from

mild to no edema were observed at intact sites. Severe necrosis (in scratches) and mild edema were observed at abraded sites.

Mucous Membrane Irritation

Copper Sulfate

The mucous membrane irritation potential of copper sulfate was studied using groups of 2 to 5 Sprague-Dawley rats (2 to 3 months old).[104] Copper sulfate was brushed onto the rat buccal mucosa continuously for 1 minute. Test concentrations ranged from 0.05% to 25% in water. The animals were killed 6 h later, and microscopic examination for surface necrosis and/or inflammatory cells of tissue excised from the application site was performed. The test material was classified as a non-irritant.

Sensitization

Animal

Copper Sulfate

Three series of maximization tests involving groups of 19 guinea pigs were performed.[106] The studies involved varying concentrations of copper sulfate at intradermal induction (0.1%, 0.05%, and 0.01%). Copper sulfate (25% in petrolatum) was applied during epicutaneous induction in all 3 series. Challenge testing, on day 21, involved 0.1%, 0.5%, and 1.0% copper sulfate in petrolatum. Test results demonstrated that copper sulfate was a grade I allergen.

The skin sensitization potential of copper sulfate was studied using groups of 2 to 5 Sprague-Dawley rats. Test concentrations ranged from 0.05% to 25% in water.[104] The test material was applied to different sites on the neck daily for 5 days. The buccal mucosa was excised and examined 24 h after elicitation. At 24 h after oral provocation 7 days later with a predetermined non-irritant preparation of the test material, granulocytes were the predominant inflammatory cells, and there was no evidence of lymphocytic infiltration. Skin sensitized to 2% copper sulfate in SLS, followed by mucosal elicitation with copper sulfate, showed no response over the range of test concentrations.

Ammonium Persulfate, Sodium Persulfate, And Potassium Persulfate

The sensitization potential of ammonium persulfate, sodium persulfate, and potassium persulfate was evaluated in the murine local lymph node assay (LLNA).[107] BALB/c mice received a dermal application of ammonium, sodium, or potassium persulfate (2 x 25µ) on each ear, at a concentration of 0.1%, 0.5%, 1%, or 5% w/v in DMSO. The vehicle DMSO served as the control. On day 6, the mice were injected i.v. with [^{3}H]-methyl thymidine. Ammonium, sodium, and potassium persulfate caused dose-dependent positive responses. The maximum stimulation indices (max. at 5% concentration) and the EC3s (%) reported were: ammonium persulfate ($SI_{max} = 6.8 \pm 1.8$; EC3 = 1.9%), sodium persulfate ($SI_{max} = 6.5 \pm 1.2$; EC3 = 0.9%), and potassium persulfate ($SI_{max} = 5 \pm 1.0$; EC3=2.4%). These results were interpreted as strong-to-moderate sensitization induced by the 3 persulfate salts.

Human

Predictive Testing

Ammonium Persulfate

A meta-analysis was performed to determine which allergens are most prevalent in the pediatric population.[108] This analysis was a systematic review of studies investigating allergens in at least 100 children enrolled. The studies selected included 49 studies (years 1966-2010) with available data on 170 allergens; a median of 2 allergens was tested in each study. Of the 94 allergens evaluated by at least 2 studies, 58 had estimates of positive reactions in at least 1% of the children. For 21 of the 58 studies, the 95% confidence interval ensured that the proportion of positive reactions was at least 1%. The top 5 allergens evaluated in at least 2 studies were: nickel sulfate, ammonium persulfate, gold sodium thiosulfate, thimerosal, and *p*-toluenediamine. For most of the allergens, the proportion of positive reactions was greater in studies published after 1995, when compared to earlier studies ($p = 0.0065$).

Provocative Testing

Ammonium Persulfate

An 8-year analysis (years 1995 to 2002) of contact allergy to ingredients of cosmetics applied to the hair was performed using female hairdressers and their clients.[109] The following 2 subgroups were the focus of this analysis: (1) patients, currently or previously working as hairdressers, diagnosed with occupational skin disease (884 patients; median age = 24 years) and (2) patients who had never worked as hairdressers, i.e., clients (1,217 patients; median age = 46 years). These patients had consulted with the participating centers of the Information Network of Departments of Dermatology (IVDK) in Germany between 1995 and 2002. Previous atopic eczema was twice as common in hairdressers, when compared to clients. An analysis of sensitization frequencies and patterns indicated a slight increase in the sensitization rate for ammonium persulfate in hairdressers (3.1% to 6.8%). The sensitization rate for ammonium persulfate in clients remained largely constant at approximately 26%.

The risk of occupational allergic contact dermatitis in 300 hairdressers was studied.[110] Most of the patients (93%) were women (mean age = 23.7 years). The patients were seen by dermatologists from 1994 to 2003, and all were patch-tested with the European Standard series and specific hairdressing products. Positive patch test results indicated a significant increase in positive patch test reactions to ammonium persulfate (7.9% to 14.3%).

Forty-seven hairdressers **(**mean age = 25 years) were involved in a study of occupational asthma and rhinitis (from 1996 to 2004).[111] The specific inhalation challenge (SIC) with ammonium sulfate (44 hairdressers) involved a 30-minute nebulization of a solution of ammonium persulfate (8 mg) in 3 ml of distilled water. A mean concentration of 1.01 + 0.11 mg/µL during the 30-minute exposure was measured. Of the 47 hairdressers, 24 patients received a diagnosis of occupational asthma, which was due to persulfate salts in 21 patients. Thirteen of the 24 patients also received a diagnosis of occupational rhinitis, which was due to persulfate salts in 11 patients. A skin-prick test with ammonium persulfate (1% and 5% weight /volume in saline) was performed on 14 of the 21 patients with a + response to the SIC, and the results were negative.

A 4-year analysis (years 2003 to 2006) of contact allergy to hairdressing allergens was performed on female hairdressers and their clients (≥ 40 years old).[112] The following 2 subgroups were the focus of this analysis: (1) hairdressers, who suffered from occupational skin disease (480 patients) and (2) clients (non-hairdressers) in whom hair cosmetics were considered a potential cause of contact dermatitis (780 patients). These patients were participants of the IVDK in Germany between 2003 and 2006, and were patch tested with 2.5% ammonium persulfate in petrolatum; 21.7% of hairdressers had positive reactions to ammonium persulfate. The total incidence of positive reactions to ammonium persulfate in clients was 2.9%.

A group of 121 hairdressers (106 women, 6 men) was patch tested with allergens of the European standard series and hairdressing series.[113] At least one positive patch test reaction was found in 69.7% of the patients tested. Ammonium persulfate was among the most frequent contact allergens (23.2% of the patients).

In another study, 729 hairdressers with dermatitis were patch-tested with allergens from the extended European baseline series and hairdressing series.[114] Of the total, 29.9% had a current or past history of atopic eczema. Patches (in Finn chambers) containing 2.5% ammonium persulfate in petrolatum were removed at 2 days, and reactions were scored according to International Contact Dermatitis Research Group criteria. Ammonium persulfate was listed among the most frequent positive allergens in the hairdressing series, with positive reactions in 10.6% of the patients.

A group of 209 dermatitis patients (mean age = 53.8 years; 94.8% female) was patch-tested with hairdressing chemicals, one of which was ammonium persulfate.[115] The most common sites of dermatitis were the scalp, face, and hands. Patches (in Finn chambers) were applied to the upper back and remained in place for 48 h. Reactions were scored 48 h to 72 h after patch application, and, subsequently, at 96 h to 168 h after patch application. Ammonium persulfate was tested at a concentration of 2.5%, and the allergic patch test rate for this ingredient was 14.4%.

Apprentice hairdressers (139; 17 to 19 years old) participated in a study to investigate the frequency of work-related skin disorders.[116] Present or past work-related skin conditions affecting the hands were associated with 43.9% of the patients. Positive patch test reactions were reported for 38.1% of the patients. Ammonium persulfate was among the most frequent allergens, with reactions at a frequency of 8.3%.

Forty-four hairdressers (40 women, 4 men; 31 to 40 years old) with hand dermatitis were patch- tested with standard and hairdressing-related allergens.[117] Clinical features of the patients evaluated included vesicles, scaly plaques,

hyperpigmentation, and erythematous macules, patches and plaques. The vesicular form and scaly plaques were most common. Thirty-three (75%) of the cases had allergic contact dermatitis. Each patch (in 8 mm Finn chamber) containing the allergen was applied to normal skin of the back for 48 h; reactions were scored at days 2 and 5. Ammonium persulfate (test concentration not stated) was among the most commonly causative allergens, yielding positive reactions in 13.63% of the patients.

Patch tests involving 164 hairdressers and hairdressing apprentices (7 males, 157 females; mean age = 23 years) were performed.[118] A diagnosis of occupational contact dermatitis was associated with 157 patients, and allergic contact dermatitis was the primary diagnosis in 71% of the patients. Ammonium persulfate was listed among the most common occupational allergens in this population, with positive reactions in 20% of the patients.

Copper Sulfate

A group of 354 eczema patients (ages not stated) was patch tested with 5% copper sulfate in petrolatum.[119] Patch tests using the A1-test strip and porous tape were used. Of the 354 patients, 6 (all females with hand eczema) had a positive reaction to 5% copper sulfate. These patients also developed a positive eczematous response to 0.25%, 0.5%, 1%, and 5% copper sulfate. There were no reactions to the vehicle.

The allergenicity of 2% copper sulfate ($CuSO_4 \cdot 5\ H_2O$) in 1,190 eczema patients (ages not stated) was evaluated according to the maximization test method.[106] Thirteen of the 1,190 patients (1.1%) had reactions, but these reactions were considered non-relevant.

Forty-six farmers (21 men, 25 women; mean age = 39 years) with dermatitis were patch tested with copper sulfate (2%) over a 4-year period.[120] Details relating to the patch test procedure were not included. Positive patch test reactions were observed in 5 patients, and all 5 reactions were classified as relevant.

Routine patch tests were screened over a period of 967 days for positive results to copper sulfate (2% in petrolatum).[121] A database comprising 2,660 patients (2,037 females/623 males; mean age = 39.5 years) was compiled. Routine patch tests were performed and reactions were scored at 72 h according to International Contact Dermatitis Research Group criteria. Ninety-four patients (3.53%) had a positive patch test reaction to copper sulfate. Of the 94, 26 (20 females/6 males; mean age = 36.4 years) were enrolled for reevaluation of their reactions. Patch tests (Finn chambers) were applied to the upper lateral back and removed 48 h later. Reactions were scored at 48 h and 72 h. Only 10 (38%) of these patients were judged positive. Nine of the 10 were judged positive after patch testing with 5% copper sulfate in petrolatum; the strongest reactions observed were scored as ++ in 4 cases. Only 2 patients had unequivocally positive reactions to 2% copper sulfate in petrolatum. No unequivocally positive results were observed with 0.6% copper sulfate in petrolatum or with copper sulfate dissolved in water at any concentration. When copper sulfate was retested at concentrations of 0.6% and 0.2%, there were 27 and 12 positive patch test reactions, respectively.

Ferrous Ammonium Sulfate and Ferrous Sulfate

Over a 2.5-year period, 623 patients with suspected hypersensitivity to metals were patch tested with a series of 21 metals, including 2% aqueous ferric chloride and 5% ferrous sulfate in petrolatum.[122] Patients with positive reactions to iron were further tested with a series that included ferrous sulfate (up to 5% in petrolatum) and ferrous ammonium sulfate (up to 5% in petrolatum). Five positive reactions and 2 doubtful reactions to the initial 2 iron salts (ferrous sulfate and ferric chloride) were recorded, and no irritant reactions were observed. Six of 7 patients were available for further testing, and the positive result could only be confirmed in 2 of them. The reactions in these 2 patients (7 and 38 years old) were described as moderate, i.e., mostly erythema and slight infiltration.

Potassium Persulfate

The following groups of patients were involved in a study for validating the specific inhalation challenge (SIC) test for the diagnosis of occupational asthma: 8 patients with occupational asthma due to persulfate salts, 8 patients with bronchial asthma (never exposed to persulfate salts), and 10 healthy subjects.[123] The SIC challenge involved increasing concentrations of potassium persulfate (5, 10, 15, and 30 g) powder mixed with 150 g of lactose. The patients tipped the mixture from one tray to another, at a distance of 30 cm from the face, for 10 minutes in an airtight challenge booth. The estimated concentration of powder in the air was 6 mg/m^3, and the mean particle size distribution was: 17% (< 0.5 µm), 63% (between 1 and 5 µm), and 20% (> 10 µm). The SIC was positive for all patients with persulfate-induced asthma and for one patient with bronchial asthma who had never been exposed to persulfate salts. It was concluded that the SIC protocol for

persulfate salts used in this study appears to be useful for the diagnosis of occupational asthma secondary to persulfate salt exposure.

A study was performed to evaluate the effects of persulfate on the nasal mucosa and on immune cells in hairdressers who were suffering from bleaching powder-associated rhinitis (BAR).[124] The study groups were as follows: 15 hairdresssers with BAR, 14 hairdressers without BAR, and 12 atopics with rhinitis, but without exposure to bleaching powder. The nasal challenge was initially performed with a 0.001% fresh solution of potassium persulfate in isotonic saline solution. After 20 minutes, nasal challenge was performed with a 0.01% solution (w/v). A total of 300 µg of each solution was sprayed into the nasal cavities. The 15 subjects with BAR experienced a post-challenge increase in nasal signs (nasal blockage and secretions) and nasal lavage albumin, and the 12 atopics with rhinitis (no exposure to bleaching powder) reacted to a lesser extent. These 2 groups of 15 and 12 subjects also had an increase in t helper type 1 cells (Th1), and a human leukocyte antigen (HLA) class II cell expression was also reported. There was no evidence of a type 1 (immediate type) reaction to potassium persulfate.

In Vitro

Ammonium Persulfate

A study was performed to investigate the effects of ammonium persulfate on human albumin, mast cells, and basophils, including the role of ammonium persulfate oxidizing activity in ammonium persulfate-induced occupational asthma.[125] Twenty-nine hairdressers with work-related respiratory signs and symptoms and 31 nonexposed, healthy controls participated in the study. There was no evidence of persulfate-albumin conjugate formation, but oxidation of tryptophan and methionine was detected in LAD2 human mast cell and KU812 human basophil cell lines *in vitro*. Ammonium persulfate induced reactive oxygen species (ROS) generation and degranulation of the LAD2 and KU812 cells. The incubation of basophils from healthy controls with ammonium persulfate *in vitro* resulted in increased CD63 expression and ROS production. In hairdressers with ammonium persulfate-induced occupational asthma (positive persulfate challenge), basophil-CD3 expression was greater than in patients with a negative challenge and healthy controls.

Ferrous Sulfate

The local lymph node assay was performed using groups of 3 young adult CBA/N mice (6 to 8 weeks old), F344 rats (6 to 8 weeks old), and adult Hartley guinea pigs.[126] In the LLNA, groups of mice received 25 µl of iron sulfate (0.5%, 1.0%, 2.5%, or 5.0% in DMSO) on the dorsum of both ears daily for 3 consecutive days. Control mice received an equal volume of vehicle alone. Rats received 100 µl of the chemical or vehicle and guinea pigs received a 200 µl application. The animals were killed at 24 h after the final exposure. Exposure to ferrous sulfate failed to induce significant lymph node responses at all concentrations.

Copper Sulfate, Ferrous Sulfate, and Zinc Sulfate

The following chemicals were tested in an LLNA using groups of 3 female BALB/c mice (6 to 8 weeks old): 10% copper(II) sulfate ($CuSO_4 \cdot 5H_2O$), 5% and 20% iron(II) sulfate ($FeSO_4 \cdot 7H_2O$), and 10% zinc sulfate ($ZnSO_4 \cdot 7H_2O$).[127] The procedure in the preceding study was used, and 25 µl of each chemical was applied to the ears. Copper(II) sulfate increased LNC proliferation, whereas, iron(II) sulfate and zinc sulfate did not.

Case Reports

Ammonium Persulfate

A 21-year-old hairdresser developed the following work-related signs: rhinorrhea, sneezing, dry cough, dyspnea, wheezing, and neck erythema.[128] Her history was negative for atopy. Patch testing revealed a ++ reaction to ammonium persulfate. In a subsequent test, a patch containing ammonium persulfate (1% in petrolatum), applied to the back caused signs of cough, dyspnea, nasal obstruction, itching at the test site, and wheals on the neck and face by 90 minutes post-application. Erythema and wheals were observed at the patch test site. Development of a systemic reaction after patch application was said to have been indicative of cutaneous absorption of ammonium persulfate.

Dermatitis was diagnosed in a 24-year-old woman who came in contact with bleaching agents while employed as a hairdresser.[129] Signs disappeared after she changed her profession. However, she had a severe anaphylactic reaction and became unconscious after private use of a hair bleach containing ammonium persulfate. Anaphylaxis also developed 3 h

after patch testing with the hairdresser battery. Also, in the rub test, ammonium persulfate (2.5%) in a 1:100 solution produced positive results.

The development of dermatitis in a 33-year-old woman after 10 years of exposure to ammonium persulfate was reported. [130] Seven months of extensive and persistent skin lesions were followed by respiratory signs that were related to occupational exposure. The Italian standard (Societa Italiana di Dermatologia Allergologica, Professioinale e Ambientale) patch test series, specific occupational patch tests for hairdressers, and an occupational challenge with ammonium persulfate were performed. Both before and after the specific bronchial challenge, clinical parameters of inflammation, eosinophil cationic protein (ECP) and exhaled nitric oxide (fractional nitric oxide concentration in exhaled breath [FE_{NO}]) were detected. Patch test results for ammonium persulfate were positive (++), and bronchial challenge with ammonium persulfate caused a significant late response (33% decrease in the forced expiratory volume in 1 second [FEV1]). A significant increase in FE_{NO} and ECP was noted after 24 h. The authors reported dermatitis, urticaria, and angioedema on uncovered skin, due to airborne contact.

A 25-year-old woman (mild smoker) had been a hairdresser and handled ammonium persulfate since the age of 20.[131] Her work-related signs included rhinitis and cough. The patient underwent the specific inhalation challenge (SIC) with ammonium persulfate (240 minutes of cumulative exposure; concentration not stated) in a 7.46 m^3 inhalation challenge room. SIC elicited a dry cough, without any significant change in forced expiratory volume in 1 second. An increase in nasal secretion eosinophils (from 78.9% to 84.9%) and peripheral blood eosinophils (from 7.2% to 8.2%) was reported.

The following respiratory signs were reported for a 32-year-old technician involved with ammonium persulfate production: asthma attacks, rhinitis, and generalized skin rash.[132] Both patch and prick tests, an inhalation challenge, and methacholine testing were performed. The inhalation challenge was performed, using a nebulizer, at doses of ammonium persulfate ranging from 0.4 µg to 0.45 mg (cumulative dose of 0.6 mg; concentrations of 0.01 to 10 mg/mL). The patient had a positive patch test reaction to ammonium persulfate (as supplied), but skin prick test results for a 10% w/v ammonium persulfate solution were negative. Isolated, late symptomatic airway obstruction was observed after a cumulative dose of 0.6 mg ammonium persulfate (inhalation challenge). Methacholine testing resulted in a significant increase in exhaled nitric oxide and in the number of eosinophils in induced sputum.

Ammonium Persulfate and Potassium Persulfate

Chronic, relapsing cheilitis was observed in a 55-year-old man who used a cleansing agent containing 20% potassium persulfate for his dental prosthesis.[133] Patch testing with both the cleansing agent (2.5% in petrolatum) and ammonium persulfate (2.5% in petrolatum) yielded positive (++) reactions.

A 45-year-old non-atopic, female water laboratory assistant developed eczema on both hands (fingerwebs).[134] Patch test results were positive (++) for 2.5% ammonium persulfate in petrolatum on days 2, 3, and 6. Results were negative for 20 control subjects.

Dermatitis on the face and fingers of both hands was observed in a 36-year-old laboratory technician at a potato flour factory.[135] Potassium persulfate was added to certain batches of potato flour designated for use in the paper industry. Patch testing with 1%, 2.5%, and 5% aqueous potassium persulfate yielded a positive (++) reaction at all 3 concentrations by day 3. Prick test results for 1% aqueous potassium persulfate were negative. Patch test results for 2.5% ammonium persulfate in petrolatum were positive (+).

A 24-year-old female hairdresser with hand dermatitis, related to her occuptation, had handled hair bleaching products containing ammonium persulfate at a salon for 7 years.[136] During the next 5 years, she worked at another salon in which hair bleaching products containing potassium persulfate, but not ammonium persulfate, were used. Hand eczema symptoms improved at the new salon. The results of a previous patch test to 2.5% ammonium persulfate were positive. When freshly prepared ammonium persulfate (0.1%) and potassium persulfate (0.1%) were evaluated in the prick test, results were positive and negative, respectively.

Two cases (48-year-old woman; 36-year-old hairdresser) of systemic reactions after skin contact with persulfates were reported.[137] The 48-year-old woman (history of atopic dermatitis and rhinitis) had used bleaching products and hair dyes. The 36-year-old woman had a history of asthma and rhinitis that was provoked by the salon use of bleaching products, and had reported contact urticaria after skin contact with bleaching products. Prick tests were performed using serial dilutions of ammonium persulfate and potassium persulfate (0.1%, 1%, and 2% in water) and patch tests were performed using 2.5% potassium persulfate in water and 2.5% ammonium persulfate in petrolatum. Prick test results were positive for

ammonium persulfate and potassium persulfate, but specific IgE was not detected in the serum. Patch testing revealed early positive reactions to both persultates in one patient.

An 18-year-old hairdressing apprentice presented with occupationally-related hand eczema and asthma.[138] Skin prick test results were positive for 1% ammonium persultate and 1% potassium persulfate. The authors concluded that both ingredients induced immediate-type allergic reactions.

Potassium Persulfate

Development of an itch, erythema of the ears, neck, and forehead, and pruritus and erythema of the hand were reported after use of a hair dye kit by a 37-year-old woman.[139] A prick test of one of the components, potassium persulfate (at 1/30 in phosphate-buffered saline [PBS]), yielded negative results. However, a saturated or 1/10 dilution of a saturated potassium persulfate PBS solution produced a 5-mm wheal reaction.

A 26-year-old female hairdresser had work-related signs of sneezing and shortness of breath.[140] Patch testing with 2.5% ammonium persulfate yielded positive reactions (erythema and vesicle formation) after 24 h, 48 h, and 72 h. There was no immediate reaction after bronchial challenge with ammonium persulfate (cumulative dose = 0.5 mg); however, the following signs were reported 210 minutes later: shortness of breath, nasal obstruction, and cough.

A 1-year history of generalized, scattered dermatitis was reported for a 45-year-old male who used potassium monopersulfate in the "shock" treatments for his spa.[141] The dermatitis appeared as red papules and patches on the trunk and extremities, and there were no lesions above the neck line. The patient noted that his dermatitis flared when the hot tub was used. Patch testing (Finn chamber) with 2.5% ammonium persulfae in petrolatum yielded a ++ reaction. When shock treatments were switched to hydrogen peroxide-based treatments, the patient's dermatitis cleared.

Gene expression in the cells in nasal lavage from hairdressers exposed to potassium persulfate was studied using the following groups: 6 hairdressers with work-related bleaching powder-associated nasal symptoms, 7 hairdressers without work-elated bleaching powder-associated symptoms, and 6 atopics.[142] Nasal challenge with potassium persulfate (0.001% w/v and 0.01% w/v) in isotonic saline solution was performed. Symptomatic hairdressers had increased expression of IL5 and IFNG (involved in allergic inflammation), but not IL13 (involved in cell-mediated immunity) during challenge. This was not so for hairdressers without work-related, bleaching powder-associated nasal symptoms. Atopics had increased expression of IL5 and IL13.

Aluminum Sulfate

A 23-year-old man developed dermatitis after application of a cream for acne and hyperpigmentation.[143] Patch testing (aluminum chambers) yielded a ++ reaction to aluminum sulfate. The investigators noted that contact sensitization to uninjected aluminum is rare. Furthermore it was noted that, most often, the diagnosis is made accidentally during patch testing, and rim margin (of aluminum chamber) reactivity has been observed in most instances.

Barium Sulfate

Exposure to barium sulfate occurs in miners of barium and its salts, workers in the lithopone industry, and in patients undergoing diagnostic roentgenography of the gastrointestinal tract.[144] Barium sulfate dust, when inhaled, leads to a benign form of pneumoconiosis (baritosis), which occurs primarily in miners and workers in the lithopone industry. Escape of barium sulfate from the digestive tract into the peritoneal cavity has been reported in patients with peptic ulcers undergoing x-ray studies. Barium granulomas have been reported in the appendix, sigmoid and peritoneum, and rectum in patients receiving barium enemas.

A 43-year-old patient was diagnosed with acute appendicitis, and barium sulfate was used in diagnostic studies, i.e., upper gastrointestinal series and barium enema.[144] During these procedures, barium sulfate entered the appendix and escaped into the mesoappendix and adjacent periappendical fat. The resulting foreign-body granuloma was said to have been due to the escape of barium sulfate.

A case of barium sulfate-granulomatosis of the lung was reported for a 67-year-old man, due to barium sulfate aspiration during an x-ray investigation of the stomach.[145] In the lung parenchyma, multiple granulomas were observed in groups of alveoli where barium sulfate had been deposited.

Barium enema examination is a frequently performed radiographic procedure, and this procedure was reported to caused barium granuloma of the rectum in 2 patients (males 75 and 78 years old).[146] Rectal intramural extravasation of barium occurs as a result of asymmetric enema balloon inflation and impaction of the enema tip against the rectal mucosa. The lesions appeared as indurated, ulcerated rectal masses that resembled carcinoma on endoscopic examination. Deep mucosal biopsy results demonstrated no malignancy or barium sulfate crystals in tissue macrophages. Radiographs showed persistent soft-tissue barium in the rectum.

A severe anaphylactic reaction was observed in a 51-year-old female cancer patient at approximately 5 to 10 minutes after starting a barium enema.[147] The barium enema mixture contained barium sulfate, sodium benzoate, potassium sorbate, citric acid, sodium saccharin, ethyl maltol, vegetable gum, sorbitol, simethicone, and natural and artificial flavors. It was stated that the anaphylactic reaction could have been an IgE-mediated hypersensitivity reaction to one of the barium sulfate suspension constituents. The patient had a history of prior sensitizing exposure to barium radiographic contrast material. No skin prick test reaction, i.e., no cutaneous hypersensitivity, to diluted sodium benzoate, potassium sorbate, or whole liquid barium sulfate suspension was detected. The patient declined further provocation testing. The authors noted that the patient's severe reaction to barium may have been partly attributable to the following 3 factors: (1) her history of atopy and prior medication allergy, (2) a prior sensitizing exposure to barium sulfate, and (3) possible increased absorption of allergens into the bloodstream through the recurrently bleeding ulcerated carcinoma of the sigmoid colon.

Two children developed hypersensitivity reactions of varying severity following upper GI series.[148] The first case involved an 11-year-old boy with documented anaphylaxis, following exposure to fish and peanuts, and multiple food intolerances. The patient experienced oral swelling and a red swollen tongue after drinking 150 ml of 45% weight/weight barium sulfate. The absence of sequelae after prior upper GI series that involved drinking barium sulfate was noted. Endoscopic biopsies from the upper and lower GI tracts established the diagnosis of eosinophilic gastroenteropathy. The second case involved a 7-year-old girl with a history of mild allergy to penicillin (hives), but no other known allergies. After drinking 150 ml of 45% barium sulfate, she developed urticaria on her face, trunk, and lower extremities.

Cases of patients with a "magenta colon" from radiologic barium have been reported every 3 to 4 years.[149] This condition can occur with either upper or lower barium contrast studies, resolves over 4 to 7 days, and can be present without visible residual barium. It was noted that most reactions are mild, but, occasionally, are severe enough to hamper determining whether there is true colitis. The severe reaction is characterized by edema, loss of all vascular markings, and redness to almost a magenta color, but without ulcerations, friability, necrosis, or exudate. Biopsies show inflammatory changes.

Calcium Sulfate Hydrate, Copper Sulfate, and Zinc Sulfate

The skin irritation potential of calcium sulfate hydrate (plaster of Paris, 20% in petrolatum), copper sulfate (1% aqueous), and zinc sulfate (1% in petrolatum) was evaluated using 12 male students (average age = 20 years) at a dental technology school.[150] Finn chambers (on Scanpor tape) were applied according to International Contact Dermatitis Research Group (ICDRG) recommendations. Reactions were scored at 2 and 3 days post-application. The following skin irritation indexes were reported: 4.2 (calcium sulfate hydrate), 9.1 (copper sulfate), and 12.5 (zinc sulfate). The skin irritation index for methyl methacrylate, which is a strong skin irritant, was 37.5.

Copper Sulfate

A 36-year-old male employee of a nursery regularly came in contact with pesticides, one of which was copper sulfate.[151] Patch testing with 2.5% aqueous copper sulfate yielded positive reactions at 48 h (++) and 72 h (+++). Re-testing with 1% aqueous copper sulfate yielded no reaction. Thus, the positive reaction to 2.5% aqueous copper sulfate was regarded as false.

Ferric Sulfate

Following a dental retraction procedure, a 20-year-old male had an ulceration that extended from the marginal gingiva to the alveolar buccal mucosa.[152] Ulceration on the palatinal tissue of the maxillary anterior region was also observed. The retraction cord had been soaked with ferric sulfate and topical application of ferric sulfate solution had resulted in leakage into neighboring tissues. It was noted that these injuries to oral tissues were induced by ferric sulfate.

Ferrous Sulfate

A ferrous sulfate tablet taken by a pregnant 17-year-old female patient became lodged in the diverticulum.[153] The corrosive action of the tablet caused a small area of localized gangrene at the fundus of Meckel's diverticulum. Perforation was not observed.

An 89-year old female patient had ulceration and swelling of the hypopharynx and cervical esophagus after a single 525 mg ferrous sulfate tablet became lodged in the hypopharynx.[154] Severe edema of the arytenoids and swelling in the aryepiglottic fold and piriform sinus were noted.

Acute bronchial damage was observed in an 84 year-old female patient after aspiration of a ferrous sulfate tablet.[155] Necrotic collagenous tissue and a mild acute inflammatory reaction were observed in the biopsy specimen.

A 68-year-old female patient with iron-deficiency anemia suffered anaphylactic reactions due to immediate hypersensitivity to ingested iron salts.[156] In a single-blind, placebo-controlled oral challenge, the patient was administered 65 mg ferrous sulfate and, then, 525 mg ferrous sulfate. At 2 h post-administration of the last dose, papules on the wrist and back were observed. The placebo challenge was negative. Prick test results for ferrous sulfate (10 mg/l) were negative. However, an intradermal test of ferrous sulfate (0.01 mg/l and 1 mg/l) yielded positive responses in the patient. Intradermal test results for 15 healthy controls were negative.

Hydroxylamine Sulfate

A photographic assistant chemist who was exposed to color film developers became sensitized to para-substituted amines and hydroxylamine sulfate.[157]

Chronic hand eczema and fingernail onycholysis were observed in a technician (age not stated) who handled several types of color-processing chemicals on the job, including a mixture of hydroxylamine sulfate and lithium chloride.[158] Patch test reactions to hydroxylamine sulfate at the following concentrations were: 1% aqueous w/v (negative at 48 h; ?+ at 96 h), 2% aqueous w/v (+ at 48 h; ++ at 96 h), and 5% aqueous w/v (+ at 48 h; ++ at 96 h). Reactions were not observed in 10 control subjects patch tested with 5% aqueous hydroxylamine sulfate.

Magnesium Sulfate

Two patients (29 ad 32 years old) were treated i.v. with magnesium sulfate for preterm labor.[159] Both patients were started with a 4 mg i.v. loading dose of magnesium sulfate. An urticarial reaction, rapid and sudden onset, was observed in both patients, and the eruption cleared when dosing with magnesium sulfate was discontinued.

A 29-year-old female presented with generalized tonic-clonic seizure at 17 h post-partum.[160] Infusion with magnesium sulfate involved a loading dose of 4 g (16 mmol) by burette, and the patient received 100 mmol over approximately 20 minutes. A peak serum magnesium level of 6.87mmol/l was reported. The absence of circulatory compromise or arrhythmias was noted. The authors stated that the toxic effects of absorbed magnesium are well-described and predictable, and include: flushing, nausea, vomiting (all early symptoms), electrocardiographic changes (PR interval and QRS prolongation at 2.5 to 5.0 mmol/l serum), loss of tendon reflexes (at 5 mmol/l serum), respiratory arrest (at 7.5 mmol/l serum), and cardiac arrest (at 12.5 mmol/l serum).[161]

Zinc Sulfate

Zinc sulfate (300 to 1,200 mg/day) was administered orally to 27 patients (11 to 38 years old) with Wilson's disease for a total period of 142 patients-years.[162] Signs of intolerance to zinc were not observed.

A 47-year-old man developed asthma symptoms 2 years after initial employment at a plant where metals were galvanized in heated zinc.[163] Skin prick test results for zinc sulfate were positive at concentrations of 1 and 10 mg/ml, but not 0.1 mg/ml. Specific inhalation challenges were performed, whereby the subject inhaled a 10 mg/ml zinc sulfate solution (nebulized) for 6 minutes. Results indicated an immediate reaction, which was a maximum decrease in forced expiratory volume in one second (FEV_1) of 23%.

Twenty-two patients with Wilson's disease (10 boys; mean age = 6.2 years) were treated orally with zinc sulfate.[164] Zinc sulfate was administered (dosage in mg elemental zinc) as follows: 25 mg twice daily until age 6; 25 mg 3 times daily between the ages of 7 and 16 years or until the child attained a body weight of 125 lb, and 50 mg 3 times daily thereafter.

Zinc sulfate was administered at least 1 h before or after the intake of food or beverages other than water. Dosing with zinc sulfate had no adverse effects on growth. Psychomotor development was described as normal. Only 1 patient complained of epigastric pain, described as temporary.

A double-blind randomized prospective, right-to-left clinical trial was performed to study the beneficial effects of zinc sulfate on the skin.[165] This study involved 47 patients with chronic hand eczema (35 females, 12 males; ages not stated), the signs of which included: pruritus, erythema, lichenification, and scaling. All patients had similar symmetrical lesions on their right and left hands. The right or left hand of each patient was selected at random for treatment with either 0.05% Clobetasol™ cream alone or 0.05% Clobetasol™ + 2.5% zinc sulfate cream twice daily for 2 weeks. No significant side-effects were reported or observed by patients in both groups.

Oral zinc sulfate therapy was studied using 31 patients with multiple, non-genital viral warts (11 men, 20 women; mean age = 26).[166] The patients were treated orally with zinc sulfate (10 mg/kg; maximum dose = 600 mg/day) for 2 months. Of the 31 patients, 18 had low serum zinc levels. Twenty-six patients completed the study. The side-effects reported, not considered serious, included nausea, mild gastric pain, and itching sensation.

Zinc

The effect of zinc ingestion on the immune response and serum lipoproteins was studied using 11 healthy adult men. The men ingested elemental zinc (150 mg) twice daily for 6 weeks.[167] Dosing was associated with a reduction in lymphocyte stimulation of the response to phytohemagglutinin as well as reduction in chemotaxis and phagocytosis of bacteria by polymorphonuclear leukocytes. A significant decrease in the serum high-density lipoprotein concentration and a slight increase in the in the low-density lipoprotein level were also reported.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Animal

Aluminum Sulfate

Aluminum sulfate **(**in saline**,** 5 mg/kg body weight**)** was injected i.p. into the abdomen of each of 7 adult male albino Wistar rats. [168] Injections were made 3 times per week over a period of 2 weeks. The 7 control rats were injected i.p. with saline (10 ml/kg body weight) according to the same procedure. The following observations were reported after dosing with aluminum sulfate: the germinal epithelium of the seminiferous tubules was thinner in places and spermatids were almost absent. Sperm numbers were low, and sperm were absent from the lumen.

A two-generation reproductive toxicity study was performed using groups of Crl:CD(SD) rats (24 males, 24 females/group; 5 weeks old).[169] The groups received 120, 600, or 3,000 ppm aluminum sulfate in drinking water for 7 weeks, and controls received drinking water. Aluminum sulfate reduced water consumption in all groups; a transient decrease in body weight gain was noted in rats receiving 3,000 ppm. Pre-weanling body weight gain was inhibited at a concentration of 3,000 ppm in F_1 and F_2 pups; liver and spleen weights were decreased at the time of weaning. Additionally, vaginal opening was slightly delayed at a concentration of 3,000 ppm. There were no test substance-related changes in other reproductive/developmental parameters, including developmental and neurobehavioral endpoints. Study results indicated an aluminum sulfate NOAEL of 600 ppm for parental systemic toxicity and reproductive/developmental toxicity. The total ingested dose of aluminum from drinking water and food (standard rat diet containing 25 to 29 ppm aluminum) combined for the 600 ppm group was calculated to be 8.06 mg aluminum/kg.

Calcium Sulfate

The teratogenicity of calcium sulfate was evaluated in experiments with mice, rats, and rabbits. The following doses of calcium sulfate were administered to pregnant adult female albino CD-1 mice: 16 mg/kg (22 animals), 74.3 mg/kg (24 animals), 345 mg/kg (24 animals), and 1,600 mg/kg (23 animals).[170] The 19 positive control mice were dosed with aspirin (150 mg/kg), and 22 sham-treated mice served as negative controls. Doses were administered orally in water (dose volume = 10 ml/kg body weight) to pregnant mice for 10 consecutive days (gestation days 6 through 15). Dosing with calcium sulfate had no clearly discernible effect on nidation or on maternal or fetal survival. In the experimental groups, the number of abnormalities seen in either soft or skeletal tissues did not differ from the number occurring spontaneously in the sham-treated controls.

In a second experiment, the following doses of calcium sulfate were administered to pregnant adult female Wistar albino rats (ages not stated) according to the preceding test procedure: 16 mg/kg (21animals), 74.3 mg/kg (23 animals), 345 mg/kg (23 animals), and 1,600 mg/kg (21 animals). The 23 positive control rats were dosed with aspirin (250 mg/kg), and 25 sham-treated mice served as negative controls. In the third experiment, the following doses of calcium sulfate were administered to pregnant adult Dutch-belted rabbits according to the same test procedure, with the exception of dosing for 13 consecutive days and use of a different positive control: 16 mg/kg (14 animals), 74.3 mg/kg (13 animals), 345 mg/kg (13 animals), and 1,600 mg/kg (14 animals). The 10 positive control rats were dosed with 6-aminonicotinamide (250 mg/kg) on day 9, and 13 sham-treated mice served as negative controls. The dosing of rats or rabbits with calcium sulfate had no clearly discernible effect on nidation or on maternal or fetal survival. In the experimental groups (rats or rabbits), the number of abnormalities seen in either soft or skeletal tissues did not differ from the number occurring spontaneously in the sham-treated controls.[170]

Copper Sulfate

The embryotoxicity/teratogenicity of copper sulfate was evaluated using 34 pregnant golden hamsters of the *Cricetus auratus* strain (24 test and 10 controls).[171] The following doses of the test solution were injected i.v. (injected volume never exceeded 1 ml/100 g body weight) on day 8 of gestation: 2.13 mg Cu/kg (16 hamsters), 4.25 mg Cu/kg (3 hamsters), 7.50 mg Cu/kg (3 hamsters), and 10.0 mg Cu/kg (2 hamsters). Control animals were injected with demineralized water. The animals were killed at day 4 or 5 post-injection. The highest dose administered (10 mg Cu/kg) was maternicidal. When compared to controls, dosing with copper sulfate caused an increase in embryonic resorptions in the remaining dose groups. The following malformations were observed in the 2.3 mg Cu/kg dose group (12 abnormal embryos): thoracic wall hernias, encephalocoeles, spina bifida, and microphthalmia. Exencephaly, hydrocephalus, abdominal hernia, and abnormal spinal curvature were observed in the 4.25 mg Cu/kg dose group (4 abnormal embryos). Histological examination of the thoracic anomalies indicated that the heart was herniated through the opening in the thoracic wall (ectopia cordis). Malformations were not reported for the 7.50 mg Cu/kg dose group. Copper sulfate was teratogenic as well as embryocidal in this study.

Pregnant female CFLP female mice (6 to 8 weeks old) were injected i.v. with copper sulfate on gestation day 7 (7 mice), day 8 (12 mice), or day 9 (6 mice) and then examined on gestation day 10.[172] Injection on day 7 induced embryolethality. The majority of surviving embryos of females injected on day 8 had anomalies of the neural tube and/or the heart. Injection on day 9 resulted in a very low incidence of anomalies. The most common malformations observed on day 10 involved failure of neural tube closure in the head region of the embryo and various types of anomalies of cardiac rotation and shape. When additional females injected on day 8 were examined on day 12, exencephaly was found in a high proportion of the fetuses examined. In an *in vitro* experiment, embryos from untreated females were explanted on day 9 and cultured in *vitro* with concentrations of copper sulfate of 5×10^{-6} M, 2.5×10^{-5} M, and 5×10^{-5} M. The lowest concentration had little obvious effect on neural tube closure. Retarded and anomalous embryonic development was noted at the intermediate dose, and the highest concentration resulted in neural tube and cardiac anomalies that were similar to those produced *in vivo*.

Five pregnant, random-bred female Wistar rats were dosed i.p. with aqueous copper sulfate (2 mg Cu/kg; dose volume = 0.1 ml/100g) on gestation day 8.[173] The control group of 5 rats received deionized water. The animals were killed on gestation day 19. Dosing with the test material had no significant effect on the incidence of fetal resorption when compared to the control group. The test material induced a higher incidence of the following fetal abnormalities, but the differences were not statistically significant: skeletal retardation, the absence of or delayed ossification of vertebrae, foreshortening of the ribs, and ectrodactyly.

Groups of 20 Fischer 344/N rats (10 males, 10 females/group) received copper sulfate at the following concentrations in feed for 92 days: 500 ppm, 1,000 ppm, 2,000 ppm, 4,000 ppm, and 8,000 ppm.[85] The control group received drinking water without the test material. Groups of 20 $B6C3F_1$ mice (10 males, 10 females/group; 6 weeks old) received copper sulfate at the following concentrations in feed for 92 days: 1,000 ppm, 2,000 ppm, 4,000 ppm, 8,000 ppm, and 16,000 ppm for 92 days. The control group received drinking water without the test material. Complete necropsies and histopathologic examinations were performed as stated for rats in the preceding experiment. Epididymal sperm motility was evaluated at necropsy and vaginal cytology was evaluated in animals during the week preceding necropsy. Copper sulfate produced no adverse effects on any of the reproductive parameters measured in rats or mice of either sex.

The structural integrity of rabbit spermatozoa after exposure to copper sulfate was evaluated in an *in vitro* study.[174] At least 500 spermatozoa per sample were evaluated, and the concentration of copper sulfate in the incubation medium ranged from 3.57 to 4.85 µg copper sulfate/ml. When compared to the control culture, decreased motility of spermatozoa was noted over the range of test concentrations. At time 0, the difference was statistically significant at concentrations

ranging from 3.70 to 4.85 µg copper sulfate/ml. After 48 h, almost all spermatozoa were dead, i.e., no motility at all concentrations. When compared to the control culture (30.60% abnormal spermatozoa), the total percentage of morphologically abnormal spermatozoa was significantly higher (46.20%; $P < 0.05$) at the highest copper sulfate concentration.

Two groups of 12 NMRI female mice (6 weeks old) were dosed orally with copper sulfate (0.2 cc, by gavage) at doses of 100 mg/kg and 200 mg/kg, respectively, once daily for 35 consecutive days.[175] Six control rats were dosed with saline (0.2 cc) according to the same procedure. The animals from each experimental group were killed at 14 and 35 days, and the ovaries were removed for light and electron microscopic examination. Only the number of antral follicles was decreased on day 14, when compared to the control group ($P = 0.043$). The higher copper sulfate dose (200 mg/kg) or a longer consumption period significantly reduced different classes of follicles and corpora lutea. The following mild ultrastructural cellular damage was observed on day 14, after dosing with 100 mg/kg: decrease in zona pellucida thickness, limited vacuolated areas, and nuclear envelop dilation. The higher dose (200 mg/kg) or longer copper sulfate administration caused more detrimental effects, described as follows: more vacuolated areas, presence of secondary lysosomes, irregularity in cell shape and segmented nuclei with condensed and marginated chromatin, and more enlarged and damaged mitochondria.

Ferrous Sulfate

The spermicidal activity of ferrous sulfate was evaluated using human semen *in vitro*.[176] Observations were made at 40 seconds, 5, 10, 15, and 20 minutes, and at 1 h. Sperm motility inhibition ranged from 32% to 95% in hypotonic solutions (0.006 M to 0.12M). At an isotonic concentration (300 mOsM, 0.15 M) , inhibition of sperm motility was slightly lower. At concentrations of 0.238 M to 0.3 M (hypertonic range), 100% inhibition was noted. Ferrous sulfate (0.238 M) resulted in complete immobilization of human sperm within 40 seconds of incubation.

Groups of 4 male Wistar rats were injected i.p. twice with 0.4 mmol/kg ferrous sulfate.[177] At 1, 2, and 4 days after the second injection, in each of the 3 groups respectively, the testis of each animal was analyzed for iron and malondialdehyde (MDA) content. Both iron and MDA content increased in parallel after injection of ferrous sulfate. On days 1 and 2 post-injection, numerous necroses were observed in different cell types of the germinal epithelium. Fewer alterations were noted at day 4. Electron microscopy results indicated the presence of up to 3 nuclei and at least 3 axonemes in some spermatids. At day 4 post-injection, the number of spermatids was reduced.

Magnesium Sulfate

The following doses of magnesium sulfate were administered to Crj:CD(SD) female rats s.c. three times per day on days 15 through 20 of gestation: 250, 500, and 1,000 mg/kg.[178] The control group and 250 mg/kg group each consisted of 19 rats. The remaining 2 dose groups each contained 20 rats. Effects of the test material on the dams and F_1 animals were examined. Dams dosed with 500 and 1,000 mg/kg had decreased food consumption, Hypolocomotion, pronation, bradypnea, and decreased body weight gain were observed in the 1,000 mg/kg dose group. There were no test material-related effects on delivery or lactation, and necropsy results were normal. Results for F_1 animals dosed with 1,000 mg/kg were as follows: low body weight, delays in differentiation (eruption of lower incisor and opening of eyelid), and reversible changes in ribs (wavy ribs). However, there were no test material-related effects on viability, functional examinations, behavioral tests, or reproductive ability. It was concluded that the NOAEL for general toxicological effects on the dams was 250 mg/kg/day (3 times per day), and that the NOAEL for reproductive ability and development were 1,000 mg/kg/day (3 times per day) and 500 mg/kg/day (3 times per day), respectively.

Manganese Sulfate

Six male albino rats (used as control group) were injected i.p. with manganese sulfate in saline (6 mg Mn/kg body weight) daily for 25 days.[92] At 48 h after the last injection, the animals were injected i.p. with saline (0.11 m mole/kg) daily for 8 days. Animals of the other control group were injected with saline throughout the experiment. There was no evidence of testis abnormalities at gross examination of all animals injected with manganese sulfate for 25 days, followed by dosing with saline. At microscopic examination, pathomorphological alterations of the testis were observed. Testicular alterations included degeneration of a few seminiferous tubules and depletion of spermatocytes from them. Intertitial cells did not appear abnormal.

Six pregnant adult female rats (2BAW strain) received single daily i.p. doses of aqueous magnesium sulfate (150 mg/kg) on days 17 through 21 of gestation.[179] The six control rats received distilled water. The animals were killed on day

21. Dosing with the test material resulted in strong mitochondrial alterations in the maternal and fetal liver, i.e., reduced numbers, rarefaction, crystolysis, swelling, and disruption.

Pregnant QS mice were given a single i.p. injection of manganous sulfate ($MnSO_4 \cdot 4H_2O$; 12.5, 25, or 50 mg Mn^{++}/kg dose) on day 8, 9, or 10 of gestation (plug day = day 0).[180] On days 8 and 9, dosing with 12.5, 25, and 50 mg/kg involved 10, 10, and 5 mice, respectively. On day 9, dosing with 12.5, 25, and 50 mg/kg involved 10, 5, and 5 mice, respectively. Control mice received saline solution. The animals were killed on day 18 of gestation. The 50 mg/kg dose was embryolethal on gestation days 8, 9, and 10. The 25 mg/kg dose was teratogenic when given on gestation day 8; a low incidence of encephaly was reported. When given on gestation day 9, the 25 mg/kg dose caused embryonic loss and prenatal growth retardation, but no malformations. The 12.5 mg/kg dose caused 2% exencephaly on day 8, slight growth retardation on day 9, and severe growth retardation and prenatal death on day 10. Control animals were normal throughout the study.

Male rat fertility following ingestion of manganese sulfate ($MnSO_4 \cdot 2H_2O$) was studied using adult male Sprague-Dawley rats. Ten rats received manganese sulfate in tap water (1,000 ppm/liter) for 12 weeks.[181] Control rats received tap water. All animals remained healthy throughout the study. Each male was caged with 2 untreated virgin females for 10 days. Female rats were killed 1 week after the removal of male rats. The total number of resorptions was significantly increased ($P < 0.025$) in females impregnated by male rats that ingested manganese sulfate. There were no significant differences in numbers of pregnant females, implantation sites, or viable fetuses when test and control groups were compared.

Zinc Sulfate

Two groups of 6 pregnant Syrian hamsters were used to evaluate the placental transfer of zinc during early embryogenesis.[182] Each animal was dosed i.v. with 2 mg $^{65}ZnSO_4$. The animals of one group were killed 24 h post-injection (day 9 of gestation); samples of whole blood and liver were collected and embryos were removed. Samples of the placenta and uterus were also obtained. In the second group, tissues were collected at 96 h post-injection (day 12 of gestation) and similar samples of maternal blood and liver were collected. All embryos and fetuses were examined for gross developmental malformations. Significant amounts of zinc were detected in embryonic tissues within 24 h post-injection. Dosing with $^{65}ZnSO_4$ did not cause any developmental malformations.

Beginning on day 1 of conception, 12 mated Charles-Foster rats were given feed containing 4,000 ppm zinc sulfate (anhydrous).[183] The females were killed on day 18 of gestation. Fetuses with placentae were removed and the endometrium was inspected for resorption sites. The number of resorption sites was negligible in both groups. The incidence of conception was found to be lower in the experimental group when compared to the control group ($p < 0.01$). Only 5 of 12 experimental mice conceived, whereas, all 12 mated control females conceived. The number of implantation sites, expressed per mated female, in experimental rats was also reduced; however, this finding was not statistically significantly different when compared to the control group. There also were no significant differences in mean placental and fetal weights when the 2 groups were compared. Stillbirth and malformed fetuses were absent in control and experimental groups.

A second experiment involved 2 groups (15 experimentals, 11 controls) of rats of the same strain, and anhydrous zinc sulfate (4,000 ppm) was added to the diet of the experimental group for 21 days. Mating between males and females was allowed in both groups at the end of the 21-day feeding period. The remainder of the experiment was performed according to the protocol used in the first experiment. Fourteen experimental and 10 control animals conceived. The difference in the incidences of conception between the 2 groups was not statistically significant. The number of resorption sites was negligible in both groups. When implantation sites were expressed per mated female or pregnant female, there was no significant difference between experimental and control groups. Mean fetal and placental weights in both groups were similar. Stillbirth and malformed fetuses were absent in control and experimental groups.[183]

Ten male Sprague-Dawley rats (ages not stated) were injected i.p. with zinc sulfate (in saline, 3 mg/kg/day) for 4 weeks.[184] The control group consisted of 10 untreated rats. Histological examination of testes revealed no differences between treated and control rats. Neither inhibition of spermatogenesis nor testicular tubular degeneration and necrosis was observed in treated animals.

Human

Ferrous Sulfate

A study was performed to determine the effect of prophylactic iron supplementation on the iron status and birth outcomes among nonanemic pregnant women.[185] A randomized, triple-blind clinical trial was performed using nonanemic pregnant women (148 women; ages between 20 and 35 years) with the following profile: hemoglobin (> 110 g/l), serum

ferritin (> 12 µg/l), and gestational age (< 20 weeks). The women were randomly assigned to receive either ferrous sulfate (60 mg elemental ion; 70 subjects) or placebo (78 subjects) until the time of delivery. At the time of delivery, the incidence of iron deficiency was significantly lower in the group that received ferrous sulfate. Also, when the 2 groups were compared, there were no significant differences in maternal hemoglobin and ferritin concentrations at the time of delivery or in infant birth weight, birth length, or length of gestation.

Magnesium Sulfate

Over a period of 14 years, 7,000 infants were born to mothers who had received magnesium sulfate parenterally because of preeclampsia or eclampsia.[186] A 50% magnesium sulfate ($MgSO_4 \cdot 7H_2O$, USP) solution was injected intramuscularly (30 to 40 g doses, during 24 h) into the gravida. This regimen was continued as long as the mother had demonstrable knee jerks, urine output of at least 100 ml during 4 h, and no depression of respiration. The serum level of magnesium in the fetus rapidly approached the maternal level, but could not be correlated with any adverse effect. Dosing did not have any observable deleterious effects on the fetus or newborn.

Five neonates were born to mothers who had been treated i.v. with magnesium sulfate for tocolysis.[187] The neonates were retrospectively reviewed to assess the presence of radiographic, clinical, and biochemical abnormalities. Two infants had radiographic bony abnormalities; one had frank rachitic changes and dental enamel hypoplasia. One of these patients as well as an additional infant had transient hypocalcemia. It was hypothetized that prolonged infusion of magnesium sulfate, especially when initiated during the second trimester, may lead to fetal parathyroid gland suppression, with consequent abnormalities resembling rickets.

The effects of maternal magnesium sulfate treatment on newborns were studied.[188] The subjects in this study were newborn infants, delivered at ≥ 34 weeks of gestation, whose mothers had received a minimum of 12 h of i.v. magnesium sulfate therapy prior to delivery. A total of 26 magnesium-exposed and 26 control infants was enrolled. The mean dose of magnesium sulfate prior to delivery was 51.2 ± 24 g, and the mean duration of therapy was 23.1 ± 120 h. The mean maternal serum magnesium level before delivery was 5.8 ± 1.1 mg/dl. Infants exposed to magnesium sulfate in utero had a higher incidence of hypotonia and lower median Apgar scores, compared to control infants ($p < 0.001$). However, there was no association between adverse outcomes and maternal serum magnesium concentrations at the time of delivery, duration of treatment, or dose of magnesium sulfate. Pneumocardiogram data were similar between magnesium sulfate-exposed and control infants (all, $p \geq 0.16$).

In a controlled trial, mothers in preterm labor were randomized as follows: magnesium sulfate tocolysis (46 mothers, 55 newborns) and saline control (28 mothers, 29 newborns).[189] Magnesium sulfate was administered as a 4-g bolus, followed by infusion of 2 to 3 g of magnesium sulfate per hour. Children with adverse outcomes had higher umbilical cord magnesium levels at the time of delivery. In regression models that controlled for confounders, which included very low birth weight, magnesium remained a significant risk factor (adjusted odds ratio = 3.7; 95% CI of 1.1 to 11.9; P = 0.03). Dosing with magnesium sulfate was associated with 11 composite adverse pediatric outcomes, which included intraventricular hemorrhage (IVH) and periventricular leucomalacia (PVL), and cerebral palsy. However, the differences in this trial were not statistically significant (magnesium sulfate: 37% [11 adverse events in 30 infants]; saline solution: 21% [6 adverse events in 29 infants] (P = 0.25).

Between January 2000 and February 2009, 6,654 women with preeclampsia were treated with an intravenous infusion of magnesium sulfate, with the goal of achieving a therapeutic range of 4 to 7 mE/L (2.0 to 3.5 mmol/L).[190] Eighty-eight infants (6% of the infants) were diagnosed with hypotonia. Lowder 1-minute and 5-minute Apgar scores, intubation in the delivery room, admission to special care nursery, and hypotonia were all significantly increased as maternal serum magnesium concentrations increased prior to birth.

Zinc Sulfate

Twenty women (ages not stated; blood zinc concentrations < 11.5 µmol/l) participated in a study designed to examine the effects of oral zinc sulfate supplementation.[191] Zinc sulfate (45 mg), in the form of an effervescent preparation, was administered orally to 7 of the women twice daily until the time of delivery. Increased urinary zinc excretion ($p < 0.005$) was noted in all 7 women after 1 week of treatment, indicating that zinc had been absorbed. During the 8-12 days of follow up, it was noted that dosing with zinc sulfate did not cause reticulocytosis. All 7 women had normal deliveries. However, the infant of 1 woman, a primigravida, showed slight signs of dysmaturity. This woman had the shortest period (5 weeks) of dosing with zinc sulfate. Five of 13 women who did not receive zinc therapy had normal deliveries.

Zinc

A randomized double-blind, placebo-controlled trial was performed to evaluate whether zinc supplementation during pregnancy is associated with an increase in birth weight.[192] Healthy pregnant women (580 subjects; mean age = 23.4 years) with plasma zinc levels below the median at enrollment in prenatal care, randomized at 19 weeks' gestational age, participated in the study. Women who were taking a non-zinc-containing prenatal multivitamin/mineral tablet were randomized to receive either a daily dose of 25 mg zinc (294 women) or a placebo (286 women) until the time of delivery. Mean daily dietary intakes of zinc were 12.8 mg and 13.1 mg in zinc and placebo groups, respectively. When zinc supplement and placebo groups were compared, there were no significant differences in Cesarean section rates, length of maternal hospital stay, or overall maternal infection rates. For all study participants, infants in the zinc supplement group had a significantly greater birth weight (126 g; $P = 0.03$) and head circumference (0.4 cm; $P = 0.02$) when compared to infants in the placebo group. The crown-heel length and chest, abdominal, arm, and thigh circumferences were not significantly different when the 2 groups were compared. In women with a body mass index of < 26 kg/m^2, zinc supplementation was associated with a 248-g higher infant birth weight ($P = 0.05$) and a 0.7-cm larger infant head circumference ($P = 0.007$). Plasma zinc concentrations were significantly higher in the zinc supplement group. It was concluded that daily zinc supplementation in women with relatively low plasma zinc concentrations in early pregnancy is associated with greater infant birth weights and head circumferences, with this effect occurring predominantly in women with a body mass index of < 26 kg/m^2.

GENOTOXICITY

In Vitro

Aluminum Sulfate

In a sister chromatid exchanges (SCE) assay using human peripheral blood lymphocytes,[193] aluminum sulfate was tested at concentrations of 10 and 20 µg/ml. Additionally, a chromosome aberrations (CA) assay involved a similar treatment protocol. When compared to controls, the 10 µg/ml concentration did not affect the frequency of SCEs and CAs. However, the 20 µg/ml concentration caused significant increases in SCEs and CAs.[194]

Aluminum Sulfate and Silver Sulfate

The genotoxicity of aluminum sulfate and silver sulfate was evaluated in the rec-assay procedure, used to detect DNA damaging activity.[195] Two strains of *Bacillus subtilis*, H17 and M45, were used. When DNA damage is produced by a chemical and subjected to cellular recombination-repair function, the growth of recombination-deficient cells is usually inhibited much more than that of wild cells. Each metal was tested at concentrations ranging from 0.005 to 0.5 M. Results for aluminum sulfate and silver sulfate were negative in this assay.[196]

Barium Sulfate

The genotoxicity of barium sulfate was evaluated using murine fibroblasts in the *in vitro* single-cell gel (comet) assay. The fibroblasts were exposed for 5 h (at 37°C) to barium sulfate at final concentrations ranging from 10 to 1,000 µg/ml. Vehicle control cultures were exposed to phosphate-buffered solution, and positive control cultures were exposed to 10 µM hydrogen peroxide. A total of 50 randomly captured comets per treatment (25 cells from each slide) were examined using a fluorescence microscope. Barium sulfate did not increase cell mortality and was not genotoxic, i.e., did not induce DNA breakage. The positive control caused a significant increase ($P = 0.02$) in tail moment, when compared to the negative control.[197] Barium sulfate (1 to 1,000 µg/mL) also was not genotoxic in human peripheral blood lymphocytes in the *in vitro* single-cell (comet) assay.[198]

Calcium Sulfate Hydrate

Gypsum (calcium sulfate hydrate) served as the negative control in a comet assay performed to evaluate the genotoxicity of 2 kinds of bentonite particles on human B lymphoblast cells *in vitro*.[199] At a concentration of 240 µg/ml, the % tail DNA of cells exposed to gypsum for 24 h was 3.07 ± 0.29. Values for native and active bentonite particles tested at this concentration in cultures exposed for 24 h were 4.29 ± 0.43 and 4.40 ± 0.43, respectively. Both values were statistically significantly different ($P < 0.05$) when compared to the mean value for gypsum. The active and native bentonite particles were classified as genotoxic in the comet assay, which can identify DNA damage such as strand breaks and alkali-labile sites.

Copper Sulfate

The genotoxicity of copper sulfate was evaluated in the Ames test using the following *Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538.[200] The concentrations tested were not stated. However, it was stated that copper sulfate was tested, with and without metabolic activation, at various dilutions (in duplicate or triplicate plates), starting from its solubility or toxicity limit. Results were negative in each strain.

Copper sulfate was also evaluated in a DNA-repair test using *Escherichia coli*.[200] The initial concentration of copper sulfate was governed either by its solubility or by its toxicity. Starting with that concentration, the test material was further diluted in nutrient broth for a total of eight 2-fold dilutions (50 µl/well, 6 wells/dilution). Cupric sulfate did not induce DNA damage in this assay and was classified as non-genotoxic.

Copper Sulfate and Ferrous Sulfate

A comparison of calf thymus DNA damage induced by copper and iron salts in the presence of H_2O_2 and ascorbate was made.[201] The ethidium bromide (EB) binding assay, based on the formation of a fluorescent complex between double-strand DNA and EB, was used to measure DNA damage. The degree of fluorescence loss indicates the extent of DNA damage. In the absence of metals, DNA-EB yielded a measurable amount of fluorescence (control). When copper sulfate or ferrous sulfate (50 µM in the presence of 2 mM H_2O_2 and 2 mM sodium ascorbate) was incubated with calf thymus DNA for 30 minutes, a loss of EB-induced DNA fluorescence was noted. Compared to the control, copper sulfate ($P < 0.01$) and ferrous sulfate ($P < 0.01$) caused significant loss of EB-induced DNA fluorescence.

Ferrous Ammonium Sulfate

The treatment of germinated conidia of the in1 mutant of *Neurospora crassa* with 0.02 mM ferrous ammonium sulfate resulted in a decrease in survival and a high level of mutagenicity.[202] The reversion frequency ($\times 10^8$) was 1 ± 0.2 in the untreated control culture and 1,840 in the presence of ferrous ammonium sulfate.

Ferrous Sulfate

Ferrous sulfate ($FeSO_4 \cdot 7H_2O$) was evaluated for genotoxicity in a sister-chromatid exchanges (SCE) assay using Chinese hamster cells.[203] Ferrous sulfate was solubilized in sterile DMSO at a concentration of 10 mg/ml before use, and, thereafter, was diluted in pre-warmed growth medium. The cells were exposed to ferrous sulfate throughout incubation with 5-bromo-deoxyuridine (BUdR, 1 µg/ml) for 2 cell cycles (28 h). During the last 2 h of incubation, Colcemid™ (0.1 µg/ml) was added. Ferrous sulfate induced 5.4 SCE per cell, compared to the control value of 3.90 ± 0.82 SCE per cell. The value for ferrous sulfate was not statistically significantly higher than the spontaneous SCE level.

The genotoxicity of ferrous sulfate was also evaluated in the Ames test.[204] Using *Salmonella typhimurium* strain TA97, ferrous sulfate was tested at doses up to 36.2 µg Fe/plate with and without metabolic activation. Results were negative with and without metabolic activation.

Ferrous sulfate was evaluated for genotoxicity in the L5178Y TK+/- mouse lymphoma assay.[204] Mouse lymphoma cells were tested in the presence and absence of metabolic activation at concentrations up to 1.5 µg Fe/ml and up to 201 µg Fe/ml, respectively. A dose-related increase (dose range: 0.804 to 1.5 µg Fe/ml) in the mutant frequency was observed with metabolic activation. Results were weakly positive without metabolic activation; at the lowest dose (20.10 µg Fe/ml) and highest dose (201 µg Fe/ml), the mutation frequencies were 25 per 10^6 survivors and 80 per 10^6 survivors, respectively

The comet assay[205] and chromosome aberrations analysis were used to determine the DNA-damaging and clastogenic effects of ferrous sulfate ($FeSO_4 \cdot 7H_2O$).[206] In the comet assay, human lymphocytes were treated with ferrous sulfate at concentrations of 1.25, 2.5, and 5 µg/mL during the quiescent phase. In the chromosome aberrations assay, the same concentrations were tested during the G_1, G_1/S, S (pulses of 1 h and 6 h), and G_2 phases of the cell cycle. All test concentrations were cytotoxic and significantly reduced the mitotic index in all phases of the cell cycle. Also, at all concentrations, chromosome aberrations were induced in G_1, G_1/S, and S (pulses of 1 h and 6 h) phases of the cell cycle. Iron sulfate also induced polyploidy in cells treated during the G_1 phase. Iron sulfate did not induce significant DNA damage in the comet assay. The results of this study indicated that iron causes alteration and inhibition of DNA synthesis only in proliferative cells, which explains the concomitant occurrence of genotoxicity and cytotoxicity at the concentrations tested in the human lymphocytes studied.

A study was performed to evaluate the capacity of ferrous sulfate to produce leukocyte DNA damage, compared to H_2O_2 and 4-hydroxynonenal (HNE), known to cause DNA damage. DNA damage in human leukocytes was evaluated using the alkaline comet assay.[205] The genotoxicity of ferrous sulfate (250 to 1,000 µM) did not differ significantly from that of H_2O_2 and HNE.[207]

Ferrous Sulfate and Magnesium Sulfate

Ferrous sulfate and magnesium sulfate were evaluated for genotoxicity in the Ames test using the following *Salmonella typhimurium* strains: TA92, TA94, TA98, TA100, TA1535, and TA1537.[208] Each test substance (in phosphate buffer) was evaluated at doses up to 100 mg/plate with metabolic activation, and results were negative in all bacterial strains tested.

In a chromosome aberrations assay using Chinese hamster ovary cells,[209] the genotoxicity of ferrous sulfate or magnesium sulfate (dissolved in physiological saline) was evaluated at concentrations up to 4 mg/ml in culture. Ferrous sulfate induced chromosomal aberrations in this assay, but magnesium sulfate did not.[208]

Ferrous Sulfate, Aluminum Sulfate,
Copper Sulfate, Magnesium Sulfate, and Manganese Sulfate

The genotoxicity of ferrous sulfate ($FeSO_4$, 7 H_2O) and other inorganic sulfates was evaluated in the SOS Chromotest using *Escherichia coli* strain PQ37, with and without metabolic activation.[210] The SOS Chromotest is a colorimetric assay that measures the expression of genes induced by genotoxic agents in *E. coli* by means of fusion with the structural gene for β-galactosidase. Test substance concentrations were as follows: ferrous sulfate ($FeSO_4$,$5H_2O$; up to 3,000 nM/ml), aluminum sulfate ($Al_2(SO_4)_3$,$12H_2O$; up to 3,000 nM/ml), copper sulfate ($CuSO_4$,$5H_2O$; up to 1,000 nM/ml), magnesium sulfate ($MgSO_4$,$7H_2O$; up to 30,000 nM/ml), and manganese sulfate ($MnSO_4$, H_2O; up to 30 nM/ml). All 5 chemicals were not genotoxic, with or without metabolic activation.

Ferrous Sulfate, Manganese Sulfate, and Zinc Sulfate

In a mitotic recombination assay,[211] the genotoxicity of the following salts was evaluated using *Saccharomyces cerevisiae strain D7*: ferrous sulfate, manganese sulfate, and zinc sulfate. Gene conversion at the *trp* locus and reverse mutation at the *l/v* locus were evaluated.[212] Each test substance was dissolved in sterile distilled water at a concentration of 0.1 M and cultures were incubated overnight. Manganese sulfate induced a strongly positive response for both conversion and reverse mutation. Ferrous sulfate induced a positive response for conversion and a weakly positive response for reverse mutation. Zinc sulfate induced weakly positive responses for conversion and reverse mutation.

Hydroxylamine Sulfate

The genotoxicity of hydroxylamine sulfate was evaluated in the dominant lethal assay using male and female ICR/Ha Swiss mice.[213] Each male mouse was dosed with the test substance or solvent control and subsequently caged with 3 untreated female virgin mice. Two doses of the test substance (102 and 112 mg/kg i.p.) were evaluated, using 7 and 9 males at the lower and higher dose levels, respectively. Of the 7 males dosed with 102 mg/kg, 1 died. None of the 9 males dosed with 112 mg/kg died. Hydroxylamine sulfate did not meet any of the screening criteria for mutagenic effects.

Magnesium Sulfate

The frequency of sister chromatid exchanges (SCEs) in cultures of human peripheral blood lymphocytes (from single donor) incubated with magnesium sulfate was evaluated.[214] Cultures containing the following concentrations of magnesium were incubated for 96 h: 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, and 1,000 µg/ml. At each experimental point and in the corresponding control (unspecified), 40 metaphases of the second mitosis were analyzed. At all concentrations tested, the frequency of SCEs in cultures incubated with magnesium sulfate did not differ significantly ($P > 0.05$) from that of the control (6.20 ± 0.43 µg/ml). It was concluded that magnesium sulfate was not genotoxic.

The genotoxicity of magnesium sulfate was evaluated in the Ames test and in a chromosomal aberrations assay.[215] In the Ames test, magnesium sulfate was evaluated in the following bacterial strains at doses up to 5,000 µg/plate, with and without metabolic activation: *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A. Magnesium sulfate did not induce an increase in the incidence of reverse mutations in any of the bacterial strains tested in this assay. A Chinese hamster lung fibroblast cell line (CHL/IU) was used in the chromosomal aberrations assay (direct and metabolic activation methods), and magnesium sulfate was evaluated at concentrations up to 5.0 mg/ml,

with and without metabolic activation. Magnesium sulfate did not induce an increase in the incidence of chromosomal aberrations or genome mutation (polyploidy) in this assay.

Manganese Sulfate

In the rec-assay,[195] manganese sulfate (test concentration = 0.05M) was evaluated for genotoxicity using *Bacillus subtilis* strains H_{17} (Rec^+, arg^- and trp^-) and M_{45} (Rec^-, arg^- and trp^-). Results were positive.[216]

The genotoxicity of manganese (II) sulfate monohydrate was evaluated in the sex-linked recessive lethal test using *Drosophila melanogaster*.[217] In one experiment, the test material was fed at a concentration of 12,500 ppm in 5% sucrose for 3 days. In the other experiment, the test material was injected at a concentration of 1,000 ppm in 0.7% aqueous NaCl. After test substance administration, Canton-S males were mated with 3 consecutive harems of *BASC* females over a 7-day period to collect germ cells treated primarily at post-meiotic stages. A lethal mutation was judged to have occurred if no wild-type males were recovered in the F_2 among at least 20 *BASC* males (or *BASC*/ + females). Manganese sulfate was not genotoxic in this assay.

Manganous sulfate ($MnSO_4 \cdot 4H_2O$, in ddH_2O) was evaluated for gentoxicity at concentrations up to 1,200 µM in the Ames test using *Salmonella tyhimurium* strain TA97.[218] The test substance was evaluated without metabolic activation, and results were negative.

The genotoxicity of manganese sulfate was evaluated in the fluctuation test[219] for mitotic gene conversion at the tryptophan-5 and histidine-4 loci in *Saccharomyces cerevisiae* strain JD1. Manganese sulfate was evaluated at concentrations up to 500 µg/ml, and results were negative.[220]

Zinc Sulfate

The genotoxicity of zinc sulfate (in Hank's balanced salt solution) was evaluated in the micronucleus test.[221] The test substance was administered to mice (4 total) at doses up to 2 x 86.3 mg/kg (at 0 h and 24 h, respectively). Bone marrow smears were prepared and 1,000 erythrocytes were scored per mouse. The percentage of micronucleated polychromatic erythrocytes was 2.9% at the highest dose; this value was not significantly different from the control.

In the HeLa DNA-synthesis inhibition test (detects strong mutagens and carcinogens, except when difficulties with metabolic activation are encountered),[222] zinc sulfate (test concentration not stated) was considered a diagnostic negative. For an agent to be classified as a diagnostic negative, the rate of DNA synthesis must be 60% or less of the control at the time of removal of the agent, and recover to control values in the next 30 to 90 minutes.[223]

The single-cell gel electrophoresis/comet assay was performed to determine the extent of possible zinc sulfate-induced DNA damage, using 6 groups of 8 Swiss albino male mice that received the following oral (intubation) doses of zinc sulfate (in distilled water): 5.70 mg/kg, 8.55 mg/kg, 11.40 mg/kg, 14.25 mg/kg, 17.10 mg/kg, and 19.95 mg/kg.[224] Negative and positive control groups received distilled water and cyclophosphamide (in distilled water), respectively. Significant DNA damage was observed at all doses when compared to controls; a clear, dose-dependent response was noted. A gradual decrease in the tail-lengths, from 48 h post-dosing and onward, was observed, indicating a time-dependent decrease in the DNA damage. It was concluded that zinc sulfate caused significant DNA damage.

A DNA microarray was used to assess transcriptional alterations in human HeLa cells after exposure to 100 µM zinc sulfate.[225] Of 9,182 human genes, expression was increased in 7 genes and decreased in 4 genes by a factor of 2. Four of the 7 upregulated genes were those coding for metallothionein isoforms.

The genotoxicity of zinc sulfate was studied using human lymphocytes and myelogenous leukemia K562 cells, in the presence of zinc and hydrogen peroxide.[226] Zinc sulfate was added to cell suspensions and untreated cultures (culture medium only) served as controls. After pretreatment with zinc sulfate, the cells were incubated with 10 µM hydrogen peroxide. Cell viability was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[227] DNA damage was assessed using the comet assay.[205] In this assay, the mean percentage of comet tail DNA is positively correlated with the level of DNA breakage and/or alkali labile sites in the cell, and negatively correlated with the level of DNA crosslinks.

Zinc sulfate caused a concentration-dependent decrease in cell viability. Except for the highest test concentration of zinc sulfate (1,000 µM), the survival of treated lymphocytes was much better than that of treated leukemia cells. For example, the viability of lymphocytes was 98% at a concentration of 40 µM zinc sulfate, and the viability of K562 cells was

42% at that concentration. The comet assay revealed that zinc sulfate did not induce DNA damage in normal lymphocytes, but DNA damage was pronounced in K5562 cells. At zinc sulfate concentrations ranging from 10 to 100 µM, a strong increase (dose-response) in DNA damage was noted. A mild increase was observed at higher concentrations. Zinc sulfate exerted a protective effect against hydrogen peroxide-induced cytotoxicity and genotoxicity in normal cells, but these effects were enhanced in cancer cells. Zinc sulfate also inhibited the repair of hydrogen peroxide-induced DNA damage in cancer cells. The authors noted that these results suggest that zinc may protect normal cells against DNA-damaging activity and increase DNA–damaging activity in cancer cells.[226]

The effect of zinc sulfate on cell proliferation was investigated using the WIL2-NS human lymphoblastoid cell line.[228] DNA damage was evaluated using the comet assay and the cytokinesis-block micronucleus cytome (CBMN-Cyt) assay. Zinc sulfate was tested at the following concentrations: 0.4, 4.0, 16.0, 32.0, and 100.0 µM. Untreated cultures (0 µM zinc sulfate; i.e., zinc-depleted cells) served as controls. Cell viability was evaluated using the MTT assay, and results indicated that cell growth and viability were decreased in zinc-depleted cells and at concentrations of 32 µM and 100 µM ($p < 0.0001$). In the comet assay, DNA strand breaks were increased in zinc-depleted cells ($P < 0.05$), when compared to cultures treated with zinc sulfate (up to 100 µM). CBMN-Cyt assay results indicated a significant increase ($P < 0.0001$) in the frequency of apoptotic and necrotic cells under zinc-deficient conditions. Increased frequencies ($P < 0.0001$) of micronuclei, nucleoplasmic bridges, and nuclear buds were induced in zinc-depleted cells. However, genome damage was reduced in cultures treated with zinc sulfate (4 µM and 16 µM), suggesting that these concentrations may be optimal for genome stability.

Zinc Sulfate and Copper Sulfate

Zinc sulfate and copper sulfate were evaluated for genotoxicity in the Ames test using *Salmonella typhimurim* strains TA98 and TA100.[229] Both chemicals were tested at doses up to 5,000 µg/plate, with and without metabolic activation, and were classified as non-genotoxic.

Zinc Sulfate and Manganese Sulfate

The genotoxicity of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) and manganese sulfate ($MnSO_4 \cdot H_2O$) was evaluated in the Ames test using *Salmonella typhimurium* strain TA102 without metabolic activation.[230] Both chemicals were tested at concentrations up to 1,000 nM/plate, and the results were negative.

In Vivo

Aluminum Sulfate

The effect of aluminum sulfate on human leukocyte chromosomes *in vitro* was evaluated.[231] Peripheral venous blood was obtained from healthy male and female blood donors of the following age groups (5 males, 5 females/group): 0 to 10 years, 21 to 30 years, and 41 to 50 years. Lymphocyte cultures were prepared and the parameters studied were: mitotic index, proliferation rate index, and frequencies of chromosomal aberrations, micronuclei, and sister chromatid exchanges.

In almost all cultures, treatment with aluminum sulfate increased the frequency of chromosomal aberrations. When compared to respective controls, a statistically significant increase in chromosomal aberrations was associated with treated lymphocytes from females in the oldest age group. The frequency of chromatid-type breaks was increased by treatment, but frequencies of translocations, dicentrics, and rearrangements were not. The frequency of SCEs was increased in all treatment sets, but was statistically significant only in females when compared to the corresponding controls. Treatment induced a significant increase in the frequency of SCEs/cell.[231]

Copper Sulfate

An analytical grade of copper sulfate was injected intraperitoneally into male, inbred Swiss mice (10 to 12 weeks old; 3 per group).[232] Control mice received distilled water. For the study of spermatogonial chromosomes, the animals were killed at 6 h, 24 h, and 48 h post-dosing. For the study of spermatocytic chromosomes, the mice were killed after 56 days of dosing. Chromatid-type gaps and deletions were observed in spermatogonial chromosomes; gaps were more frequent than breaks. A single case of chromatid exchange was also reported. These effects were time-dependent, with a peak at 24 h post-dosing. Only the results noted at 24 h and 48 h post-dosing differed significantly from the control. When compared to controls, an increased incidence of polyploid and aneuploidy cells and autosomal and X/Y univalents was reported for spermatocytic chromosomes. Two cases of translocation multivalents were also induced. None of the individual results in

the treated series differed significantly from the corresponding control value, although the total aberrations frequency was statistically significant. It was concluded that copper sulfate was genotoxic in germ line cells.

The genotoxicity of copper sulfate ($CuSO_4 \cdot 5H_2O$) was evaluated in the following *in vivo* test systems:[233] In the bone marrow chromosome aberrations assay, copper sulfate ($CuSO_4 \cdot 5H_2O$) was injected, i.p. and/or s.c., into inbred Swiss mice at doses of 5, 10, and 20 mg/kg body weight. The acute exposure durations were 6 h, 24 h, and 48 h. For chronic exposure, the highest dose of 20 mg/kg was divided into 5 equal parts. Each part was injected i.p. repeatedly 5 times, and there was a 24 h gap between treatments. The animals were killed 24 h after the last injection. In the sperm abnormality assay, each dose was fractionated into 5 equal parts. Each part was injected i.p. repeatedly 5 times, with an interval of 24 h between treatments. The animals were killed 35 days after the first injection. In the micronucleus assay, each dose was injected i.p. twice, at an interval of 24 h. The animals were killed 6 h after the second injection.

Treatment with copper sulfate ($CuSO_4 \cdot 5H_2O$) produced dose-related increases in micronuclei, bone marrow chromosomal aberrations, and sperm cell abnormalities. The chromosomal aberrations included chromatid gaps, isochromatid gaps, chromatid breaks, fragments, double minutes, exchanges, and rings. Gaps were more frequent than breaks. The highest percentage of micronuclei was reported for polychromatic erythrocytes and, the lowest, for white cells. The micronuclei were either dot or ring-shaped, and size varied from 1/7 to 1/12 of the cell size. The sperm abnormalities included varied head shape, different modes of tail attachments, and double-headed and double-tailed sperm cells.[233]

The genotoxicity of copper sulfate was evaluated in the micronucleus assay using groups of 6 Swiss albino mice per dose administered.[234] The animals were injected i.p. with the test substance ($Cu_2SO_4 \cdot 5H_2O$) at doses ranging from 1.1 to 6.6 mg/kg body weight. The animals were killed at 6 h, 12 h, and 24 h post-dosing. When compared to the negative control, copper sulfate induced a significant increase ($P < 0.05$) in the frequency of chromosomal aberrations in bone marrow cells at all doses administered. The aberrations induced were mainly of the chromatid type. Chromosomal breaks were significantly enhanced only at the highest dose (6.6 mg/kg).

Two mouse bone-marrow micronucleus assays were performed according to a procedure similar to that in the preceding study.[235] In each assay, male CBA mice were dosed i.p. with copper sulfate ($Cu_2SO_4 \cdot 5H_2O$) as follows: 6.6 mg/kg (assay 1: 2 groups, 7 to 8 mice/group; assay 2: 2 groups, 6 mice/group), 13.2 mg/kg (5 mice), and 19.8 mg/kg (6 mice). In assay 1, animals in one of the 6.6 mg/kg dose groups (7 mice) were killed 24 h after dosing; animals in the other group (8 mice) were killed 48 h after dosing. The two 6.6 mg/kg groups in assay 2 were treated similarly. Additionally, 2,000 and 1,000 polychromatic erythrocytes per mouse were evaluated in the 1st and 2nd assays (all dose groups), respectively. In both assays, copper sulfate failed to induce micronuclei in the bone marrow. The authors noted that the inactivity of copper sulfate in this micronucleus assay makes the clastogenic effects observed in the preceding study difficult to explain. Furthermore, they noted that the ages and sexes of the test animals were basically the same in both studies and that it is unlikely that the sample of copper sulfate that yielded positive results was contaminated with a mutagen, although purity data on copper sulfate were not provided. With this in mind, the authors mentioned the possibility of a strain-specific bone-marrow response, for which no precedent exists.

Alkaline single cell gel electrophoresis (comet assay) was used to study single-stranded DNA breaks induced by copper sulfate.[236] Groups of 5 Swiss male albino mice received the following oral doses (by intubation) of copper sulfate: 1.25, 2.50, 5.0., 7.50, 10.0, and 12.50 mg/kg. Samples of whole blood were collected at 24 h, 48 h, and 72 h during week 1 and week 2 post-dosing. Study results indicated statistically significant, dose-dependent DNA damage at all doses, compared to controls. Further evaluation of samples revealed a reduction in the decrease in mean comet tail-length, indicating reduced repair efficiency capacity, compared to controls.

Ferrous Sulfate

The genotoxicity of ferrous sulfate was evaluated in the micronucleus test using groups of 6 ddY mice.[237] The test material (in water) was injected i.p. at a single dose of 25, 50, 100, and 180 mg/kg. An untreated control group was included in the study. The animals were killed at 24 h post-dosing. Ferrous sulfate did not induce micronuclei in bone marrow erythrocytes and was classified as negative in this assay.

The combined effects of dietary iron and ascorbic acid on genotoxicity were studied by measuring the frequency of micronuclei in the bone marrow cells of C3H/He weanling mice (number not stated).[238] The mice were fed a diet containing ferrous sulfate, at a dose of 100 or 300 mg/kg diet, which was supplemented either with or without ascorbic acid (15 g/kg diet). The results indicated that the high ferrous sulfate diet caused an increased frequency of micronucleated polychromatic erythrocytes (MnPCEs), compared to the low ferrous sulfate diet. Ascorbic acid supplementation in the low iron diet did not

affect on the incidence of MnPCEs and, in the high ferrous sulfate diet, protected against the increased frequency of MnPCEs.

Manganese Sulfate

For the chromosomal aberrations assay, groups of 5 Swiss albino mice (*Mus musculus* L. strain; 8 to 10 weeks old) were dosed orally with manganese sulfate ($MnSO_4 \cdot H_2O$; 61, 20.5, or 10.25 mg/100 g body weight) over a period of 3 weeks.[239] Negative control animals received distilled water. For the micronucleus test, groups of 5 mice received the same 3 oral doses (2 of same dose per animal). For the sperm head abnormality assay, groups of 5 mice received the same 3 oral doses for 5 days. Five-hundred sperms per animal were scored for abnormalities.

In the chromosomal aberrations assay, all 3 doses of the test substance produced chromosome breaks and increased the frequencies of micronucleated PCEs and NCEs, compared to controls. Abnormal sperm heads induced at the 3 doses were double heads, amorphous, round, spear, hookless, and giant size. A dose-response study of results from the 3 genotoxicity assays indicated a statistically significant trend. The frequencies of chromosomal aberrations in bone marrow cells, micronuclei, and sperm head abnormalities were statistically significantly increased. The authors noted that these effects were probably mediated by Mn^{2+} ions produced directly from manganese sulfate in gastric juices.

Human

Ferrous Sulfate

A study was performed to evaluate the effects of iron and vitamin C on oxidative damage to DNA in healthy subjects (20 women, 20 men; mean age = 30.3 years).[240] The subjects were co-supplemented with ferrous sulfate (14 mg/day) and ascorbic acid (either 60 mg/day or 260 mg/day). The subjects were divided into 2 groups, i.e., subjects with plasma vitamin C levels of 71.9 µmol/l (Group 1) and 50.4 µmol/l (Group 2), respectively. After 6 weeks of supplementation, a significant increase in several oxidative DNA base damage products and in total oxidative damage in DNA extracted from white blood cells was observed in Group 1. These results were not reported after 12 weeks. In Group 2, pre-supplemental levels of oxidative DNA damage were higher and decreased after supplementation with ferrous sulfate and ascorbate. The authors noted that because oxidative DNA damage has been suggested as a risk factor for the development of cancer, the implications of increased levels of oxidative DNA damage in well-nourished subjects after ferrous sulfate/ascorbate supplementation are of concern, in light of the frequent use of dietary supplements containing both ferrous sulfate and ascorbate.

Antigenotoxicity

Magnesium Sulfate

The effect of magnesium sulfate on metal-induced mutagenicity was evaluated in the Ames test using *Salmonella typhimurium* strain TA97.[218] The preincubation mutagenicity test was performed in triplicate by adding the following, in that order, to tubes incubated for 30 minutes: 500 µl buffer or ddH_20, freshly prepared metal dilutions (50 µl), bacterial cell culture (100 µl), and $MgSO_4$ (50 µl). One of the following metals was included in one of the tubes (final volume = 700 µl), which was incubated for 30 minutes: Co^{++} (up to 800 µM), Fe^{++} (up to 1,000 µM), Mn^{++} (up to 1,200 µM), Zn^{++} (up to 1,000 µM), and Cd^{++} (up to 200 µM). Magnesium sulfate inhibited the mutagenicity of Fe^{++}, Mn^{++}, and Zn^{++}, had only a slight effect on the mutagenicity of Co^{++}, and had no effect on the mutagenicity of Cd^{++}.

Enhancement of Genotoxicity

Ammonium Sulfate

The mutagen ethyl methanesulfonate (20 mM) was added to V79 hamster cell cultures.[241] After 1 h, 300 µl of ammonium sulfate was added to yield a final osmolality of 500, 750, 1,000, or 1,500 mOsm/kg. TG^r mutations were enhanced by ammonium sulfate. The authors suggested that hypertonic salt post-treatment led to conformational changes in the DNA, which resulted in an increase in TG^r mutations and chromosomal aberrations.

CARCINOGENICITY

Animal

Ammonium Sulfate

The carcinogenicity of ammonium sulfate was studied using groups of 50 F344/DuCrj rats (5 weeks old; 25 males, 25 females/group).[84] The animals were fed ammonium sulfate at dietary concentrations of 1.5% and 3.0% for 104 weeks. Control animals were fed a diet that did not contain ammonium sulfate. In all dietary groups, ammonium sulfate did not have any significant influence on the incidences of tumors in any of the organs or tissues examined. Ammonium sulfate was classified as noncarcinogenic in his study.

Calcium Sulfate Hydrate

Gypsum (calcium sulfate hydrate) and other fibrous dusts (chrysotile, glass fibers, nemalite, and palygorcite) and granular dusts (actinolite, biotite, hematite, pectolite, sanidine, and talcum) were injected i.p. into groups of 40 Wistar rats (ages not stated).[242] Pure saline was injected into 80 control Wistar rats. The test dusts were suspended in saline solution at concentrations up to 25 mg/2 ml, and most of the groups received four 25 mg doses i.p. The rats were observed until spontaneous death or until the animals were killed. The time required to produce the first tumor in the group dosed with gypsum was 546 days, and the tumor rate in this group was 5%. Nearly all of the tumors were sarcomatous mesotheliomata. The tumor rate in animals dosed with palygorcite was 65%. Tumors were not observed in the saline control group.

Calcium Sulfate and Magnesium Sulfate

The following types of man-made fibers were administered intratracheally to groups of 20 hamsters (1 fiber per group): calcium sulfate (diameter = 1.0 µm; length = 17.8 µm) and magnesium sulfate (diameter = 0.45 µm; length = 22.4 µm).[243] Intratracheal administration involved a dose of 2 mg per animal weekly for 5 weeks (total of 10 mg/animal). Tumors were observed in 9 of 20 hamsters dosed with magnesium sulfate and in 3 of 20 hamsters dosed with calcium sulfate. Tumors were not observed in the control group. The primary sites of the tumors were not only in the pleural cavity, but also in the intracelial organs, kidney, adrenal gland, bladder, and uterus. Only a few tumors were identified as mesotheliomas at histological examination. The following changes were observed in the lungs: fibrosis, pleural thickening, and chronic inflammatory changes. However, these changes appeared to have been too mild to promote the development of pneumoconiosis.

Copper Sulfate

A case-control study was performed to examine possible associations between occupational and environmental risk factors and renal cell cancer (RCC).[244] The study consisted of 100 histologically verified cases of RCC and 200 controls. Regarding all exposure variables under study, 2 levels of duration were defined as short and prolonged, for less than 10 years or more, respectively. The highest risk estimates for RCC were found for prolonged exposure to organic solvents (odds ratio [OR] of 2.2; 95% confidence interval [CI] of 1.0-4.8). Prolonged exposures to pesticides (OR = 2.2; 95% CI of 0.8-4.7) and copper sulfate (OR = 2.7; 95% CI of 1.3-5.5) were also associated with increased risk for RCC. The author noted that these data suggest an association between RCC and exposure to organic solvents, pesticides, and copper sulfate. A risk gradient as a function of exposure duration was found for organic solvents ($p = 0.044$) and copper sulfate ($p = 0.036$), but not for pesticides.

Hydroxylamine

The carcinogenicity of hydroxylamine was evaluated using groups of female C3H/NeN mice (4 weeks old).[87] The following groups received the test substance, in drinking water: 12 weeks (8 rats), 20 weeks (8 rats; hydroxylamine for 12 weeks, then water for 8 weeks) , 36 weeks (8 rats; hydroxylamine for 12 weeks, then water for 18 weeks), and 52 weeks (5 rats; hydroxylamine for 52 weeks). Groups of 4 to 5 rats (controls) received water. The 4 positive control mice received urethane (10 mM) in drinking water for 17 weeks. Exposure to hydroxylamine did not have a great influence on body or liver weights, but caused remarkable splenomegaly, a decrease in the red blood cell count, and an increase in the white blood cell count. These effects were reversible. Approximately 50% of the mice that received hydroxylamine for 52 weeks had sizable areas of bone formation in the spleen. This finding was not observed in mice receiving the test substance for shorter periods of time. Hydroxylamine did not induce tumor formation in any of the groups. It was noted that spontaneous mammary tumors were not observed, even in animals that survived for 2 years. All 4 positive control (urethane) mice had numerous lung adenomas at the end of 17 weeks.

Manganese Sulfate

The carcinogenicity of manganous sulfate ($MnSO_4 \cdot 4H_2O$) was evaluated using groups of 40 mice of the A/Strong strain (20 males, 20 females; 6 to 8 weeks old).[245] In the 3 test groups, each animal was injected i.p. with the test substance (in 0.85% saline solution) 3 times per week for a total of 24 injections. The 3 dose groups received the maximum tolerated dose (MTD), a 1:2 dilution of the MTD, and a 1:5 dilution of the MTD. The total dose per mouse (3 injections total per week) in the 3 groups amounted to 660 mg/kg, 330 mg/kg, and 132 mg/kg, respectively. The mice were killed at 30 weeks after the last injection and lungs were removed for the counting and histopathologic examination of lung nodules. Other organs examined at necropsy were as follows: liver, intestines, thymus, kidney, spleen, salivary gland, and endocrine glands. The 4 control groups were defined as follows: (1) mice receiving 24 i.p. injections of either 0.85% saline solution or tricaprylin alone; (2) animals given a single i.p. injection of carcinogen urethane (20 mg/mouse); and (3) untreated mice maintained along with the test groups. A comparison of the tumor responses in untreated and vehicle control mice indicated that the occurrence of lung tumors was not significantly affected by the injections. No tumors other than lung adenomas were observed in the controls. Manganous sulfate produced a significant ($p < 0.05$) increase in the lung tumor response when compared to appropriate controls. There was a well-defined dose-response between the middle and high doses, though the low dose produced tumor responses that were similar to the middle dose. The authors noted that the dose of manganous sulfate that was required for the 1 tumor per mouse response was 3.3 mmoles/kg. Neoplasms other than lung tumors were not observed in any of the 3 manganous sulfate dose groups.

Manganese (II) sulfate mononhydrate was evaluated in a National Toxicology Program (NTP) 2-year carcinogenicity study involving F344/N rats and $B6C3F_1$ mice (41-day old rats and mice).[246] Groups of 70 male and 70 female rats were fed diets containing 0,1,500, 5,000, or 15,000 ppm manganese (II) sulfate monohydrate. Feeding at these concentrations resulted in daily ingestion of 60, 200, or 615 mg/kg body weight (males) or 70, 230, or 715 mg/kg body weight (females). When compared to the control group, the survival of male rats fed 15,000 ppm was significantly lower. The deaths of males in the control and exposure groups were attributed to a variety of spontaneous neoplastic and nonneoplastic lesions. However, the greater number of deaths in the 15,000 ppm group was due to increased incidences of advanced renal disease that was related to ingestion of manganese (II) sulfate monohydrate. The survival of exposed females was similar to that of the controls. There were no clinical findings or differences in hematology and clinical chemistry that were related to dosing with the test material. At both 9-month and 15-month interim evaluations, tissue concentrations of manganese were significantly elevated in the livers of male and female rats exposed to 5,000 ppm and 15,000 ppm concentrations of the test material, with an accompanying depression of hepatic iron.

The 15,000 ppm concentration was associated with a marginal increase in the average severity of nephropathy in male rats, which was accompanied by significantly increased incidences of mineralization of the blood vessels and glandular stomach, parathyroid gland hyperplasia, and fibrous osteodystrophy of the femur. These lesions were identified as manifestations of renal failure, uremia, and secondary hyperparathyroidism. The reduced survival of male rats fed the 15,000 ppm concentration was attributed to the increased incidence of advanced renal disease. No increase in the incidence of neoplasms in male or female rats was attributed to the ingestion of diets containing manganese (II) sulfate monohydrate.[246]

Groups of 70 male and 70 female mice were fed diets containing 0, 1,500, 5,000, or 15,000 ppm manganese (II) sulfate monohydrate. Feeding at these concentrations resulted in an average daily ingestion of 160, 540, or 1,800 mg/kg body weight (males) or 200, 700, or 2,250 mg/kg body weight (females). When compared to the control group, survival rates of exposed male and female mice were similar. No clinical findings were attributed to dosing with manganese (II) sulfate monohydrate. There were no clinical findings or differences in hematology and clinical chemistry that were related to dosing with the test material. At 9-month and 15-month interim evaluations, tissue concentrations of manganese were significantly elevated in the livers of animals exposed to concentrations of 5,000 ppm or 15,000 ppm. Hepatic iron levels were significantly lower in female mice (9-month interim evaluation). At the 15-month interim evaluation, this finding was reported for male mice exposed to 5,000 ppm or 15,000 ppm and female mice of all exposure groups. When compared to controls, the incidences of thyroid follicular dilatation and hyperplasia were significantly higher in male and female mice exposed to 15,000 ppm. Follicular cell adenomas occurred in one male mouse (15-month interim evaluation) and in 3 male mice (at the end of study) exposed to 15,000 ppm, but not in the control or lower exposure groups. Follicular cell adenomas were also observed in 2 controls, 1 female mouse exposed to 1,500 ppm, and in 5 female mice exposed to 15,000 ppm at the end of the study. The authors noted uncertainty as to whether the slightly increased incidence of follicular cell adenoma was related to the ingestion of manganese (II) sulfate monohydrate.[246]

The conclusions for this NTP carcinogenicity study were stated as follows: There was no evidence of carcinogenic activity of manganese (II) sulfate monohydrate in male or female F344/N rats receiving 1,500, 5,000, or 15,000 ppm. There was equivocal evidence of carcinogenic activity of manganese (II) sulfate monohydrate in male and female $B6C3F_1$ mice,

based on the marginally increased incidences of thyroid gland follicular cell adenoma and the significantly increased incidences of follicular cell hyperplasia.[246]

Magnesium Sulfate

The tumorigenicity of magnesium sulfate fibers was evaluated using 20 female Syrian hamsters (ages not stated).[247] Each 500 mg of fiber was suspended in 50 ml of sterilized saline with 0.25 g of sodium carboxymethylcellulose to delay fiber sedimentation. The magnesium sulfate fiber suspension was sonicated and then injected intratracheally (0.2 ml/animal) once per week for 5 weeks. Vehicle alone was administered to 20 control hamsters according to the same procedure. At 2 years post-administration, the animals were killed and necropsy performed. Due to solubility, magnesium sulfate fibers could not be detected in the lung tissue of hamsters at 2 years post-administration. There were 9 tumor-bearing animals in the group dosed with magnesium sulfate fibers, and the tumors were defined as follows: adrenal gland (a neuroblastoma, a cortical adenoma, and A & B cell tumor), pleural mesothelioma (2 epithelial types), kidney (a malignant histocytoma and an anaplastic tumor), lung (1 tumor, unspecified cell type), uterus (1 leiomyosarcoma), and bladder (1 leiomyoma). Malignant histocytoma of the kidney and leiomyosarcoma of the uterus were observed in the same hamster. Tumors were not observed in vehicle control hamsters.

Zinc Sulfate

A group of 15 adult male Sprague-Dawley rats (50 days old) received zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) in drinking water at a dose of 227 mg Zn per liter for 20 weeks.[248] The 15 control rats did not receive the test material. At week 20, the animals were killed and prostate tissue was excised. Dosing with the test material induced prostate intraepithelial neoplasm (PIN) in both lobes (incidences of 46.7% and 40%, respectively). This difference in PIN prevalence was found to be statistically significant for both lobes ($P = 0.01$ and 0.03, respectively). Microscopic examination did reveal prostate adenocarcinoma. PIN was not observed in control animals.

Human

Calcium Sulfate Hydrate

A multicancer site, multifactor case-control study was performed to generate hypotheses about possible occupational carcinogens.[249] Interviews were carried out with eligible cases, males in the 35- to 70-year age range. The interview was designed to obtain detailed lifetime job histories and information on potential confounders. Each job history was translated into a history of occupational exposures, and these exposures were analyzed as potential risk factors in relation to the sites of cancer included. For each site of cancer analyzed, the controls were selected from among the other sites in the study. Odds ratio (OR) estimates and corresponding 90% confidence intervals for each association were based on logistic regression models. Model 1 included the variables age, ethnic group, socioeconomic status, smoking, blue/white collar job history, asbestos exposures, and the database set of confounders unique to each association, except for other inorganic dusts. The cumulative exposure index was cut at the median to create an exposure level that was referred to as substantial. In determining the OR, exposure was defined as substantial exposure. For the 55 cases exposed to gypsum dust, an OR of 1.4 for nonadenocarcinoma lung cancer (NAC) was reported. The authors noted that the associations between NAC and gypsum were reduced once all cofounders were included in the regression model, although the odds ratio using model 1 (OR_1) for gypsum was suggestively high.

Co-carcinogenicity

Ferrous Sulfate

Ten weanling Sprague-Dawley rats were fed a 0.55% ferrous sulfate diet for 15 days prior to the first of 23 subcutaneous injections of the carcinogen 1,2-dimethylhydrazine.[250] The incidences of colon and small intestine tumors (adenocarcinomas) were not statistically significantly different from that observed in the 14 animals fed control diet.

Anticarcinogenicity

Zinc Sulfate

The effect of zinc sulfate on 1,2-dimethylhydrazine (DMH)-induced carcinogenesis was evaluated using the following 4 groups of 6 male Sprague-Dawley rats:[251] Group 1 (normal controls; received water and diet *ad libitum* and

dosed s.c. with 1 mM EDTA-saline); Group 2 (weekly s.c. injections of DMH (in 1 mM EDTA-normal saline [pH 6.5] at doses of 30 mg/kg body weight for 16 weeks); Group 3 ($ZnSO_4 \cdot 7H_2O$ [in drinking water *ad libitum*] at dose of 227 mg/L drinking water); and Group 4 (combined treatment of DMH as well as zinc in a manner similar to the protocol for groups II and IV, respectively). Significantly increased levels of reduced glutathione (GSH) and glutathione reductase (GR) were observed in rats treated with DMH. The administration of zinc sulfate to DMH-treated rats significantly decreased the tumor incidence, tumor size, and aberrant crypt foci number, with simultaneous enhancement of lipid peroxidation, SOD, catalase, and glutathione-*S*-transferase. Furthermore, the levels of GSH and GR were also decreased after zinc sulfate supplementation involving DMH-treated rats. Further, the administration of zinc sulfate to DMH-treated rats restored normalcy in the colonic histoarchitecture, and these rats exhibited no signs of neoplasia. A significant decrease in tissue concentrations of zinc in the colon after DMH treatment was detected. Zinc supplementation resulted in recovery to near normal levels of zinc. It was concluded that zinc sulfate had a positive, beneficial effect against chemically (DMH)-induced colonic preneoplastic progression in rats.

OTHER EFFECTS

Immunosuppression

Ferrous Sulfate

An *in vitro* model (Mishell-Dutton culture) for evaluation of the humoral immune response of mice spleen cells to sheep red blood cells (SRBC) was used to study the immunosuppressive effect of ferrous sulfate.[252] Ferrous sulfate (0.1 mM) in Mischell-Dutton culture significantly decreased ($p \leq 0.01$) the SRBC antibody forming cell (AFC) response by approximately 63% of the control (phosphate buffer solution) value.

Effect on Erythropoiesis

Aluminum Sulfate

A study was performed to investigate the effects of chronically-administered aluminum sulfate (in physiological saline) on erythropoiesis using two groups of 12 male Wistar rats (12 weeks old).[253] One of the groups was injected i.p. with aluminum sulfate solution (50 µmol of Al/kg body weight; dose volume/injection = 1 ml/kg). Injections were made 5 times per week for 3 months. The other group (control) was injected i.p. with physiological saline solution (1 ml/kg). When compared to the control group, aluminum sulfate caused a significant decrease in hemoglobin concentration (32% decrease) and hematocrit (24% decrease). Serum iron decreased in the test group, whereas total iron binding capacity did not change.

Effect on Melanogenesis

Zinc Sulfate

A study was performed to determine whether excess zinc ions affect hair follicle melanogenesis *in vivo*. Zinc sulfate ($ZnSO_4 \cdot 7H_2O$) was administered orally (continually in drinking water) to C57BL/6 α/α mice (3 to 5 mice; 6 to 8 weeks old) during spontaneous and depilation-induced hair follicle cycling.[254] The test material was administered at a concentration of 20 mg/ml in drinking water (mean daily dose = 1.2 ± 0.53 ml). Control mice (3 to 5 animals) received drinking water. Oral dosing with zinc sulfate induced a bright brown lightening of new hair shafts produced during anagen, but did not induce an electron paramagnetic resonance (EPR)-detectable switch from eumelanogenesis to phaeomelanogenesis. Additionally, the total content of melanin in the skin and hair shafts during the subsequent telogen phase (i.e., after completion of a full hair cycle) was significantly reduced ($P = 0.0005$) in mice dosed with zinc sulfate. When compared to control mice, melanin granules in precortical hair matrix keratinocytes, hair bulb melanocytes, and hair shafts of zinc sulfate-treated mice were reduced and poorly pigmented. Over the course of several hair cycles, lasting hair shaft depigmentation was observed. It was concluded that high-dose, oral zinc sulfate was a potent downregulator of eumelanin content in murine hair shafts *in vivo*.

Effect on Cell Proliferation

Zinc Sulfate

The influence of 14 µM zinc sulfate (14 µM = average serum zinc concentration) on the physiology of normal human kerationcytes was evaluated *in vitro*.[255] After 9 days of incubation, zinc sulfate-treated cells showed enhanced proliferation at microscopic examination, when compared to untreated control cells. The treatment of keratinocytes with zinc

sulfate resulted in the formation of typical cell monolayers, which differed from the untreated controls by the degree of confluence. Treated cells formed complete monolayers, but the controls displayed approximately 40 to 50% confluence at the same time.

Modulation of Hormonal Effect

Zinc Sulfate

The anabolic effect of 17-β-estradiol in osteoblastic MC3T3-E1 cells was studied. These cells were cultured for 3 days in medium containing either vehicle (unnamed) or 17-β-estradiol (10^{-11} to 10^{-9} M).[256] Alkaline phosphatase activity and the cellular protein concentration were increased in the presence of 17-β-estradiol. At a concentration of 10^{-9} M 17-β-estradiol, significant elevation of cell numbers and cellular DNA content was noted. The 17-β-estradiol (10^{-10} or 10^{-11} M)-induced stimulation of alkaline phosphatase activity and cellular protein concentration were significantly increased in the presence of 10^{-5} M zinc sulfate.

Lipid Peroxidation

Ferrous Ammonium Sulfate

The addition of ferrous ammonium sulfate (200 μM) to a suspension of whole rat brain homogenate in Krebs buffer caused oxidative injury.[257] Tissue vitamin E dropped sharply over a 30-second interval and then recovered marginally for 5 minutes. After 5 minutes, vitamin E levels dropped to a low and constant level. Additionally, after 5 minutes, thiobarbituric acid reactive substances (a color test for lipid peroxidation) indicated a statistically significant ($P \leq 0.05$) increase that continued for the remainder of the 30-minute experiment. At 15 minutes after addition of ferrous ammonium sulfate, a statistically significant decrease ($P \leq 0.05$) in reduced protein thiols was observed. The authors noted that these results suggest that, in this model of iron-initiated lipid peroxidation, the endogenous antioxidant vitamin E is initially depleted before membrane lipids and membrane-bound proteins show evidence of oxidative injury.

SUMMARY

The inorganic sulfates reviewed in this safety assessment function mostly as astringents, opacifying agents, skin conditioning agents, and viscosity increasing agents in cosmetic products. In addition to these, other ingredient functions associated with this group include cosmetic biocide (zinc sulfate) and skin bleaching agent (calcium sulfate hydrate). Furthermore, sodium bisulfate functions only as a pH adjuster and ferrous ammonium sulfate functions only as a pesticide in cosmetics.

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2013, the following inorganic sulfates are being used in cosmetic products: aluminum sulfate, ammonium sulfate, barium sulfate, calcium sulfate, copper sulfate, magnesium sulfate, manganese sulfate, potassium sulfate, sodium bisulfate, and zinc sulfate. Results from a survey of ingredient use concentrations provided by the Personal Care Products Council in 2013 indicate that these ingredients are being used at concentrations up to 49% (magnesium sulfate, in bath capsules diluted for bath use). The next highest maximum use concentration reported is 37%, for barium sulfate in lipstick (leave-on product).

Considerable binding of aluminum by blood cells was observed in rats dosed i.v. with aluminum sulfate. Barium was detected in the bone and lungs of rats after inhalation of barium sulfate. Manganese sulfate was distributed systemically (brain uptake included) in rats and rhesus monkeys after inhalation exposure.

Following topical application of zinc sulfate or copper sulfate to human skin *in vitro*, an increase in zinc and copper concentrations in whole skin and in the epidermis was observed.

After oral dosing of pregnant rats with $^{59}FeSO_4$, large amounts of ^{59}Fe were transferred from maternal plasma to the fetuses late in gestation. Magnesium sulfate and manganese sulfate crossed the placenta and entered the fetal brain and other tissues, following s.c. injection into pregnant rats. In another study, the feeding of rats with ferrous sulfate resulted in the accumulation of iron in the liver, spleen, and kidneys in a dose-dependent manner. More than 9% of i.v.-administered

hydroxylamine sulfate was cleared from the blood of rats almost immediately after injection; it appeared that the blood contained an acid-labile derivative of hydroxylamine, acetohydroxamic acid. Urinary excretion was very low and did not exceed 1% of the administered dose in rabbits dosed i.v. with zinc sulfate. In rats dosed i.p. with zinc sulfate, the injected zinc had been completed excreted from the tissues by 3 weeks.

The bioavailability of iron in ferrous sulfate preparations, film-coated and enteric-coated tablets and oral solution, was studied in humans. Bioavailability of iron in the enteric-coated preparations was low, relative to that of the film-coated products and the oral solution. In eclamptic patients dosed i.v. or intramuscularly with magnesium sulfate, cumulative renal excretions ranged from 38% to 53% of the injected dose at the end of 4h. Urinary excretion of inorganic sulfate (30.2 ± 17.2% of administered dose) was noted in healthy male subjects during the first 24h after oral dosing with magnesium sulfate. Excretion during the next 48h was negligible. In psoriasis patients treated orally with zinc sulfate, 75% of the doses ingested had been excreted upon completion of the 2-month study.

Following acute inhalation exposure to increasing concentrations of aluminum sulfate or copper sulfate, differences in mortality between experimental and control mice increased linearly with increasing exposure concentration. In guinea pigs exposed (inhalation, acute) to ammonium sulfate, copper sulfate, or sodium sulfate, the order of irritant potency in the respiratory tract was: ammonium sulfate > ammonium bisulfate > copper sulfate. Guinea pigs exposed to ammonium sulfate (inhalation, acute) did not develop labored breathing and there were no significant differences in respiratory frequency. Copper sulfate produced acute inflammatory responses in the lungs of rats at a dose of 5 µg/rat. None of the mice exposed to zinc sulfate aerosol ($\geq$ 1.2 mg/m^3) died.

At concentrations up to 1.0 mg/m^3 during acute inhalation exposure, neither ammonium sulfate nor sodium bisulfate caused a significant reduction in specific airway conductance and flow rates in human subjects. Acute inhalation exposure to ammonium sulfate (100 µg/m^3) produced little or no evidence of adverse health effects in another group of human subjects. The acute inhalation exposure of human subjects to ferric sulfate aerosol (75µg/m^3) did not cause significant changes in respiratory system resistance or forced expiratory volume.

The following acute oral toxicity LD_{50} values were reported: aluminum sulfate (> 9,000 mg/kg, mice and rats), ammonium sulfate (3,040 mg/kg body weight, rats), barium sulfate (307 g/kg, rats), copper sulfate (369 mg/kg [mice]; 794 mg/kg [rats]), ferrous sulfate (1,025 mg/kg [mice]; 2,625 mg/kg [rats]), hydroxylamine sulfate (545 mg/kg, rats), manganese sulfate (2,330 mg/kg [mice]; 2,150 mg/kg [rats]), and zinc sulfate (422 mg/kg [mice]; 1,710 mg/kg [rats]).

Following acute dermal exposure to 0.5 g/kg and 0.1 g/kg hydroxylamine sulfate (under plastic cover) in rabbits, 90% and 20% of the rabbits died, respectively. There were no mortalities in rabbits exposed to 1.0 g/kg hydroxylamine sulfate under gauze.

The following inorganic sulfates were inhaled repeatedly in studies involving rats: ammonium sulfate, barium sulfate, calcium sulfate, copper sulfate, magnesium sulfate, manganese sulfate, and zinc sulfate. No remarkable adverse effects were observed. Particularly exposure to ~ 300 mg/m^3 ammonium sulfate, among the higher exposure concentrations evaluated, for up to 14 days did not induce death or any detectable toxicological effects in rats. There were no significant changes in pulmonary function in subjects exposed to ammonium sulfate aerosol (1.0 ± 0.05 µm) for 3 consecutive weeks.

Toxic effects observed in repeated dose oral toxicity studies on aluminum sulfate, ammonium sulfate, copper sulfate, and ferrous sulfate were as follows: A significant decrease in the number of red blood cells was observed in rats that received 30 mM aluminum sulfate in sodium citrate for 6 months. A significant increase in kidney and/or liver weights (without any effects on histopathological parameters) was observed in rats that received 3% ammonium sulfate in the diet for 52 weeks. When administered in drinking water at concentrations of 3,000 to 30,000 ppm, copper sulfate was lethal to rats and mice within 2 weeks. Intestinal mucosal irritation was observed in the lower GI tract of rats that received ferrous sulfate (25 mg/kg/day) in drinking water for up to 12 months. The incidence of gastrointestinal side effects in infants that received ~3 mg ferrous sulfate/kg/day for 3 months was no greater than in infants that received placebo therapy.

Following administration of 20 mM hydroxylamine sulfate, in drinking water, to mice for 52 weeks, bone formation was observed in the spleens of approximately 50% of the animals. The maximum no-effect-level of zinc sulfate was 3,000 ppm in mice that received zinc sulfate in the diet at concentrations up to 30,000 ppm for 13 weeks.

In a neurotoxicity study involving mice, the administration of 2.5% aluminum sulfate in tap water for up to 12 months caused a reduction in the neuronal expression of the GRP78 stress-response protein, a finding that was similar to what has previously been observed in Alzheimer's disease. The results of a study in which monkeys were exposed to manganese sulfate (10 to 15 mg/kg/week) for 272 ± 17 days suggested that chronic exposure may have detrimental effects on behavior, cognition, and motor function.

Intravitreally-injected copper sulfate (30 µg/eye) produced ocular chalcosis, the signs of which included hemorrhage and iridial ischemia. Copper sulfate was classified as non-irritating to the skin and buccal mucosa of rats at concentrations up to 25% aqueous. Topical application of hydroxylamine sulfate (under occlusion) at doses up to 1.0 g/kg caused skin irritation and edema. The occurrence of edema was described as random and not dose-related. Reactions ranging from mild to no edema at intact sites and severe necrosis and mild edema at abraded sites were observed after application of sodium bisulfate under an occlusive patch.

Copper sulfate was classified as allergenic in the guinea pig maximization test at challenge concentrations up to 1% in petrolatum. Rats sensitized to 2% copper sulfate, followed by buccal mucosal elicitation with copper sulfate, showed no response over the range of test concentrations (up to 25% aqueous). A low incidence of sensitization was observed in dermatitis/eczema patients patch tested with copper sulfate, ferrous ammonium sulfate, or ferrous sulfate. Sensitization reactions to inorganic sulfates have also been identified in various case reports.

The following inorganic sulfates were evaluated in animal reproductive and developmental toxicity studies: aluminum sulfate, calcium sulfate, copper sulfate, ferrous sulfate, magnesium sulfate, manganese sulfate, sodium sulfate, and zinc sulfate. Results relating to reproductive and developmental toxicity potential were mixed. Studies involving ferrous sulfate or zinc sulfate supplementation during pregnancy were negative for adverse effects on fetuses. Results were mixed regarding adverse outcomes in the infants of mothers dosed with magnesium sulfate during pregnancy.

Inorganic sulfates evaluated for genotoxicity in *in vitro* or *in vivo* assays were as follows: aluminum sulfate, barium sulfate, calcium sulfate hydrate, copper sulfate, ferrous ammonium sulfate, ferrous sulfate, hydroxylamine sulfate, magnesium sulfate, manganese sulfate, silver sulfate, and zinc sulfate. Results in both *in vitro* and *in vivo* assays were mixed.

Ammonium sulfate was classified as noncarcinogenic in rats that received dietary concentrations up to 3% for 104 weeks. When gypsum (calcium sulfate hydrate) dust in saline was injected i.p. (four 25 mg doses) into 40 rats, a tumor rate of 5% was reported and nearly all of the tumors were sarcomatous mesotheliomata. Tumor formation was not induced in groups of 8 rats exposed to hydroxylamine in drinking water for up to 52 weeks. Manganese sulfate produced a significant ($p < 0.05$) increase in the lung tumor response in groups of 40 mice, when compared to appropriate controls, in a study that involved i.p. injections (doses up to 660 mg/kg) 3 times per week for a total of 24 injections. In an NTP 2-year carcinogenicity study, there was no evidence of carcinogenic activity of manganese (II) sulfate monohydrate in male or female F344/N rats receiving 1,500, 5,000, or 15,000 ppm. There was equivocal evidence of carcinogenic activity of manganese (II) sulfate monohydrate in male and female $B6C3F_1$ mice, based on the marginally increased incidences of thyroid gland follicular cell adenoma and the significantly increased incidences of follicular cell hyperplasia.

The tumorigenicity of magnesium sulfate fibers was evaluated using 20 hamsters. The fiber (500 mg) suspension was injected intratracheally once per week for 5 weeks. Dosing resulted in 9 tumor-bearing animals. A group of 15 rats received zinc sulfate in drinking water (227 mg/liter) for 20 weeks. Prostate intraepithelial neoplasm was observed in both lobes of the prostate (incidences of 46.7% and 40%, respectively).

In a case-control study (100 cases; 200 controls), an association between renal cell cancer and exposure to copper sulfate was made. A risk gradient as a function of exposure duration was found for copper sulfate ($p = 0.036$).

Figure 2. Formulas of the ingredients reviewed in this safety assessment.[5]

Aluminum Sulfate	$Al_2(SO_4)_3$
Ammonium Sulfate	$(NH_4)_2SO_4$
Barium Sulfate	$BaSO_4$
Calcium Sulfate	$CaSO_4$
Calcium Sulfate Hydrate	$CaSO_4 \cdot xH_2O$
Copper Sulfate	$CuSO_4$
Ferric Sulfate	$Fe_2(SO_4)_3$
Ferrous Ammonium Sulfate	$Fe(NH_4)_2(SO_4)_2$
Ferrous Sulfate	$FeSO_4$
Hydroxylamine Sulfate	$(NH_2OH)_2 \cdot H_2SO_4$
Magnesium Sulfate	$MgSO_4$
Manganese Sulfate	$MnSO_4$
Potassium Sulfate	K_2SO_4
Silver Sulfate	Ag_2SO_4
Sodium Bisulfate	$NaHSO_4$
Sodium Sulfate	Na_2SO_4
Zinc Sulfate	$ZnSO_4$

Table 1. Definitions and functions of the ingredients in this safety assessment.[5]
(The italicized text below represents additions made by CIR staff.)

Ingredient/CAS No.	Definition	Function
Aluminum Sulfate 10043-01-3 17927-65-0	is the inorganic salt that conforms to the formula. *Aluminum Sulfate is the aluminum salt of sulfuric acid.*	Antiperspirant Agents; Cosmetic Astringents; Deodorant Agents; Drug Astringents - Skin Protectant Drugs
Ammonium Sulfate 7783-20-2	is the inorganic salt that conforms to the formula. *Ammonium Sulfate is the di-ammonium salt of sulfuric acid.*	Viscosity Increasing Agents - Aqueous
Barium Sulfate 7727-43-7	is the inorganic salt that conforms to the formula. *Barium Sulfate is the barium salt of sulfuric acid.*	Opacifying Agents
Calcium Sulfate 10034-76-1 10101-41-4	is the inorganic salt that conforms to the formula. *Calcium Sulfate is the calcium salt of sulfuric acid.*	Abrasives; Bulking Agents; Opacifying Agents
Calcium Sulfate Hydrate 13397-24-5	is the inorganic salt that conforms to the formula. *Calcium Sulfate Hydrate is the hydrated calcium salt of sulfuric acid.*	Abrasives; Anticaking Agents; Binders; Cosmetic Astringents; Opacifying Agents; Skin Bleaching Agents; Skin-Conditioning Agents - Occlusive; Surface Modifiers
Copper Sulfate 7758-98-7	is the copper salt of sulfuric acid that conforms to the formula. *Copper Sulfate is the copper (II) salt of sulfuric acid.*	Not Reported
Ferric Sulfate 10028-22-5	is the inorganic salt that conforms to the formula. *Ferric Sulfate is the iron (III) salt of sulfuric acid.*	Skin-Conditioning Agents - Humectant
Ferrous Ammonium Sulfate 10045-89-3	is the inorganic salt that conforms to the formula. *Ferrous Ammonium Sulfate is the iron (II) ammonium salt of sulfuric acid.*	Pesticides
Ferrous Sulfate 7720-78-7 7782-63-0 (heptahydrate)	is the inorganic salt that conforms to the formula. *Ferrous Sulfate is the iron (II) salt of sulfuric acid.*	Not Reported
Hydroxylamine Sulfate 10039-54-0	is the amine salt that conforms to the formula. *Hydroxylamine Sulfate is the di(hydroxylamine) salt of sulfuric acid.*	Antioxidants
Magnesium Sulfate 18939-43-0 7487-88-9	is the inorganic salt that conforms to the formula. *Magnesium Sulfate is the magnesium salt of sulfuric acid.*	Bulking Agents
Manganese Sulfate 10034-96-5 10124-55-7 7785-87-7	is the inorganic salt that conforms to the formula. *Magnesium Sulfate is the magnesium salt of sulfuric acid.*	Skin-Conditioning Agents - Miscellaneous
Potassium Sulfate 7778-80-5	is the inorganic salt that conforms to the formula. *Potassium Sulfate is the potassium salt of sulfuric acid.*	Viscosity Increasing Agents - Aqueous
Silver Sulfate 10294-26-5	is the inorganic salt that conforms to the formula. *Silver Sulfate is the silver (I) salt of sulfuric acid.*	Not Reported
Sodium Bisulfate 7681-38-1	is the inorganic salt that conforms to the formula. *Sodium Bisulfate is the sodium salt of hydrogen sulfate.*	pH Adjusters
Zinc Sulfate 7446-19-7 (monohydrate) 7446-20-0 (heptahydrate) 7733-02-0 (anhydrous)	is the inorganic salt that conforms to the formula. *Zinc Sulfate is the zinc (II) salt of sulfuric acid.*	Cosmetic Astringents; Cosmetic Biocides; Oral Care Agents

Table 1. Definitions and functions of the ingredients in this safety assessment.[5]
(The italicized text below represents additions made by CIR staff.)

Ingredient/CAS No.	Definition	Function
Sodium Sulfate 7727-73-3 (decahydrate) 7757-82-6	is the inorganic salt that conforms to the formula. *Sodium Sulfate is the sodium salt of sulfuric acid.*	Viscosity Increasing Agents - Aqueous
Ammonium Persulfate 7727-54-0	is the inorganic salt that conforms to the formula. *Ammonium Persulfate is the tetra-ammonium salt of sulfuric acid.*	Oxidizing Agent
Potassium Persulfate 7727-21-1	is the inorganic salt that conforms to the formula. *Potassium Persulfate is the potassium salt of peroxydisulfate.*	Oxidizing Agent
Sodium Persulfate 7775-27-1	is the inorganic salt that conforms to the formula. *Sodium Persulfate is the sodium salt of peroxydisulfate.*	Oxidizing Agent

Table 2. Properties of Inorganic Sulfates.[6]

Molecular Weight	342.15 (aluminum sulfate) 132.14 (ammonium sulfate) 233.39 (barium sulfate) 136.14 (calcium sulfate) 284.05 (ferrous ammonium sulfate) 151.91 (ferrous sulfate) 164.14 (hydroxylamine sulfate) 120.37 (magnesium sulfate) 151.00 (manganese sulfate) 174.26 (potassium sulfate) 311.80 (silver sulfate) 120.06 (sodium bisulfate) 142.04 (sodium sulfate) 161.47 (zinc sulfate)
Form	white crystals (aluminum sulfate) orthorhombic crystals (ammonium sulfate) polymorphous crystals (barium sulfate) orthorhombic crystals (calcium sulfate) rhombic crystals (copper sulfate) blue-green crystals (ferrous ammonium sulfate) yellow crystalline powder (ferrous sulfate) crystals (hydroxylamine sulfate) efflorescent crystals (magnesium sulfate) red efflorescent crystals (manganese sulfate) white crystals (potassium sulfate) crystals (silver sulfate) crystals (sodium bisulfate) efflorescent crystals (sodium sulfate) powder or granules (zinc sulfate)
Solubility	soluble in H_2O; insoluble in alcohol (aluminum sulfate) soluble in H_2O; insoluble in alcohol (ammonium sulfate) soluble in H_2SO_4; insoluble in H_2O (barium sulfate) slightly soluble in H_2O; insoluble in organic acids (calcium sulfate) practically insoluble in water (copper sulfate) soluble in H_2O; insoluble in alcohol (ferrous ammonium sulfate) soluble in H_2O; insoluble in alcohol (ferrous sulfate) soluble in H_2O (hydroxylamine sulfate) soluble in H_2O; sparingly soluble in alcohol (magnesium sulfate) soluble in H_2O; insoluble in alcohol (manganese sulfate) soluble in H_2O; insoluble in alcohol (potassium sulfate) slowly soluble in H_2O (silver sulfate) soluble in water (sodium bisulfate) soluble in water (sodium sulfate) soluble in water; practically insoluble in alcohol (zinc sulfate)

Table 3. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[10,11]

	Aluminum Sulfate		Ammonium Sulfate		Barium Sulfate	
	# of Uses	Conc. (%)	# of	Conc. (%)	# of	Conc. (%)

			Uses		Uses	
Exposure Type						
Eye Area	29	NR	NR	NR	84	0.01-18.6
Incidental Ingestion	2	0.07	NR	NR	123	0.04-37
Incidental Inhalation- Sprays	7*	NR	2**	NR	16	15
Incidental Inhalation- Powders	13	0.2	2***	NR	35	0.034-15.8
Dermal Contact	76	0.2-0.35	2	0.04-0.06	206	0.0035-20
Deodorant (underarm)	1	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	2	0.035-0.19	NR	0.55
Hair-Coloring	NR	2	3	0.5-3.5	NR	0.62
Nail	1	NR	NR	NR	24	0.001-3
Mucous Membrane	2	0.07	NR	0.04-0.06	125	0.04-37
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	55	0.07 to 0.35	2	NR	348	0.001-37
Rinse off	27	2	5	0.035-3.5	2	0.0035-0.99
Diluted for (bath) Use	NR	NR	NR	NR	NR	0.94
Totals/Conc. Range	88	0.07 to 2	7	0.035-3.5	359	0.001-37
	Calcium Sulfate		**Copper Sulfate**		**Magnesium Sulfate**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	26	5.8-14	NR	NR	23	0.4-2.1
Incidental Ingestion	2	1.8-3	NR	NR	4	NR
Incidental Inhalation- Sprays	7	NR	9	NR	121	0.5 -11
Incidental Inhalation- Powders	13	NR	9	0.042***	97	1
Dermal Contact	79	1-20	16	0.042	368	0.00001-49
Deodorant (underarm)	1	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	68	0.01-15
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	1	0.0001	NR	NR	2	NR
Mucous Membrane	2	1.8-3	1	NR	48	0.00001-49
Baby Products	NR	NR	NR	NR	NR	0.7
Duration of Use						
Leave-On	52	0.0001-20	9	0.042	321	0.002-11
Rinse off	27	2-9	2	0.042	59	0.00001-25
Diluted for (bath) Use	NR	NR	NR	NR	31	0.1-49
Totals/Conc. Range	84	0.0001-20	16	0.042	510	0.00001-49
	Manganese Sulfate		**Potassium Sulfate**		**Sodium Bisulfate**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	2	0.25-1.5	10	0.02	NR	NR
Incidental Ingestion	NR	NR	8	0.02	NR	NR
Incidental Inhalation- Sprays	NR	13.2**	28	0.005-0.01**	NR	NR
Incidental Inhalation- Powders	NR	0.58-36.6***	27	0.02-0.04***	NR	NR
Dermal Contact	7	0.25-36.6	50	0.00015-0.04	5	0.0013
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	0.1-13.2	4	0.001-0.02	NR	NR
Hair-Coloring	3	0.5	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	9	0.02	5	0.0013
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	6	0.25-36.6	49	0.00015-0.04	NR	NR
Rinse off	3	0.1-26.7	5	0.001-0.016	5	0.0013
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	10	0.1-36.6	64	0.00015-0.04	5	0.0013

Table 3. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[10,11]

	Sodium Sulfate		**Zinc Sulfate**		**Ammonium Persulfate**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)

Exposure Type						
Eye Area	11	0.000046-0.0064	NR	NR	1	NR
Incidental Ingestion	NR	0.00015-0.83	NR	0.05	NR	NR
Incidental Inhalation- Sprays	38	0.00015-0.0088	10	0.44**	1**	NR
Incidental Inhalation- Powders	34	0.005	10	0.07-1***	NR	NR
Dermal Contact	272	0.00001-96.8	45	0.057-1	1	NR
Deodorant (underarm)	2	0.0001-0.0027	NR	NR	NR	NR
Hair - Non-Coloring	76	0.00095-2	16	0.44	1	NR
Hair-Coloring	209	1-2.7	NR	NR	36	4.2-21.1
Nail	11	0.001-9.1	NR	NR	NR	NR
Mucous Membrane	190	0.00015-96.8	2	0.057	NR	NR
Baby Products	7	0.29	NR	NR	NR	NR
Duration of Use						
Leave-On	74	0.00001-9.1	23	0.07-1	2	NR
Rinse off	458	0.00015-8	30	0.057	36	4.2-22.1
Diluted for (bath) Use	42	0.14-96.8	NR	NR	NR	NR
Totals/Conc. Range	612	0.00001-96.8	63	0.057-1	38	4.2-22.1

	Potassium Persulfate		**Sodium Persulfate**		
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	
Exposure Type					
Eye Area	NR	NR	NR	NR	
Incidental Ingestion	NR	NR	2	NR	
Incidental Inhalation- Sprays	1**	NR	NR	NR	
Incidental Inhalation- Powders	NR	NR	NR	NR	
Dermal Contact	NR	NR	NR	NR	
Deodorant (underarm)	NR	NR	NR	NR	
Hair - Non-Coloring	1	NR	NR	NR	
Hair-Coloring	64	10.3-72	43	5-8	
Nail	NR	NR	NR	NR	
Mucous Membrane	NR	NR	2	NR	
Baby Products	NR	NR			
Duration of Use					
Leave-On	1	NR	NR	NR	
Rinse off	64	10.3-72	45	5-8	
Diluted for (bath) Use	NR	NR	NR	NR	
Totals/Conc. Range	65	10.3-72	45	5-8	

NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on Product Uses.

*It is possible that these products may be sprays or powders, but it is not specified whether the reported uses are sprays or powders.

**It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.

***It is possible that these products may be powders, but it is not specified whether the reported uses are powders.

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

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2013 FDA VCRP Data

Aluminum Sulfate

03F - Mascara	3
03A - Eyebrow Pencil	1
03B - Eyeliner	3
03C - Eye Shadow	21
03G - Other Eye Makeup Preparations	1
07A - Blushers (all types)	1
07B - Face Powders	6
07E - Lipstick	2
07I - Other Makeup Preparations	3
08G - Other Manicuring Preparations	1
10B - Deodorants (underarm)	1
12C - Face and Neck (exc shave)	3
12D - Body and Hand (exc shave)	2
12F - Moisturizing	2
12H - Paste Masks (mud packs)	27
12J - Other Skin Care Preps	5
Total	**79**

Calcium Sulfate Hydrate

Total	**0**

Copper Sulfate

10A - Bath Soaps and Detergents	1
12C - Face and Neck (exc shave)	5
12D - Body and Hand (exc shave)	3
12H - Paste Masks (mud packs)	1
12I - Skin Fresheners	1
Total	**11**

Ferric Sulfate

Total	**0**

Ferrous Ammonmium Sulfate

Total	**0**

Ferrous Sulfate

Total	**0**

Hydroxylamine Sulfate

Total	**0**

Magnesium Sulfate

02A - Bath Oils, Tablets, and Salts	27
02B - Bubble Baths	2

02D - Other Bath Preparations	2
03C - Eye Shadow	5
03D - Eye Lotion	5
03G - Other Eye Makeup Preparations	13
04E - Other Fragrance Preparation	1
05A - Hair Conditioner	20
05F - Shampoos (non-coloring)	7
05G - Tonics, Dressings, and Other Hair Grooming Aids	18
05H - Wave Sets	2
05I - Other Hair Preparations	21
07A - Blushers (all types)	1
07B - Face Powders	1
07C - Foundations	74
07D - Leg and Body Paints	1
07E - Lipstick	4
07F - Makeup Bases	12
07H - Makeup Fixatives	1
07I - Other Makeup Preparations	18
08B - Cuticle Softeners	1
08G - Other Manicuring Preparations	1
10A - Bath Soaps and Detergents	9
10E - Other Personal Cleanliness Products	4
11A - Aftershave Lotion	6
11E - Shaving Cream	1
12A - Cleansing	8
12C - Face and Neck (exc shave)	31
12D - Body and Hand (exc shave)	12
12F - Moisturizing	39
12G - Night	13
12H - Paste Masks (mud packs)	8
12I - Skin Fresheners	1
12J - Other Skin Care Preps	36
13A - Suntan Gels, Creams, and Liquids	4
13B - Indoor Tanning Preparations	2
Total	**411**

Calcium Sulfate

03A - Eyebrow Pencil	1
03B - Eyeliner	3
03C - Eye Shadow	21
03G - Other Eye Makeup Preparations	1
07A - Blushers (all types)	1
07B - Face Powders	6
07E - Lipstick	2
07I - Other Makeup Preparations	3

08G - Other Manicuring Preparations	1
10B - Deodorants (underarm)	1
12C - Face and Neck (exc shave)	3
12D - Body and Hand (exc shave)	2
12F - Moisturizing	2
12H - Paste Masks (mud packs)	27
12J - Other Skin Care Preps	5
Total	**79**

Ammonium Sulfate

05F - Shampoos (non-coloring)	2
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	1
06F - Hair Lighteners with Color	1
06G - Hair Bleaches	1
12D - Body and Hand (exc shave)	2
Total	**7**

Barium Sulfate

03A - Eyebrow Pencil	2
03B - Eyeliner	17
03C - Eye Shadow	53
03D - Eye Lotion	3
03F - Mascara	3
03G - Other Eye Makeup Preparations	6
04C - Powders (dusting and talcum, excluding aftershave talc)	6
07A - Blushers (all types)	18
07B - Face Powders	16
07C - Foundations	39
07D - Leg and Body Paints	1
07E - Lipstick	123
07G - Rouges	6
07H - Makeup Fixatives	2
07I - Other Makeup Preparations	11
08A - Basecoats and Undercoats	1
08D - Nail Extenders	1
08E - Nail Polish and Enamel	21
08G - Other Manicuring Preparations	1
10A - Bath Soaps and Detergents	2
12C - Face and Neck (exc shave)	6
12D - Body and Hand (exc shave)	2
12F - Moisturizing	5
12J - Other Skin Care Preps	2
13A - Suntan Gels, Creams, and Liquids	1
13B - Indoor Tanning Preparations	1
13C - Other Suntan Preparations	1

Total	**350**

2013 FDA VCRP Data

Manganese Sulfate

03D - Eye Lotion	2
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	3
12C - Face and Neck (exc shave)	1
12F - Moisturizing	2
12J - Other Skin Care Preps	1
Total	**9**

Potassium Sulfate

03D - Eye Lotion	9
03G - Other Eye Makeup Preparations	1
05A - Hair Conditioner	3
05G - Tonics, Dressings, and Other Hair Grooming Aids	1
07E - Lipstick	8
10E - Other Personal Cleanliness Products	1
12C - Face and Neck (exc shave)	8
12D - Body and Hand (exc shave)	2
12F - Moisturizing	9
12G - Night	8
12H - Paste Masks (mud packs)	1
12J - Other Skin Care Preps	3
Total	**54**

Silver Sulfate

Total	**0**

Sodium Bisulfate

10A - Bath Soaps and Detergents	2
10E - Other Personal Cleanliness Products	3
Total	**5**

Sodium Sulfate

01A - Baby Shampoos	5
01C - Other Baby Products	2
02A - Bath Oils, Tablets, and Salts	18
02B - Bubble Baths	20
02D - Other Bath Preparations	4
03D - Eye Lotion	1
03F - Mascara	9
03G - Other Eye Makeup Preparations	1
05A - Hair Conditioner	19
05C - Hair Straighteners	1
05F - Shampoos (non-coloring)	48
05G - Tonics, Dressings, and Other Hair Grooming Aids	2

05I - Other Hair Preparations	1
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	207
06D - Hair Shampoos (coloring)	2
07C - Foundations	1
07D - Leg and Body Paints	1
08A - Basecoats and Undercoats	1
08C - Nail Creams and Lotions	2
08E - Nail Polish and Enamel	5
08G - Other Manicuring Preparations	3
10A - Bath Soaps and Detergents	101
10B - Deodorants (underarm)	2
10E - Other Personal Cleanliness Products	47
11E - Shaving Cream	1
11G - Other Shaving Preparation Products	1
12A - Cleansing	26
12C - Face and Neck (exc shave)	3
12D - Body and Hand (exc shave)	9
12F - Moisturizing	21
12I - Skin Fresheners	1
12J - Other Skin Care Preps	7
13B - Indoor Tanning Preparations	2
Total	**574**

Zinc Sulfate

05A - Hair Conditioner	7
05F - Shampoos (non-coloring)	8
05I - Other Hair Preparations	1
07C - Foundations	6
10A - Bath Soaps and Detergents	2
11G - Other Shaving Preparation Products	2
12A - Cleansing	9
12C - Face and Neck (exc shave)	8
12D - Body and Hand (exc shave)	2
12H - Paste Masks (mud packs)	2
12J - Other Skin Care Preps	6
Total	**53**

Ammonium Persulfate

03G - Other Eye Makeup Preparations	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	1
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	10
06F - Hair Lighteners with Color	3
06G - Hair Bleaches	17
06H - Other Hair Coloring Preparation	6

Total	38

Potassium Persulfate

05G - Tonics, Dressings, and Other Hair Grooming Aids	1
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	14
06F - Hair Lighteners with Color	4
06G - Hair Bleaches	39
06H - Other Hair Coloring Preparation	7
Total	**65**

Sodium Persulfate

06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	4
06F - Hair Lighteners with Color	3
06G - Hair Bleaches	33
06H - Other Hair Coloring Preparation	3
09A - Dentifrices	2
Total	**45**

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Final Report on the Safety Assessment of Sodium Sulfate[1]

Sodium Sulfate is used as a viscosity increasing agent in cosmetic formulations, at concentrations that are reportedly as high as 97%. No evidence of systemic toxicity was seen in oral exposure studies in animals, although there was moderate ocular irritation in rabbits when a granular sodium carbonate–Sodium Sulfate mixture was instilled. No developmental or reproductive toxicity was reported in rats or mice; there was an increase in birth weight in the mice. Sodium Sulfate was negative in mutagenesis assays. In several studies in which Sodium Sulfate was given with other agents, the results depended on the carcinogenicity of the other agents. Clinical data indicated no significant adverse effects following dermal, oral, or inhalation exposure. Because some irritation was seen under patch test conditions, it was concluded that the concentration should be limited to a level known to produce only a very small frequency of irritation if used in a leave-on application. Accordingly, Sodium Sulfate was found to be safe for use in rinse-off formulations, and *safe at concentrations up to 1% in leave-on formulations.*

INTRODUCTION

The following is a compilation of studies concerning the testing of Sodium Sulfate (CAS No. 7727-73-3 for the decahydrate form and 7757-82-6 for the anhydrous form).

A comprehensive review of literature published from 1920 to 1972 concerning sulfates (Franklin Institute Research Laboratories 1973) is available through the National Technical Information Service (NTIS). The review had been used by the Select Committee on Generally Recognized as Safe (GRAS) Substances in affirming the status of Sodium Sulfate (as well as other sulfates) as a GRAS compound (FDA 1978).

CHEMISTRY

Definition and Structure

Sodium Sulfate (anhydrous) is the inorganic salt with the chemical formula Na_2SO_4 (USP 1995). The empirical formula for Sodium Sulfate in the *International Cosmetic Ingredient Dictionary* and *Handbook* is $H_2SO_4 \cdot 2Na$ (Wenninger, Canterbery, and McEwen 2000). The decahydrate form has the chemical formula $Na_2SO_4 \cdot 10H_2O$.

Synonyms include: disodium sulfate; sulfuric acid, disodium salt (Wenninger, Canterbery, and McEwen 2000); Glauber's salt (Taylor 1988); natriumsulfat; salt cake; sodium sulphate; thenardite; trona (RTECS 1995; Lewis 1993); bisodium sulfate; Caswell No. 793; and disodium monosulfate (Chemline 1995).

Physical and Chemical Properties

Some of the physical properties and chemical properties are listed in Table 1.

The decahydrate solution of Sodium Sulfate has a neutral pH (Budavari 1989). Sodium Sulfate reacts with aluminum, and emits toxic fumes of SO_x and Na_2O when heated to decomposition (Sax 1979; Lewis 1993).

CTFA lists the following specifications for cosmetic grade Sodium Sulfate (anhydrous): 3 ppm maximum Arsenic (as As) 20 ppm maximum Lead (as Pb), and 30 ppm maximum Selenium (as Se) (Nikitakis and McEwen 1990). The Sodium Sulfate sample must closely match the Cosmetic, Toiletry, and Fragrance Association (CTFA) Spectrum—IR with no indication of foreign materials (Nikitakis and McEwen 1990). These specifications are similar to those listed in the *Food Chemicals Codex (FCC)*, except that the FCC restricts lead to a maximum of 10 ppm (National Academy of Sciences 1981).

Method of Manufacture

Sodium Sulfate occurs naturally as the minerals mirabilite and thenardite (Budavari 1989). It can also be prepared by the neutralization of sulfuric acid with sodium hydroxide (Rothschild 1990).

USE

Purpose in Cosmetics

Sodium Sulfate is used in cosmetic formulations as a viscosity increasing agent—aqueous (Wenninger, Canterbery, and McEwen 2000).

Scope and Extent of Use in Cosmetics

United States

As of January 1997, there were 28 reported uses of Sodium Sulfate in cosmetic formulations (FDA 1997). See Table 2. Concentrations of use are no longer reported to the FDA (1992). Data submitted to Cosmetic Ingredient Review (CIR) indicated that one company uses Sodium Sulfate at 0.5% in facial toner and lotion, 3.5% in liquid bubble bath, 82.0% in powder bubble bath, and 96.3% in bath powder (CTFA 1996a). Another company

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TABLE 1
Properties of Sodium Sulfate

Property	Characteristic	Reference
Molecular weight	142.04 Da (anhydrous) 322.20 Da (decahydrate)	Budavari 1989; Sax 1979; Lewis 1993a
Appearance	White crystals or powder, odorless (anhydrous)	Sax 1979
Melting point	888°C (anhydrous) 33°C (decahydrate)	Sax 1979; Lewis 1993b
Density	2.671 (anhydrous) 1.46 (decahydrate)	Sax 1979; Lewis 1993a Budavari 1989
Solubility	Soluble: water, glycerin Insoluble: alcohol	Sax 1979; Lewis 1993a

reported use at 1% to 5% in liquid hand soap and body wash soap, and 0.1% to 1% in shampoos (CTFA 1996b).

International

Sodium Sulfate is listed in the *Comprehensive Licensing Standards of Cosmetics by Category (CLS)*. Sodium Sulfate, which conforms to the specifications of the *Japanese Standards of Food Additives* and/or the *Japanese Standards of Cosmetic Ingredients*, has precedent for unrestricted use in all CLS cosmetic categories except eyeliners for which there has been no use precedence. Sodium Sulfate, anhydrous, which conforms to the standards of the *Japanese Cosmetic Ingredient Codex* has precedent for unrestricted use in all CLS categories except eyeliners and lip and oral preparations (Rempe and Santucci 1997).

Noncosmetic

Sodium Sulfate is recognized as a GRAS ingredient (FDA 1980; Rothschild 1990). Its use as a food additive is not restricted by the World Health Organization's (WHO) Joint Expert Committee on Food Additives (JECFA), except that intake is limited by its laxative action (FAO/WHO 1994).

TABLE 2
Frequency of use of Sodium Sulfate (FDA 1997)

Product category	No. formulations in category	No. containing Sodium Sulfate
Bath oil, tablets, and salts	117	1
Bubble baths	186	11
Bath soaps and detergents	341	1
Cleansing	630	2
Body and hand (excluding shaving)	776	3
Moisturizing	743	5
Night	185	1
Skin fresheners	181	1
Other skin care preparations	683	3
1997 total		**28**

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

Human absorption, distribution, metabolism and excretion studies are reported in the Clinical Assessment of Safety section of this report.

Oral

Krijgsheld et al. (1979) used male Wistar rats (300–330 g body weight [bw]) to investigate the absorption of inorganic sulfate following oral administration of $Na_2{}^{35}SO_4$. One set of animals had a permanent catheter placed in the right atrium to collect blood samples. The samples were analyzed by liquid scintillation to determine plasma ^{35}S concentrations. Groups of six animals were dosed under light anesthesia by gastric tube with 600 μCi/kg bw $Na_2{}^{35}SO_4$ in 2 ml water. One group received only the tracer dose. Another two groups received, in addition to the tracer, either 1.0 or 5.0 mmol nonradioactive Na_2SO_4. Feed and water were provided ad libitum.

Radioactivity was detected in the plasma 15 minutes after administration of the tracer dose. A peak activity of >4000 cpm was reached 1.5 to 2 hours following administration. By 10 hours, 50% of the maximum plasma concentration remained; by 19 hours only 10% of the maximum plasma radioactivity remained. In animals that also received nonradioactive Sodium Sulfate, the peak radioactivity was again reached at 1.5 to 2 hours post administration. However, the amount of radioactivity in the plasma decreased as the dose of nonradioactive Sodium Sulfate increased, indicating that the "fractional absorption of (labeled) Sodium Sulfate decrease(d) as the administered dose increase(d)."

The urinary excretion of sodium sulfate was studied in a different group of rats (Krijgsheld et al. 1979). Animals were treated via a gastric tube with 50 μCi/kg bw $Na_2{}^{35}SO_4$ in 2 ml water to which was added either varying amounts of sodium

chloride or between 0.25 to 5.0 mmol nonradioactive Na_2SO_4. Control rats received water with sodium chloride added. The rats were placed in metabolic cages and urine was collected for as long as 7 days following oral sulfate administration. Rats that received the two high sulfate doses (2.5 and 5.0 mmol) developed diarrhea that started 4 hours after administration and lasted for 4 hours; removal of the feces thus resulted in some loss of urine. Blood samples from the aorta were obtained 2 hours after oral administration from rats dosed with 0.5, 2.5, or 5.0 mmol Na_2SO_4 and were analyzed for sulfate concentration.

When the radioactive Sodium Sulfate was administered with either saline or the two lowest doses of nonradioactive Sodium Sulfate (0.25 or 0.5 mmol), about 90% of the administered dose was recovered in the urine within 24 hours. (The researchers attributed the remaining 10% to incorporation into unidentified macromolecules in the body.) When the amount of nonradioactive Sodium Sulfate was increased to 1.0, 2.5, and 5.0 mmol/rat, the percentage of radioactivity recovered in the 24-hour urine decreased to 73%, 67%, and 56%, respectively. Serum sulfate concentrations of 1.34, 1.95 and 2.13 mmol/L were found in blood samples from animals dosed with 0.5, 2.5, or 5.0 mmol nonradioactive Sodium Sulfate, respectively. Corresponding untreated controls had serum sulfate concentrations of 0.77 mmol/L, whereas those animals treated with varying amounts of sodium chloride and water (vehicle control) had concentrations between 0.57 and 0.66 mmol/L.

Its detection in the plasma soon after administration and the urinary excretion of 90% of the administered radioactivity within 24 hours (when low doses of nonradioactive sulfate were also added), indicating almost complete absorption of the dose from the gut. The researchers considered that orally administered Sodium Sulfate was rapidly absorbed in rats.

The results of Krijgsheld et al. (1979) corroborated those reported by Hwang (1966) in which 57% to 74% of an orally administered dose of 2800 mg/kg $Na_2{}^{35}SO_4$ was recovered in the 24-hour urine of four rats.

Parenteral

Dziewiatkowski (1949) conducted a study in which 1 mg $Na_2{}^{35}SO_4$ was administered intraperitoneally (IP) to 14 male and 13 female adult rats (180–330 g). Animals were killed at various times postdosing and tissue samples were collected. Approximately 67% of the administered ^{35}S was excreted in the urine within 24 hours. By 120 hours, 85% was recovered in the urine; when fecal excretion was included, 95% of the administered dose had been recovered. Rapid elimination was noted in the blood, liver, and brain with almost complete elimination by 48 hours. However, a notable rise in the ^{35}S concentration was noted at 8 hours in bone and at 24 hours in bone marrow. Elimination was slow in these two tissue samples with significant concentrations noted 120 hours after administration.

Odeblad and Boström (1952) used autoradiography to measure the incorporation of ^{35}S Sodium Sulfate into different organs of rats and rabbits. Five adult rats (200 g) received subcutaneous (SC) injections of 100 μCi ^{35}S as "carrier free" Na_2SO_4 diluted with 0.1 mg nonradioactive Na_2SO_4 in 0.2 ml distilled water/100 g bw. One adult rabbit was injected with 2.0 mCi ^{35}S as Na_2SO_4 in 4 ml of distilled water, containing 0.2 mg carrier. All animals were killed 48 hours after dosing and various organs were removed. In rats, "very large amounts" of radioactivity were detected in the epithelium of the esophagus and ileum, in the cornea, and in the cartilage of the trachea. In the rabbit, "large amounts" of radioactivity were detected in the tunica intima and tunica media of the aorta, and in the respiratory epithelium and cartilage plates of the lungs. The researchers considered ^{35}S to be taken up by tissues where sulfomucopolysaccharides are present.

Boström and Aqvist (1952) reported that $Na_2{}^{35}SO_4$ administered IP to rats was incorporated in small amounts into the chondroitin sulfuric acid of the costal cartilage within 24 hours, and in trace amounts into taurine isolated from the liver within 8 hours. The researchers reported that the exogenous sulfate was not incorporated into methionine or cysteine. Exogenous sulfate was taken up primarily into mucopolysaccharides.

In a study by Dohlman (1957), $Na_2{}^{35}SO_4$ was administered intravenously (IV) to rabbits which were then killed at various times postdosing; eyeballs were enucleated and analyzed for radioactive sulfur content. The radioactive sulfur was rapidly taken up by the eyeball with high concentrations being detected in the uvea. Turnover rates were also high in the uvea but slow in the cornea and lens. By day 3 after dosing, concentrations remained high in the inner layers of the cornea and sclera but were low in the uvea, retina, and the pia and dura of the optic nerve head. No radioactivity was detected within the aqueous humor or vitreous body.

$Na_2{}^{35}SO_4$ was injected into the femoral artery of a dog (Balchum, Dybicki, and Meneely 1960). After 100 minutes, 0.6% was retained in the trachea, 0.2% in the lungs, 4.6% in the liver, 0.26% in the spleen, 0.3% in the kidneys, and 0.6% in the brain.

Effects on Enzymes and Serum Parameters

Intravenous injection of Sodium Sulfate at <400 mg/L of blood into a 15-kg dog increased biliary volume and biliary salt excretion twofold (Chabrol and Maximin 1929).

Sodium Sulfate injected intravenously at 175 mg/kg into a rabbit produced a 22% drop in serum calcium concentrations in 4 hours. Inorganic phosphorus concentrations were decreased by 34% at 1.75 hours, but returned to normal at 4 hours. No changes were noted in the serum magnesium concentration (Brookfield 1934).

Fasted and fed female Golden labradors were infused with 3 parts of 5% creatinine and 2 parts of Sodium Sulfate (50 mM) at rates of 0.75 and 1.0 ml/min. The glomerular filtration rate was increased by 30% to 50% over initial values in fasted dogs. Sodium Sulfate administration increased the phosphate-filtered

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load as measured from heparinized plasma; this effect was not noted when 5% creatinine alone was administered (Foulks 1955).

Kowarski, Kowarski, and Berman (1961) demonstrated that the addition of 1% Sodium Sulfate to milk fed to rats decreased calcium ionization and reduced calcium absorption from the gut. Calcium retention was reduced by 50%.

Duhm, Deuticke, and Gerlach (1969) reported that the addition of Sulfate to cultures of human erythrocytes in plasma inhibited, by almost 80%, the spontaneous degradation of 2,3-diphosphoglycerate. This effect of Sulfate was also noted in erythrocytes incubated in glucose-free media and in hemolysates under conditions in which no synthesis of 2,3-diphosphoglycerate occurred. High 2,3-diphosphoglycerate concentrations in vivo reduced the affinity of hemoglobin for oxygen and thus favored the release of oxygen in tissues.

Drug Interaction

Acetaminophen

Slattery and Levy (1977) reported that Sodium Sulfate increased the LD_{50} of IP acetaminophen in Swiss mice from 425 to 575 mg/kg. Groups of 10 mice (25–30 g) had received single IP injections of 300 to 800 mg/kg acetaminophen together with an equimolar amount of Sodium Sulfate. (Control groups received acetaminophen with varying amounts of sodium chloride.)

In a follow-up study using Sprague-Dawley rats, Galinsky, Slattery, and Levy (1979) demonstrated that plasma acetaminophen concentrations decreased and plasma acetaminophen sulfate concentrations increased more rapidly in Sodium Sulfate–treated rats as compared to controls that were given an identical amount of sodium in the form of sodium chloride. The researchers considered that the decreased acetaminophen toxicity caused by Sodium Sulfate dosing was due to accelerated elimination of acetaminophen. Similarly, Lin and Levy (1986) reported that concomitant administration of inorganic sulfate (delivered as Sodium Sulfate) to Sprague-Dawley rats increased by 1.5-fold the total clearance of large doses of acetaminophen (300 mg/kg), and increased by twofold the fraction of that dose eliminated as acetaminophen sulfate, when compared to rats that had not received supplemental sulfate. Clearance was limited by the activity of sulfotransferase enzymes that are responsible for acetaminophen sulfate formation.

Subsequent studies by Hjelle, Brzeznicka, and Klaassen (1986) using adult male CF-1 mice found that administration of either Sodium Sulfate (4 mmol/kg) or *N*-acetylcysteine (NAC) increased serum sulfate and hepatic adenosine 3′-phosphate 5′-phosphosulfate concentrations. The mice (23–32 g) received IP doses of 400 or 600 mg/kg acetaminophen (2.5 and 4 mmol/kg) dissolved in either Sodium Sulfate or NAC vehicle. No significant change in acetaminophen sulfation or elimination was noted with administration of NAC or Sodium Sulfate. However, unlike NAC, Sodium Sulfate did not attenuate the marked decrease in glutathione in the liver observed after acetaminophen administration. Also, NAC decreased covalent binding of tritium derived from [3H]-acetaminophen to liver protein. Sodium Sulfate did not. Sodium Sulfate did not protect against acetaminophen-induced hepatotoxicity whereas lethality was reduced in NAC-treated animals.

Selenium

Groups of five weanling Sprague-Dawley rats were fed diets containing 500 and 1000 mg Sodium Sulfate/kg feed in conjunction with 5, 10, and 20 mg Se/kg feed. Mortality was 60% and 100% in mice treated with 10 and 20 mg selenium, respectively, regardless of the Sodium Sulfate dosage; no deaths were found in the 0 and 5 mg selenium groups. The concurrent treatment with Sodium Sulfate did not significantly alter the course of selenite toxicity (i.e., feed intake, daily weight gain, testis weight, hepatic hemorrhage and necrosis, renal necrosis, arrested spermatogenesis). The main effect of the SO_4 was increased liver copper concentrations (Kezhou et al. 1987).

Effect on DDT Absorption

A group of six male Sprague-Dawley rats (230–330 g) was treated via feeding tube with 80 mg/kg ^{14}C-DDT in a volume of 10 ml/kg of cathartic (15% Sodium Sulfate containing 20% acacia). One hour later each rat received a second dose of the Sodium Sulfate cathartic without DDT at the rate of 10 ml/kg. A second dose of DDT with cathartic was given after 24 hours. A control group of rats was treated with distilled water containing 20% acacia. Feces and urine were collected during the experiment and analyzed for radioactivity by liquid scintillation. Rats were killed 24 hours after the second dose of DDT. Perirenal and peritesticular adipose tissue samples were collected and analyzed by gas chromatography. Although the difference was not of statistical significance, all Sodium Sulfate–treated rats had adipose DDT concentrations (95 ppm) below the control group (137 ppm). Once the values were corrected for contamination of urine with loose feces (resulting from Sodium Sulfate treatment), the liquid scintillation values corresponded with the adipose tissue measurements. It was estimated that 60.8% of the administered DDT was recovered in the feces of the Sodium Sulfate group rats versus 57.5% for the control rats (Keller and Yeary 1980).

ANIMAL TOXICOLOGY

Short-Term Oral Toxicity

A group of six weanling male Sprague-Dawley rats fed either 0.88, 8.64, or 138 mmol Sodium Sulfate/kg basal diet for up to 4 weeks had no significant differences in weight gain, feed intake, feed-gain ratio, or water intake as compared to control rats. Hemoglobin, red blood cell count, white blood cell count, serum protein, alkaline phosphatase, and inorganic phosphate concentrations were also comparable to values for the control group. No changes were observed in gastrointestinal organ weights or

in the length or color of the small intestine (Moinuddin and Lee 1960).

Acute Inhalation Toxicity

Amdur et al. (1978) found no adverse pulmonary effects in 10 guinea pigs exposed for 1 hour to 0.90 mg/m^3 Sodium Sulfate (particle size 0.1 μm). No change in resistance was noted. A slight decrease in compliance was observed; it was not statistically significant. Sodium Sulfate was the least irritating of the sulfate aerosols tested (ranked in decreasing order: ammonium sulfate > ammonium bisulfate > copper sulfate > sodium sulfate).

Sackner et al. (1981) performed a variety of studies to investigate the effects of sulfate aerosols on cardiopulmonary function in dogs and tracheal mucous velocity of sheep. In the studies described below, statistical analysis compared the response to sulfates against the response to sodium chloride (control).

In a brief exposure study, five intubated anesthetized dogs breathed aerosol generated from a 0.1% Sodium Sulfate solution (particle size 0.1–0.2 μm) for 7.5 minutes. The aerosol generated had a mass concentration of 1.0 mg/m^3. Measurements of lung volume and mechanics were made before exposure and at 5, 15, 30, 60, 120, and 180 minutes after exposure termination. After completion of the final measurements, the animals were exposed for 7.5 minutes to aerosol generated from a 1.0% Sodium Sulfate solution (particle size 0.1–0.2 μm). This aerosol had a mass concentration of 8.0 mg/m^3. Lung mechanics measurements were made at 5, 15, and 30 minutes following termination of the second exposure. No significant alterations in total respiratory resistance, static lung compliance, functional residual capacity, specific total respiratory conductance, and specific lung compliance were noted in the animals exposed to Sodium Sulfate.

In an intermediate exposure study, five intubated anesthetized dogs breathed aerosols generated from 0.5% Sodium Sulfate solution for 4 hours. The aerosol had a mass concentration of 5.0 mg/m^3. Measurements of lung volume, breathing mechanics, and hemodynamics were made before, hourly during, and for 2 hours after exposure. "No significant alterations" were noted (Sackner et al. 1981).

In studies of tracheal mucous velocity, Sackner et al. (1981) exposed six sheep for 20 minutes to aerosol generated from a 0.1% Sodium Sulfate solution. (The solutions used in the sheep study had the same particle size and mass concentration as described in the dog studies.) No significant change was noted in tracheal mucous velocity measurements taken at 30, 60, 120, and 180 minutes after exposure termination when compared to baseline values. The exposure did not significantly alter tracheal mucous velocity. In a second study, five sheep were exposed for 4 hours to an aerosol generated from a 0.5% solution of Sodium Sulfate. Measurements made before, at the end of, and 2 hours after termination of exposure produced tracheal mucous velocity values of 14.3, 11.9, and 12.0 mm/min, respectively. The differences in the values were not statistically significant.

In a study by Schlesinger (1984) comparing the irritancy potential of inhaled sulfate aerosols, the following ranking was determined: sulfuric acid > ammonium bisulfate > ammonium sulfate, (equivocal to) Sodium Sulfate. Five rabbits had been exposed for 1 hour to a maximum concentration of almost 2000 μg/m^3 Sodium Sulfate aerosol and measurements were made of bronchial mucociliary clearance. No significant adverse effects were reported.

Acute Parenteral Toxicity

In addition to the inhalation studies described in the earlier section, Sackner et al. (1981) also performed intravenous studies in which anesthetized dogs were injected with 1 mg of Sodium Sulfate in 10 ml sterile water. Measurements of breathing mechanics, functional residual capacity, pulmonary and carotid arterial pressures, cardiac output and arterial blood gases were done at 15, 30, 45, and 60 minutes following the IV injection. After the final measurement was taken, 10 mg Sodium Sulfate in 10 ml water was injected and the same parameters were again measured. Finally, 100 mg Sodium Sulfate in 10 ml water was injected and the same parameters at the same time intervals were measured again. A nondose dependent alteration in pulmonary function was noted. Specifically, 10 mg Sodium Sulfate, "produced a maximal fall in specific lung compliance of 11% 15 minutes after injection ($p < .05$)". This effect was not noted with either the 1 or 100 mg dose. The 10 mg dose of Sodium Sulfate also produced a, "rise in cardiac output of 11% at 60 minutes and a maximum increase of stroke volume of 22% at 45 minutes after injection ($p < .05$)." No significant hemodynamic changes resulted from the 1 or 100 mg dose.

Ocular Irritation/Toxicity

Griffith et al. (1980) classified a sodium carbonate–Sodium Sulfate granular mixture (1:1 *w/w*) as causing moderate ocular irritation. The test material was applied directly to one cornea of three albino rabbits at volumes of 0.01, 0.03, and 0.1 ml. Irritation was graded on days 1, 2, 3, 4, 7, and 14 following treatment. The reactions were scored using the Draize scale that allows a maximum score of 110. The average maximum scores noted were 11, 17, and 36 for the 0.01, 0.03, and 0.1 ml doses, respectively. These reactions took between 4 and 21 days to return to normal.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Oral

In validation of an in vivo developmental toxicity screen, Seidenberg, Anderson, and Becker (1986) administered various chemicals to pregnant ICR/SIM mice (32–36 g) by oral intubation on days 8 through 12 of gestation. Sodium Sulfate, 2,800 mg/kg/day, was administered in a water vehicle to 28 mice. Animals were housed individually; feed and water were available ad libitum. Mice were weighed on days 7 and 13 to

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determine maternal weight gain. The dams were allowed to deliver and neonates were examined, counted, and weighed on the day of birth and day 3. No maternal toxicity was observed in the Sodium Sulfate dose group; average weight gain was 8.6 g (compared to 8.5 g for nontreated controls that had received water via intubation). The Sodium Sulfate group had 24 litters with no resorptions (control had 25 with no resorptions). Survival of neonates was 100% between days 1and 3 in the Sodium Sulfate group. The average neonatal weight at birth for the Sodium Sulfate group (1.80 g) was significantly greater than that for control neonates (1.72 g; $p < .05$, assessed by a two-tailed analysis for variance). By day 3, neonates of the Sodium Sulfate group had an average weight of 2.58 g compared to 2.42 g for control neonates.

In a subsequent publication discussing the validity of the above described developmental screen, Seidenberg and Becker (1987) considered the slight but significant increase in neonatal body weight on day 1 to be a positive result for Sodium Sulfate. However, in outlining the protocol for the screen, it was stressed that "overt maternal toxicity is required"; such a dose was not reached in the Sodium Sulfate group. The researchers admitted, "a teratogen may produce a positive response in the developmental toxicity screen without inducing overt toxicity in dams." However, noting the lack of published teratogenic data via the oral route for Sodium Sulfate, the researchers were unable to interpret whether the results were valid or a false positive.

Parenteral

Sodium Sulfate induced a low incidence (6%) of skeletal anomalies in mice when injected subcutaneously on a single day of gestation (Arcuri and Gautieri, 1973).

Knight, Van Wart, and Roe (1978) studied the effects of salicylamide on the sequential uptake and loss of radiosulfate by maternal and fetal rat tissue. On day 17 of gestation, a control group of 24 pregnant Holtzman rats (230–250 g), maintained since gestation day 6 on a 25% casein diet, was injected intramuscularly with $Na_2{}^{35}SO_4$ at a dose of 25 μCi/100 g bw ($Na_2{}^{35}SO_4$ in water, 100 μCi/ml). (The experimental groups, which had been maintained on casein diets supplemented with varying amounts of salicylamide, also received an injection of Sodium Sulfate on day 17.) Dams were killed sequentially at intervals up to 24 hours postinjection. A blood sample was obtained at the time of killing, the maternal liver was extracted, and fetuses were grossly examined. Homogenates of each fetus and placenta, as well as maternal liver and serum, were analyzed by a scintillation counter. No malformations were noted in any of the 108 fetuses of the control group; there were six (5.55%) resorptions. In control rats, the uptake and retention of radiosulfate per unit weight of placenta or per placenta varied inversely with the number of placentas per dam or the placental weight. Uptake by the fetus was maximal after 2 hours, followed by a rapid decline in the following hour; the loss rate was slow. Uptake by the fetus was significantly correlated with maternal serum concentrations.

GENOTOXICITY

Sodium Sulfate at concentrations up to 275 μg/well was negative in the microscreen assay (Rossman et al. 1991). The assay measures prophage induction into *Escherichia coli* as an indicator of DNA damage to the bacteria.

Sodium Sulfate was among several salts tested for enhanced transformation of Syrian hamster embryo cells (HEC) by a simian adenovirus, SA7. A concentration of 7.0 mM Sodium Sulfate produced an enhancement 1.2 times that of the untreated control. (The enhancement was expressed as the ratio between the transforming frequency of treated, surviving cells and the transforming frequency of control cells.) The results for Sodium Sulfate were considered negative as a concentration >0.9 mM was necessary to produce the effect (Casto, Meyers, and Dipaolo 1979).

COCARCINOGENICITY

Yamamoto et al. (1973) explored whether supplemental administration of Sodium Sulfate would restore the carcinogenicity of *N*-hydroxy-*N*-2-fluorenylacetamide (*N*-OH-FAA) despite the presence of the inhibitor *p*-hydroxyacetanilide. Groups of rats were maintained for 16 weeks on feed containing: (1) 0.0213% (0.89 mmole/kg) *N*-OH-FAA; (2) carcinogen plus 0.89% (59 mmoles/kg) *p*-hydroxyacetanilide (a 66 molar excess); or (3) carcinogen plus inhibitor plus 2.52% (178 mmoles/kg, 3 molar equivalents) Sodium Sulfate. Following dosing, animals were maintained on untreated feed for an additional 10 weeks. Animals were killed at the end of the experiment and necropsy performed. Three animals from each group were housed in metabolism cages; urine was collected separately over a 24-hour period and analyzed for inorganic sulfate. Hepatomas were observed in all 10 animals of group 1, in four of the 20 rats of group 2, and in none of the 20 animals of group 3. Further, hyperplastic nodules were neither observed in four animals from group 2 nor in 11 animals from group 3. Sodium Sulfate appeared to inhibit the carcinogenicity of *N*-OH-FAA or increase the inhibitory affect of *p*-hydroxyacetanilide.

A second experiment was conducted by Yamamoto et al. (1973) using a higher dose (0.032%, 1.34 mmoles/kg) of *N*-OH-FAA as well as one-third the Sodium Sulfate amount of the above described study (0.84%, 59 mmoles/kg, 1 molar equivalent). Again rats were maintained for 16 weeks on treated feed followed, this time, by an additional 16 weeks on control feed. Hepatomas were noted in all 5 animals that received the carcinogen alone, in 6 of 12 animals that received the carcinogen plus inhibitor, in 5 of 6 animals that received the carcinogen, inhibitor, and 1 equivalent of dietary Sodium Sulfate, and in 4 of 12 animals that received the carcinogen, inhibitor plus 3 equivalents of Sodium Sulfate. With the greater amount of carcinogen used in this second study, Sodium Sulfate had no additional effect on the actions of *p*-hydroxyacetanilide.

Animals that received the carcinogen alone excreted free sulfate in the urine, whereas in animals that also received *p*-hydroxyacetanilide the sulfate was mostly conjugated. Groups

that also received 1 or 3 equivalents of Sodium Sulfate had greater concentrations of total and free urinary sulfate (Yamamoto et al. 1973).

Blunck and Crowther (1975) studied the Sodium Sulfate activation of the carcinogen (and azo dye) 3'-methyl-4-dimethylaminoazobenzine (MeDAB). Groups of 15 male Sprague-Dawley rats were fed for 16 weeks diets containing either 0.06% MeDAB or 0.06% MeDAB plus 0.84% Sodium Sulfate. Another group of five rats received feed containing only 0.84% Sodium Sulfate. The amount of feed available was restricted to that of the cage of rats consuming the least (approximately 10 g/rat/day for the first 2 weeks, then gradually increased to 17 g/rat/day by week 27). Rats had free access to tap water. After the treatment, the animals were fed a basal diet for 8 weeks. At this time, two rats from the MeDAB-dosed groups and one from the Sodium Sulfate–alone group were killed and the livers examined. The remaining rats were returned to their respective treatment diets for several 4-week periods, with a week between each period, during which they were fed basal diet. The study was terminated after 41 weeks. In a delayed-start second experiment, groups of five rats were maintained on the same dosing protocol as described for a total of 27 weeks. Pooling the results of the two experiments, 16 of the 20 rats given MeDAB, 18 of the 20 given MeDAB and Sodium Sulfate, and all 10 rats given Sodium Sulfate survived the initial 16-week dosing. At the end of the study, rats were killed and the livers examined. It was noted that Sodium Sulfate shortened the latent period (from 27 to 17 weeks), but did not affect the rate of neoplasm development. In addition, the relative risks of developing multiple neoplasms and metastatic neoplasms were increased with Sodium Sulfate supplementation. No liver abnormalities were noted in rats of the Sodium Sulfate alone or basal diet (control) groups.

Cohen and Bryan (1978) reported coadministration of Sodium Sulfate with the inhibitor *p*-hydroxyacetanilide (at an equimolar ratio with Sodium Sulfate) partially restored the leukemogenicity of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide (NFTA) in mice. Female Swiss mice were maintained for 14 weeks on the following diets: (1) 0.05% NFTA alone; (2) NFTA plus *p*-hydroxyacetanilide; (3) NFTA, *p*-hydroxyacetanilide and Sodium Sulfate; (4) NFTA plus Sodium Sulfate; or (5) *p*-hydroxyacetanilide plus Sodium Sulfate. Treatment was followed by 16 weeks of control diet. A control group of 30 mice was fed a diet containing 1.88% Sodium Sulfate for 1.5 weeks and then the concentration was reduced to 0.94% Sodium Sulfate for the remaining 12.5 weeks. (The Sodium Sulfate dose was reduced so as to remain equimolar with the dose of *p*-hydroxyacetanilide that had to be halved due to toxicity.) An identical amount/protocol for Sodium Sulfate administration was used for mice of the respective treatment groups.

Leukemia was noted in 19 of 25 surviving animals of the NFTA group, in 3 of 23 animals of the NFTA plus *p*-hydroxyacetanilide group, in 20 of 24 animals of the NFTA plus Sodium Sulfate group, and in 12 of 25 of animals of the NFTA, *p*-hydroxy-acetanilide, plus Sodium Sulfate group. Mean cumulative chemical consumption for the Sodium Sulfate control group was 6.5 g/mouse. Twenty-five mice of this group survived to week 10. After a 30-week latent period, one animal of the control group developed leukemia. Papillomas of the stomach were not observed in the Sodium Sulfate control group (two were noted in the group receiving NFTA alone, and four in the group receiving NFTA plus Sodium Sulfate). Similar observations were noted in animals that received *p*-hydroxyacetanilide plus Sodium Sulfate; the only exception was that leukemia (in 1 of 19 animals) was noted after an 18-week latent period (Cohen and Bryan 1978).

Samelson, Nelson, and Nyhus (1985) reported that Sprague-Dawley rats with acid stool pH, produced by consumption of Sodium Sulfate had significantly ($p < .05$) fewer colon tumors was injections of dimethylhydrazine (DMH) than rats treated with DMH alone. A group of 23 rats was fed a diet supplemented with 50 mg Sodium Sulfate/20 g pellet. After 4 weeks of this diet, weekly SC injections of DMH base (15 mg/kg) were given to all rats for 16 weeks. Animals were killed 8 weeks after the last injection. The final number of colon tumors was as follows: no tumors in the untreated control group; 77 tumors in the group receiving DMH alone; and, 53 tumors in the group receiving Sodium Sulfate and DMH. A mean score of 3.5 tumors/rat was observed for the DMH-alone group and a mean of 2.3 tumors/rat was found for the Sodium Sulfate plus DMH group.

CLINICAL ASSESSMENT OF SAFETY

Absorption, Distribution, Metabolism, Excretion

Oral

Cocchetto and Levy (1981) investigated absorption of Sodium Sulfate in humans as measured by recovery of free sulfate in the urine. Five healthy males (66–79 kg bw) were orally dosed with 18.1 g of decahydrate Sodium Sulfate (56.3 mmol, equivalent to 8.0 g of the anhydrous salt), in either a single dose or four equally divided hourly doses. (The Sodium Sulfate was dissolved in 50 ml of warm water and ingested following a low-fat breakfast.) With a minimum of 1 week between treatments, the dosing protocol was repeated but reversed and those who had previously received a single dose now received the divided doses and vice versa. Urine was collected over 0 to 24, 24 to 48, and 48 to 72 hour periods. All subjects experienced severe diarrhea following the single dose of Sodium Sulfate, starting 2 hours following ingestion and lasting up to 24 hours. Panelists who received divided dosings experienced mild to no diarrhea.

The baseline individual average excretion rate of inorganic sulfate (determined by collection of three 24-hour urine samples prior to sulfate treatment) ranged from 13 to 25 mmol/24 h with the two individuals with the lowest body weights having the lowest baseline values. Although the baseline excretion of free sulfate was unaffected by changes in urine flow rate, the baseline excretion rate of total sulfate (including organically bound sulfate) increased almost linearly with increasing flow rate. This effect was also observed following sulfate administration.

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Following Sodium Sulfate administration, the cumulative amounts of free sulfate excreted in the 24-, 48-, and 72-hour urine were significantly greater than the amount of free sulfate excreted in the same time periods in control experiments ($p < .01$). On average, 24 hours postdosing, 36.4% of the sulfate administered in a single dose (standard deviation [*SD*] 15.4%) and 43.5% of the divided dose (*SD* 12.0%) had been recovered. By 48 hours, an average of 49.5% of the single (*SD* 15.6%) and 53.1% of the divided dose (*SD* 7.5%) had been recovered. And by 72 hours, 53.4% of the sulfate administered in the single dose (*SD* 15.8%) and 61.8% administered in the divided dose (*SD* 7.8%) was recovered. The researchers noted "considerably less inter-individual variation" in urinary recovery of free sulfate following the divided dose.

A subsequent study by Morris and Levy (1983) in which eight panelists (six males, two females) ingested 9 g Sodium Sulfate (decahydrate) within a 1-hour period resulted in increased serum inorganic sulfate concentrations. The mean values were 0.410 mM prior to Sodium Sulfate intake, and 0.513 mM following ingestion of the test material ($p < .001$). Urinary excretion of inorganic sulfate also increased after ingestion of Sodium Sulfate. The renal clearance of endogenous creatinine was not affected.

Intravenous

Six normal human panelists received a 1-L infusion of 4% Sodium Sulfate, resulting in a decrease of urinary pH from 6.05 to 4.32 with a doubling of ammonia and titratable acid excretion. In 10 patients with renal disease and normal serum bicarbonate concentrations (>25.1 meq/L), the infusion resulted in a rise of urine pH without a change in ammonia or titratable acid excretion. Therefore, net acid excretion fell. In the same 10 patients with renal disease but with serum bicarbonate concentrations below 20 meq/L, the Sodium Sulfate infusion produced results similar to those in the six normal patients (Seldin et al. 1967).

Six males (aged 55–70 years) with normal renal function received an IV dose of 1.1 Mbq (30 μCi) radioactive Sodium Sulfate and collected urine for 72 hours. By 24 hours, 88% of the $Na_2{}^{35}SO_4$ dose was excreted in the urine; 87% was excreted by 72 hours. Heparinized blood samples were collected from another two panelists (one of each sex) every minute for the first 15 minutes after injection, and then every 15 minutes until 3 hours following injection. By extrapolating the early phase of the plasma disappearance curve, the researchers predicted that 1% to 2% of the administered Sodium Sulfate remained in the plasma 24 hours after injection (Burke and Staddon 1983).

Effect on Drug Absorption

Acetaminophen

Eight healthy adults received on separate occasions, 1 g acetaminophen; 1 g acetaminophen and 18 g Sodium Sulfate (decahydrate); 1 g acetaminophen and 10 g activated charcoal; and 1 g acetaminophen and 10 g activated charcoal and 18 g Sodium Sulfate, in random order. The Sodium Sulfate was administered such that at zero time, 4.5 g Sodium Sulfate USP was ingested in 50 ml water, followed by 4.5 g in 100 ml water at 2, 4, and 6 hours. Urine was collected for 48 hours and analyzed for acetaminophen, its metabolites, and inorganic sulfate. The panelists tolerated the various treatments well, except for instances of loose stools following Sodium Sulfate ingestion. Sodium Sulfate did not interfere with the absorption of acetaminophen by charcoal and, likewise, charcoal did not affect the absorption of Sodium Sulfate. Sodium Sulfate did not increase the formation of acetaminophen sulfate. This finding was consistent with expectations as the researchers noted administration of inorganic sulfate increases acetaminophen sulfation only when endogenous sulfate supplies are markedly depleted (i.e., very large doses of acetaminophen would be needed). The researchers considered that a combination of activated charcoal and Sodium Sulfate can be useful in the treatment of acetaminophen overdose (Galinsky and Levy 1984).

Other Drugs

Mattila, Takki, and Jussila (1974) reported that ingestion of 20 g Sodium Sulfate as two 10-g doses, 30 minutes apart resulted in diarrhea in 11 healthy panelists. Isoniazid (INH) was given with the first dose and sulfafurazole and acetylsalicylic acid were given with the second dose. Blood samples were taken at 30 minutes after the first dose (just prior to ingestion of the second dose) and at 30, 60, 120, and 240 minutes following the second dose. A control study had been conducted 3 days prior in which the drugs were administered following the same protocol but without Sodium Sulfate. Sodium Sulfate reduced serum concentrations and urinary excretion of INH and reduced the absorption rate and urinary excretion of sulfafurazole. The absorption of acetylsalicylic acid from a slow-release tablet was unaffected. The absorption of acetylsalicylic acid was slightly reduced in seven panelists when a single dose of Sodium Sulfate was administered.

Campbell et al. (1985) studied the effect(s) of Sodium Sulfate ingestion on methyldopa metabolism. Twenty-four panelists were randomized to ingest either 13.24 mg/kg Sodium Sulfate with 3.5 mg/kg methyldopa powder or methyldopa alone. One week later, the subjects were given the alternate treatment. Urine was collected for 24 hours following dosing. Sodium Sulfate ingestion increased the concentration of methyldopa sulfate (from 50.1% to 66.0%) and decreased the concentration of free methyldopa (from 27.3% to 17.1%) in the urine. A positive correlation ($r = .545$, $p < .01$) between platelet phenol sulfotransferase (PST) activity and the percentage of drug excreted as methyldopa sulfate was noted with concurrent intake of methyldopa and Sodium Sulfate. This relationship was not noted when methyldopa was taken alone ($r = -.340$, $p > .10$). PST catalyzes the metabolism of methyldopa sulfation; 3′-phosphoadenosine-5′-phosphosulfate (PAPS) serves as the sulfate donor for the PST reaction. No gastrointestinal problems associated with Sodium Sulfate ingestion were noted.

Oral Toxicity

In a study to determine the role of fecal pH on the risk of colon cancer, 27 patients with a history of colonic polyps received a mean dose of 4 g/day of Sodium Sulfate for 14 days (Kashtan et al. 1990). A control group of 25 patients received placebo. The panelists were instructed to self-adjust the daily dose (not to exceed 6 g/day) such that two to three soft stools were produced each day. No adverse effects were noted.

Inhalation Toxicity

Sackner, Ford, and Kim (1979) exposed for 10 minutes five healthy and five asthmatic adults to 1, 2, and 3 mg/m^3 Sodium Sulfate aerosol with a mass median aerodynamic diameter (MMAD) of 0.5 μm. Respiratory parameters were measured for up to 1 hour following exposure. Mean values for the measured respiratory parameters were similar to the values obtained for exposure to equivalent amounts of sodium chloride (control). Two asthmatics had a 15% to 20% fall in forced exhalation volume (FEV_1); however, the response did not worsen with exposure to higher concentrations. In a subsequent experiment, six normal and six asthmatic adults were exposed for 10 minutes to 3 mg/m^3 Sodium Sulfate aerosol. Lung function measurements were made for 3 hours following exposure. Again, mean values for Sodium Sulfate when compared to sodium chloride indicated no adverse effect on pulmonary function. An immediate 15% to 20% fall in FEV_1 was noted in two of six asthmatics after breathing either Sodium Sulfate or sodium chloride.

Kelada and Euinton (1978) found no abnormality attributable to long-term occupational exposure to Sodium Sulfate dust in 119 workers from five sodium sulfate surface solution mines. Dust exposure concentrations ranged from <5 mg/m^3, 40 mg/m^3 in the main plant, and up to 150 mg/m^3 during loading of the final product. The workers had between 2 months to 31 years of exposure. The workers were not distinguishable from the general population with regards to parameters measured in the cardiorespiratory, gastrointestinal, or hepatorenal systems. Lung function, serum sulfate, calcium and electrolytes were within normal limits. There were no significant differences in the serum sulfate concentrations of workers with >10 years experience as compared to those from workers with <10 years experience.

Dermal Irritation

A single 24-hour occlusive patch containing an effective Sodium Sulfate concentration of 9.7% (10% aqueous solution of a bath bead formulation containing 97.0% Sodium Sulfate) was applied to 19 panelists. No reactions were noted in 18 panelists. One panelist had a reaction scored as ± (first nonzero grade on scale from 0 to 4±) (CTFA 1985).

An effective Sodium Sulfate concentration of 0.1168% (2% solution of a bar soap flake formulation containing 5.84% Sodium Sulfate) was applied in three 24-hour occlusive patches to the lateral arm of 13 panelists. Mild irritation (score 0.5–1.0: maximum possible score 4.00) was noted in 11 panelists. Of these, seven reacted to all three exposures, two reacted to the second and third exposure, and two reacted to the third patch only. The group average was 0.410 and the formulation was classified as mildly irritating (Hill Top Research Inc. 1989).

An effective Sodium Sulfate concentration of 1.8% was applied in a 4-hour patch on each of 4 days to 20 panelists. The test material was a children's powdered bubble bath and the exposure concentration was 200 times the expected consumer use level. Sites were scored after the fourth exposure. Thirteen panelists had no incidence of erythema and 11 had no incidence of dryness. Mild erythema and dryness (scored ±, the first nonzero grade) were noted in seven and eight panelists, respectively. Dryness in the twentieth panelist was scored 1 on a 0 to 2+ scale (CTFA 1990).

A 24-hour occlusive patch containing an effective Sodium Sulfate concentration of 0.004% (a 0.25% aqueous solution of a cleansing bar base containing 1.75% Sodium Sulfate) was applied to the back of 35 panelists on each of 21 consecutive days. Components of the formulation other than water included: sodium alkyl glyceryl sulfonate (10–60%), stearic acid (1–20%), lauric acid (1–15%), sodium lauroyl sarcosinate (1–10%), unsulfonated alcohols (1–8%), and sodium chloride (1–5%). Reactions were noted after various exposures in all panelists. Most reactions indicated moderate irritation (score 2 or 2.5 on a scale to 4). Mild to slightly irritating reactions (score between 0.5–1.0) and severely irritating reactions (score of 3) were noted in three panelists, respectively. The group mean score was 1.571 and the formulation was considered mildly irritating (Hill Top Research, Inc. 1985).

Dermal Sensitization

An effective Sodium Sulfate concentration of 1.01% (1.25% aqueous solution of a bubble bath containing 80.8% Sodium Sulfate) was tested in a repeated insult patch test on 61 panelists. The concentration tested was a 100-fold exaggeration of normal use levels. The first induction patch was left in place on the back for 48 hours and the remaining eight patches were applied for 24 hours of exposure. Every third patch (i.e., patches 1, 4, 7 and 2, 5, 8) was applied to the same site on the back. Following a 3-week nontreatment period, panelists were challenged on a previously unexposed site with a 48-hour patch. One panelist had a single incidence of mild erythema after exposure to induction patch 4. No reactions were observed at challenge (CTFA 1976).

SUMMARY

Sodium Sulfate is a GRAS ingredient that is used in cosmetic formulations as a viscosity increasing agent. In 1997 there were 28 reported cosmetic uses. Data from two sources indicated use at a variety of concentrations, with a maximum use of almost 97% in bath formulations. Sodium Sulfate is rapidly absorbed and excreted following oral intake.

No significant adverse effects were noted in rats following short-term oral dosing or in anesthetized dogs or conscious sheep following brief or intermediate inhalation exposures. A granular

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sodium carbonate–Sodium Sulfate mixture produced moderate ocular irritation in rabbits.

No developmental changes were noted in rat fetuses whose dams had received an intramuscular injection of Sodium Sulfate on gestation day 17. An oral-dose study found increased neonate birth weight in fetuses of mice which had received Sodium Sulfate during gestation.

Sodium Sulfate was negative in mutagenicity assays. Results of various oral cocarcinogenicity assays were dependent on the carcinogen administered with Sodium Sulfate (and an inhibitor).

Clinical studies reported no significant adverse effects following oral or inhalation exposure to Sodium Sulfate. Mild-to-no irritation and no sensitization were noted in dermal studies that tested Sodium Sulfate–containing bath formulations at exaggerated-use concentrations and conditions.

DISCUSSION

In assessing the safety of Sodium Sulfate, the CIR Expert Panel relied on its GRAS status to preclude the need for many studies. Further, the submission of clinical dermal irritation and sensitization data by the cosmetics industry addressed the Panel's concerns about the lack of such studies in the published literature. The submitted data showed Sodium Sulfate induced no-to-mild irritation and no sensitization when tested in bath formulations. The Panel decided that these data were sufficient to conclude that Sodium Sulfate was safe as used in rinse-off formulations.

However, because some of these formulations produced irritation under patch test conditions, the Panel restricted the use of Sodium Sulfate in leave-on products. Results from a clinical sensitization study were considered particularly useful because the testing protocol specified repeated prolonged exposure. An induction period in which nine 24-hour insult patches containing 1.01% Sodium Sulfate were applied noted one isolated incidence of mild erythema in 1 of 61 panelists. The Panel rounded the figure to 1% to arrive at the limit for use in leave-on products.

CONCLUSION

Based on the available data, the CIR Expert panel concludes Sodium Sulfate to be safe as used in rinse-off formulations, and safe up to 1% in leave-on formulations.

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Final Report on the Safety Assessment of Ammonium, Potassium, and Sodium Persulfate[1]

Ammonium, Potassium, and Sodium Persulfate are inorganic salts used as oxidizing agents in hair bleaches and hair-coloring preparations. Persulfates are contained in hair lighteners at concentrations up to 60%, in bleaches and lighteners at up to 22% and 16%, respectively, and in off-the-scalp products used to highlight hair strands at up to 25%. They are used in professional product bleaches and lighteners at similar concentrations. Much of the available safety test data are for Ammonium Persulfate, but these data are considered applicable to the other salts as well. Acute dermal, oral, and inhalation toxicity studies are available, but only the latter are remarkable, with gross lesions observed in the lungs, liver, stomach, and spleen. In short-term and subchronic feeding studies the results were mixed; some studies found no evidence of toxicity and others found local damage to the mucous membrane in the gastrointestinal tract, but no other systemic effects. Short-term inhalation toxicity was observed when rats were exposed to aerosolized Ammonium Persulfate at concentrations of 4 mg/m^3 and greater. Ammonium Persulfate (as a moistened powder) was not an irritant to intact rabbit skin, but was sensitizing (in a saline solution) to the guinea pig. It was slightly irritating to rabbit eyes. Ammonium Persulfate was negative in the Ames test and the chromosomal aberration test. No significant evidence of tumor promotion or carcinogenicity was observed in studies of rats receiving topical applications of Ammonium Persulfate. The persulfates were reported to cause both delayed-type and immediate skin reactions, including irritant dermatitis, allergic eczematous dermatitis, localized contact urticaria, generalized urticaria, rhinitis, asthma, and syncope. The most common causes of allergic dermatitis in hairdressers are the active ingredients in hair dyes, and Ammonium Persulfate has been identified as a frequent allergen. A sensitization study that also examined the incidence of urticarial reactions was performed with 17.5% Ammonium, Potassium, and Sodium Persulfate under occlusive patches. At this concentration and exposure conditions, a mixture of these Persulfates was not sensitizing, and application of Ammonium, Potassium, and Sodium Persulfate did not result in an urticarial reaction. In normal use (i.e., not occluded and rinsed off), it was expected that a concentration greater than 17.5% would also be safe. Given the clinical reports of urticarial reactions, however, manufacturers and formulators should be aware of the potential for urticarial reactions at concentrations of Persulfates greater than 17.5%. Based on the available data, the Cosmetic Ingredient Review (CIR) Expert Panel concluded that Ammonium, Potassium, and Sodium Persulfate are safe as used as oxidizing agents in hair colorants and lighteners designed for brief discontinuous use followed by thorough rinsing from the hair and skin.

INTRODUCTION

Ammonium, Potassium, and Sodium Persulfate are inorganic salts used as oxidizing agents in hair bleaches and hair coloring preparations. The following report reviews the safety data on these ingredients.

CHEMISTRY

Definition and Structure

Ammonium Persulfate (CAS No. 7727-54-0) is the inorganic salt that conforms to the formula $(NH_4)_2S_2O_8$ (Wenninger, Canterbery, and McEwen 2000). It is also known as Ammonium Peroxydisulfate; Peroxydisulfuric Acid, Diammonium Salt (Wenninger, Canterbery, and McEwen 2000); Diammonium Persulfate; Diammonium Peroxydisulfate (Registry of Toxic Effects of Chemical Substances [RTECS] 1994); Ammoniumperoxodisulfate; Ammoniumperoxydodisulfate; and Ammoniumperoxysulfate (Cosmetic, Toiletry, and Fragrance Association [CTFA] 1994).

Potassium Persulfate (CAS No. 7727-21-1) is the inorganic salt that conforms to the formula $K_2S_2O_8$ (Wenninger, Canterbery, and McEwen 2000). It is also known as Peroxydisulfuric Acid, Dipotassium Salt (Wenninger, Canterbery, and McEwen 2000); Potassium Peroxydisulfate; and Dipotassium Persulfate (RTECS 1994).

Sodium Persulfate (CAS No. 7775-27-1) is the inorganic salt that conforms to the formula $Na_2S_2O_8$ (Wenninger, Canterbery, and McEwen 2000). It is also known as Sodium Peroxydisulfate and Peroxydisulfuric Acid, Disodium Salt (Wenninger, Canterbery, and McEwen 2000).

Physical and Chemical Properties

Ammonium Persulfate is a yellow to white crystalline material that has a slight acrid odor (Nikitakis and McEwen 1990). It has a molecular weight of 228.20 Da and readily dissolves in water (Budavari 1989). Water solubility values are 559 g/l at 20°C, pH 2 to 2.5 at 250 g/l, and 510 g/l at 25°C, pH 4 to 6 for 1% solution (CTFA 1994). Ammonium Persulfate

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decomposes at 120°C (Lewis 2000). Dangerous decomposition products of Ammonium Persulfate are sulfur dioxide and sulfur trioxide (CTFA 1994). Ammonium Persulfate is a strong oxidizing agent, and aqueous solutions of this ingredient are acidic and lose active oxygen with time, especially at elevated temperatures (Budavari 1989; Nikitakis and McEwen 1990). CTFA specifications for Ammonium Persulfate list the maximum allowable concentration for sulfated ash as 0.05% (Nikitakis and McEwen 1990). The following impurities and their maximum concentrations were also listed: arsenic (3 ppm), iron (5 ppm), and lead (20 ppm).

Potassium Persulfate is a white, odorless, crystalline material with a molecular weight of 270.3 Da (Budavari 1989). Like Ammonium Persulfate, it loses oxygen with time and with greater rapidity at higher temperatures, completely decomposing at 100°C. Potassium Persulfate is soluble in about 50 parts water and is acidic in aqueous form. This ingredient is incompatible with combustible materials, organic materials and other oxidizable materials, sulfur, metallic dust, aluminum dust, chlorates, and perchlorates.

Sodium Persulfate is a white crystalline powder with a molecular weight of 238.13 Da. It gradually decomposes, and decomposition is promoted by moisture and higher temperatures (Budavari 1989). This ingredient is soluble in water, and decomposes in alcohol (Lewis 2000).

Manufacture and Production

Ammonium Persulfate and Potassium Persulfate are prepared by electrolysis of concentrated solutions of ammonium sulfate and potassium sulfate, respectively (Lewis 1999). Merget et al. (1996) reported that Ammonium Persulfate is produced by anodic oxidation of a concentrated ammonium sulfate solution, and that Sodium Persulfate is made by conversion of Ammonium Persulfate with lye.

In 1986, a cosmetic supplier/manufacturer sold 141 tons of bleaching powder, corresponding to 5.5 million applications (CTFA 1987).

USE

Cosmetic

Ammonium, Potassium, and Sodium Persulfate are oxidizing agents used in hair bleaches, hair-coloring preparations, and/or hair lighteners with color (Wenninger, Canterbery, and McEwen 2000) and are used to decolorize or lighten hair (CTFA 1995a). The product formulation data submitted to the Food and Drug Administration (FDA) in 1998 reported that Ammonium Persulfate was used in a total of 30 cosmetic product formulations, Potassium Persulfate was used in 36 formulations, and Sodium Persulfate in 26 formulations (Table 1) (FDA 1998).

TABLE 1
Cosmetic product formulation data on Ammonium, Potassium, and Sodium Persulfate (FDA 1998)

Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
	Ammonium Persulfate	
Hair dyes and colors	1572	1
Hair bleaches	113	23
Other hair-coloring preparations	59	5
Other skin care preparations	692	1
1998 total for Ammonium Persulfate		**30**
	Potassium Persulfate	
Hair straighteners	63	1
Hair dyes and colors	1572	2
Hair lighteners with color	6	1
Hair bleaches	113	27
Other hair-coloring preparations	59	5
1998 total for Potassium Persulfate		**36**
	Sodium Persulfate	
Hair straighteners	63	1
Hair dyes and colors	1572	2
Hair lighteners with color	6	1
Hair bleaches	113	21
Other hair-coloring preparations	59	1
1998 total for Sodium Persulfate		**26**

Concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA 1992). One data submission to Cosmetic Ingredient Review (CIR) states that liquid and gel lighteners for general (all over the head) hair lightening contain ≤12% (on-head) Persulfates, usually comprised of ≤4% Ammonium and ≤8% Potassium Persulfate, and off-the-scalp products used to highlight hair strands generally contain ≤25% (on-head) Persulfates, usually comprised of ≤10% Ammonium and ≤15% Potassium Persulfate (CTFA 1995a). Professional-use lightening products contain the same maximum on-head concentrations as just given, but often contain a mixture of Ammonium (5%), Potassium (15%), and Sodium (5%) Persulfates.

Another submission to CIR by CTFA states that Ammonium, Potassium, and Sodium Persulfates are used in hair lighteners at a concentration of 60% and that the three Persulfates are contained in bleaches and lighteners at concentrations of 12% to 22% (use concentration of 4% to 8%) and 2% to 16% (use concentration of 1% to 6%) (CTFA 1995b). This submission also stated that the Persulfates are used in bleaches and lighteners that are professional products involving off-scalp use (on the hair shaft only), and in these products the Persulfates may be used at a concentration of 10% to 18%. Product formulation data submitted to the FDA in 1984 stated all three ingredients were used at concentrations greater than 50% (FDA 1984).

In general, the strong oxidizing action of persulfates is used to accelerate the bleaching process of peroxide hair bleaches (Fisher 1985a). These ingredients make the hair "porous," making it more receptive to dyes or toners that provide the final hair shade.

International

Ammonium, Potassium, and Sodium Persulfates are used in Europe to decolorize or lighten hair (CTFA 1995a).

None of these ingredients are listed in the *Comprehensive Licensing System (CLS)* categories, in which ingredients are listed that have a precedent for use in Japan (Santucci 1999). According to Notification 990 of the Pharmaceutical and Medical Safety Bureau of the Japan Ministry of Health and Welfare, issued September 29, 2000, these ingredients are not prohibited or restricted in its use beyond a basic obligation of manufacturers to use all ingredients in a manner which guarantees safety (Japan Ministry of Health and Welfare 2000).

Noncosmetic

Ammonium Persulfate is cleared for use as a bleaching agent for food starch at <0.075%; as an industrial starch modifier and as an alkaline starch reactant at ≤0.3% and ≤0.6%, respectively; in adhesives; as a component of paper and paperboard in contact with aqueous, fatty, and dry foods; and in cellophane and water-insoluble hydroxyethylcellulose film (Rothschild 1990). Ammonium Persulfate is used as a reducer and retarder in photography, as an oxidizer for copper (Budavari 1989), and as an etchant for printed circuit boards (Lewis 1997). It is also used in electroplating, the manufacture of other persulfates, deodorizing and bleaching oils, aniline dyes; preserving foods, depolarizer in batteries, and washing infected yeast.

Potassium Persulfate is cleared for use in certain types of coatings for fresh citrus fruits; in adhesives; in acrylate ester copolymer coating; in resinous and polymeric coatings at 1%; as a component of paper and paperboard in contact with aqueous, fatty, and dry foods; in closures with sealing gaskets for food containers when <1% by weight of the gasket composition; and in rubber articles intended for repeated use (Rothschild 1990). Potassium Persulfate is used as a reducing agent in photography, as an analytical reagent, as a polymerization promoter, in pharmaceuticals, in the modification of starch, as a flour maturing agent, and in de-sizing of textiles (Lewis 1997).

Sodium Persulfate is cleared for use as components of paper and paperboard in contact with aqueous, fatty, and dry food; in closures with sealing gaskets for food containers when <1% by weight of the gasket composition; and at a concentration of less than 1% in can-end cements for resinous and polymeric coatings (Rothschild 1988). Additionally, it is cleared as a denuding agent of mucous membranes in tripe. Sodium Persulfate is used as a bleaching agent for fats, oils, fabrics, and soaps. It is also used in battery depolarizers and in emulsion polymerization (Lewis 1997).

GENERAL BIOLOGY

Immunological Effects

The histamine-releasing potential of Ammonium Persulfate was investigated using skin slices obtained from Dunkin-Hartley guinea pigs, CFY rats, and Rhesus monkeys (Mahzoon, Yamamoto, and Greaves 1977). Triplicate samples (one to three slices per sample) were incubated with 1 to 1000 μg/ml Ammonium Persulfate for 15 to 30 minutes. No significant histamine release was observed at any of the concentrations tested with guinea pig or monkey skin. With the rat skin, 1000 μg/ml Ammonium Persulfate released 20% to 24% of the histamine from the skin.

Parsons, Goodwin, and Safford (1979) reported that both Ammonium and Potassium Persulfate caused histamine release from isolated rat peritoneal mast cells and from guinea pig skin in vitro and in vivo. In studies with mast cells, both persulfates (0.33 to 2.7 mg/ml) caused dose-dependent releases of histamine. Histamine release induced by Potassium Persulfate was characterized by degranulation of the mast cell with no disruption of the cell membrane. However, with Ammonium Persulfate, alterations in the granules were observed but no apparent degranulation or disruption of the cell membrane occurred. In in vitro studies with slices of guinea pig skin, Potassium Persulfate, but not Ammonium Persulfate, appeared to release histamine selectively. At concentrations ranging from 0.1 to 8 mg/0.5 ml, Ammonium Persulfate induced a dose-related mean histamine release of 1.11% to 14.42% and Potassium Persulfate induced a dose-related mean release of 0.45% to 24.33%. In

in vivo studies, intradermal injections of Potassium Persulfate (4 to 16 mg/ml saline) into guinea pigs caused a dose-dependent release of histamine. Because pretreatment with mepyramine maleate reduced histamine release, the investigators speculated that the vascular permeability changes were due in part to an indirect action mediated by histamine released from skin mast cells. However, because histamine release was not completely inhibited by mepyramine maleate, mediators other than histamine are probably also involved. The investigators concluded that Potassium Persulfate induced the release of histamine by a slow, dose-dependent, noncytolytic mechanism, whereas Ammonium Persulfate appeared to work through both this mechanism and a rapid cytolytic mechanism.

Human polymorphonuclear neutrophil granulocytes (PMNs) were treated with 0.1 to 10 mM Ammonium Persulfate and activated with different stimuli (Köller, Hilger, and König 1996). Stimulation with Ca^{2+} ionophore A23187 (which bypasses membranous signal transduction) resulted in a dose-dependent decrease in the amount of total generated leukotriene B_4 (LTB_4); the decrease was significant at all test concentrations. A similar decrease was also observed with Sodium Persulfate. A decrease in LTB_4 was also observed after incubation with Ammonium Persulfate and activation with the tripeptide formylmethionylleucylphenylalanine (fMLP) (which activates cellular responses via ligand-receptor coupling) and sodium fluoride (which directly stimulates heterotrimeric G proteins). Lymphocytes/monocytes/basophils were also treated with 0.1 to 10 mM Ammonium Persulfate. A dose-dependent histamine release was observed without additional cellular stimulation; the amount of released histamine ranged from 6% to 20% at 1 mM to 40% at 10 mM Ammonium Persulfate. Coincubation of basophils with fMLP resulted in a significant histamine release with 10 mM Ammonium Persulfate, but not at lower concentrations.

The stability of leukotrienes in a cell-free system was examined: PMNs were stimulated with the Ca^{2+} ionophore, LTB_4-enriched supernatants were obtained, and Ammonium Persulfate was then added. LTB_4 was significantly decreased at concentrations of 1 and 10 mM. The addition of Ammonium Persulfate to resting cells also resulted in a significant decrease in LTB_4.

The effect of priming PMNs with Ammonium Persulfate was also examined. Cells were pretreated with Ammonium Persulfate, washed, and stimulated with the Ca^{2+} ionophore, fMLP, or sodium fluoride. An increase in leukotriene release was observed when the cells were stimulated with fMLP or sodium fluoride; the priming effect was primarily achieved by stimulation with fMLP. The increase in leukotriene formation was generally greatest at a concentration of 0.1 mM Ammonium Persulfate. The priming effect of Ammonium Persulfate was not observed after stimulation with the Ca^{2+} ionophore (Köller, Hilger, and König 1996).

Effect on Smooth Muscle Tone

The effect of Ammonium Persulfate on smooth muscle tone was examined using an in vitro guinea pig tracheal preparation (Mensing, Marek, and Baur 1996). Ammonium Persulfate (9×10^{-5} to 9×10^{-2} M) dilated the trachea and caused a concentration-dependent decrease in intratracheal pressure. The acutely elicited tracheal muscle dilatation was mediated by nitric oxide.

Effects on Cardiomyocytes

The effects of Ammonium Persulfate on the calcium uptake in cardiomyocytes isolated from the hearts of male Sprague-Dawley rats was investigated (Kaminishi, Yanagishita, and Kako 1989). Ammonium Persulfate caused both a concentration- and time-dependent increase in the number of cells in contracture. A concentration of 55 mM Ammonium Persulfate caused contracture of 50% of the cells following 90 minutes of exposure. The ^{45}Ca concentration in the cardiomyocytes decreased in proportion to the concentration of Ammonium Persulfate. The half-maximal decrease was observed at a concentration of 20 mM. The investigators concluded that Ammonium Persulfate "... inhibited intracellular uptake of calcium and accelerated calcium release, thus raising the cytosolic calcium concentration and causing cell contracture."

Antimicrobial Activity

Loveless, Spoerl, and Weisman (1954) reported that 2000 μg/ml Potassium Persulfate reduced the growth of *Saccharomyces cerevisiae* by about 50%, but had no effect on average cell size.

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

The oral LD_{50} of Ammonium Persulfate, when intubated at a concentration of 200 mg/ml, was 820 mg/kg (Smyth et al. 1969) for rats. When administered in distilled water to male rats and as a 25% *w/v* solution in tap water to female Sprague-Dawley rats, the oral LD_{50} was 600 mg/kg and 495 mg/kg, respectively (CTFA 1994). The oral LD_{50} values of Ammonium and Potassium Persulfate were 689 and 802 mg/kg, respectively, for rats (American Conference of Governmental Industrial Hygienists, Inc. [ACGIH] 1986).

Dermal

The dermal LD_{50} of Ammonium Persulfate was 2 g/kg when applied to the intact skin of 10 Sprague-Dawley rats and 10 g/kg when applied undiluted to four male rabbits (CTFA 1994).

Inhalation

The LC_{50} of Ammonium Persulfate was 2.95 mg/l (the maximum attainable dust concentration via gravimetric method) for Sprague-Dawley rats with a 4-hour exposure time (CTFA 1994). Ninety-seven percent of the particles were <10 μm in diameter. The LC_{50} of Ammonium Persulfate in a 25% water suspension was 520 mg/l for male rats with a 1-hour exposure

time. At necropsy 14 days after dosing, gross lesions were observed in the liver, stomach, lungs, and spleen.

Parenteral

The intravenous minimum lethal dose and the intraperitoneal LD_{50} of Sodium Persulfate for rabbits were 178 and 226 mg/kg, respectively (ACGIH 1986).

Short-Term Toxicity

Oral

Groups of 10 male CR-CD rats were fed 100, 300, or 600 ppm Ammonium Persulfate in the diet for 28 days (CTFA 1994). No deaths occurred during dosing and no gross lesions were observed at necropsy. The lowest-observed-adverse-effect level (LOAEL) was 600 ppm.

Inhalation

Groups of six Sprague-Dawley rats were exposed to 1, 4, 9, 17, and 20 mg/m^3 of aerosolized Ammonium Persulfate for 23.5 hours a day for 7 days (Last et al. 1982). The mass median aerodynamic diameter of the aerosol ranged from 0.8 to 1.3 μm. Control groups of rats were exposed to filtered air. No significant changes were observed with 1 mg/m^3 Ammonium Persulfate. However, at concentrations of 4 to 20 mg/m^3, Ammonium Persulfate caused a significant reduction in body weight and a significant increase in the wet weight of the right apical of the lung lobe. The greatest increase in wet weight was 164% with 20 mg/m^3 Ammonium Persulfate. However, no change in the wet–to–dry weight ratio was observed at any of the concentrations tested. Protein and DNA concentrations were significantly increased in the lungs, and tracheal mucus glycoprotein secretion rates tended to be greater than that observed in the control animals. The investigators attributed these changes to pulmonary edema and/or inflammation.

Subchronic Oral Toxicity

No signs of toxicity were observed when six dogs were fed a diet of flour containing 15 g/45 kg Ammonium Persulfate 6 days a week for 3 months (Arnold 1949).

No gross or microscopic alterations were seen in rats and dogs fed Ammonium Persulfate–treated flour or bread in the diet for 5 or 16 months, respectively (BGChemie 1994).

Rats were fed 30 mg/kg/day Sodium Persulfate for 13 weeks (BGChemie 1994). Local damage to the mucous membrane of the gastrointestinal tract occurred, but other systemic effects were not observed. No adverse effects were observed with administration of 30 mg/kg/day Sodium Persulfate for 13 weeks or 100 mg/kg/day for 8 weeks with subsequent administration of 500 mg/kg/day for 5 weeks (BGChemie 1994).

Dermal Irritation

To determine the irritation potential of 99% pure Ammonium Persulfate, 0.5 g moistened with 0.1 ml of water was applied under an occlusive patch to the intact and abraded skin of three white Russian rabbits for 4 hours (BGChemie 1994). Slight edema, which disappeared within 24 hours, was observed in intact skin, whereas moderate to severe erythema, moderate edema, and scab formation followed by cicatrization were observed at the abraded sites. Ammonium Persulfate was considered nonirritating to intact skin.

The dermal irritation potential of Ammonium Persulfate was determined according to Organisation for Economic Cooperation and Development (OECD) Guideline No. 404 using six male and female New Zealand White rabbits (CTFA 1994). No irritation was noted within 72 hours following application.

Ammonium Persulfate (dose not specified) was applied to an intact and abraded site on six rabbits, and the sites were scored by the Draize method at 24 and 72 hours (CTFA 1994). Ammonium Persulfate was not irritating.

Dermal Sensitization

The sensitization potential of Ammonium Persulfate was determined in an optimization test (OECD Guideline No. 406) using 10 male and 10 female Pirbright White guinea pigs (BGChemie 1994). All of the animals reacted to intradermal administration of a 0.1% solution in physiological saline, whereas 16 of the animals reacted to epicutaneous application of a 1% solution in demineralized water. Ammonium Persulfate was considered sensitizing to the guinea pig.

Inhalation Sensitization

Wass and Belin (1990) developed an in vitro method for predicting sensitizing properties of inhaled chemicals. Sodium Persulfate (50 μl) was mixed with a lysine-containing peptide (500 μl) at neutral pH and 37°C. The reaction was monitored by means of high-performance liquid chromatography. A peptide reactivity index was determined, ranging from 0, for no detectable reaction, to 10, for complete reactivity. In general, simple acids, bases, and solvents did not react with the peptide, whereas chemicals known for their sensitizing and asthma-inducing properties, such as isocyanates, anhydrides, and chloramine-T, did react. The peptide reactivity index was 0 for Sodium Persulfate.

Ocular Irritation

Ammonium Persulfate, 0.1 g, was instilled into the conjunctival sac of the eye of three white Russian rabbits (BGChemie 1994). Severe diffuse reddening and swelling with hypersecretion subsided within 72 hours; however, clouding of the cornea was still present at this time. The irritation index was 10.5 and Ammonium Persulfate was considered slightly irritating to the eye.

The ocular irritation potential of Ammonium Persulfate was determined according to OECD Guideline No. 405 using nine New Zealand White rabbits; the eyes of six animals were not rinsed whereas the eyes of three animals were rinsed 30 seconds after instillation (CTFA 1994). Ammonium Persulfate caused

slight to mild conjunctivitis and iritis in the unrinsed eyes and was considered minimally irritating to these eyes. Ammonium Persulfate was practically nonirritating to rinsed eyes.

In a Draize test using eight rabbits, Ammonium Persulfate (dose not specified) was not irritating to the eye (CTFA 1994).

GENOTOXICITY

Ammonium Persulfate, 1 to 1000 μg/plate, was evaluated for mutagenic activity in an Ames test using *Salmonella typhimurium* strains TA1535, TA1537, and TA1538 (Huntingdon Research Centre 1977). Tests were performed with and without metabolic activation and in triplicate. Positive and negative controls were used. Ammonium Persulfate was not mutagenic at any of the concentrations tested.

Ammonium Persulfate was evaluated for mutagenic potential in the Ames test at concentrations up to 10.0 mg/plate using *S. typhimurium* strains TA92, TA94, TA98, TA100, TA1535, and TA1537 (Ishidate et al. 1984). Tests were conducted both with and without metabolic activation with S9 mix and in duplicate. Ammonium Persulfate was not mutagenic in either protocol at any of the concentrations tested.

Ammonium Persulfate was also negative in the chromosomal aberration test (Ishidate et al. 1984; Ishidate 1988). Chinese hamster fibroblasts exposed to Ammonium Persulfate at concentrations up to 0.25 mg/ml for 48 hours had no increase in the incidences of polyploid cells or cells with structural aberrations.

Salmonella strain TA97 was incubated in triplicate at either 25°C or 37°C with Ammonium Persulfate (concentration not specified) for 30 minutes at pH 5.0 (Pagano, Zeiger, and Stark 1990). Following incubation, the mean number of his^+ revertants was determined. Ammonium Persulfate was toxic but not mutagenic at both temperatures.

TUMOR PROMOTION AND CARCINOGENICITY

In a skin tumor-promotion test, a single topical application of 20 nmol dimethylbenzanthracene (DMBA) in 0.2 ml acetone was applied to the shaved backs of 20 female Sencar mice, followed 1 week later by biweekly applications of 200 mg/ml Ammonium Persulfate for 51 weeks (Kurokawa et al. 1984). Positive- and vehicle-control groups of mice were also initiated with DMBA, followed by treatment with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and acetone, respectively. All of the animals were examined for tumors weekly and body weight was recorded monthly. At necropsy, samples of the skin and major organs were removed and prepared for microscopic examination.

No significant change in body weight or mean survival time was observed for the mice treated with Ammonium Persulfate. At week 52, three of the mice had skin tumors. None of the acetone-treated mice developed tumors, whereas all of the mice treated with TPA had skin tumors. It was noted that there was a relatively high incidence of tumors of the mammary glands, lungs, and uterus in the treated group, but such incidences were also observed in both the positive- and negative-control groups. The investigators concluded that Ammonium Persulfate was inactive as a skin tumor promoter.

The carcinogenic potential of Ammonium Persulfate was also investigated (Kurokawa et al. 1984). Twenty female Sencar mice were topically treated with 200 mg/ml Ammonium Persulfate twice a week for 51 weeks. A control group of mice was treated with acetone alone. All of the mice were examined for skin tumors weekly, and the skin and major organs were examined microscopically at the end of the study. No significant change in body weight or mean survival time was observed for the mice treated with Ammonium Persulfate. Two mice developed epidermal hyperplasia at week 51, whereas none of the mice treated with acetone had skin tumors. As seen in the tumor-promotion study, the incidence of tumors of the mammary glands, lungs, and uterus was similar in both the treated and the vehicle-control groups. The investigators concluded that Ammonium Persulfate was not a dermal carcinogen.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation and Sensitization

The most common causes of allergic contact dermatitis in hairdressers are the active ingredients in hair dyes (Fisher 1989). Ammonium Persulfate has been identified as a frequent allergen in hairdressers' hands (Beck 1990).

The sensitization potential of Ammonium, Potassium, and Sodium Persulfate was determined in a study that was initiated with 57 subjects, 2 males and 55 females, and completed by 46 subjects, 2 males and 44 females (Jordan 1998). For induction, a lightener/developer mixture with 17.5% Ammonium, Potassium, and Sodium Persulfate was applied to the left inner forearm under an occlusive patch for 4 hours. The mixture without the Persulfates was used as a control. The patches were originally to be applied to the same sites three times a week for 3 weeks. However, due to strong irritant reactions to the vehicle, patches 3 through 9 were applied for 1 hour instead of 4 hours, and the sites were rotated on the same forearm. Following a 2-week nontreatment period, two challenge applications, applied 48 hours apart, of occlusive patches containing 0.2 ml of 2% Ammonium, 2% Potassium, and 2% Sodium Persulfate were applied to the right inner forearm, examined after 1 hour, replaced, and removed at 24 hours. The sites were evaluated at 1 and 48 hours. One subject had an "irritant response that precluded the use of the same site for the second period," so the second set of patches was applied at an adjacent site on the same arm for 30 minutes using 1% of each Persulfate.

Eight subjects were premanently removed from the study during induction because of irritation. Ammonium, Potassium, and Sodium Persulfate were not sensitizers (Jordan 1998).

Sodium Persulfate was tested at concentrations of 10, 100, and 5000 ppm in a human patch test using 26 subjects

(E.I. DuPont de Nemours and Company 1992). Each dose was placed under an occlusive patch four times a week for 3 weeks. After a 1-week nontreatment period, each subject was challenged with the same concentration as used for induction. No sensitization reactions were observed in subjects of the 10- and 100-ppm treatment groups. However, 5 of 26 subjects treated with 5000 ppm Sodium Persulfate developed grade 4 skin reactions, which included redness, induration, swelling, papules, and vesicles, following the challenge application. These five subjects were rechallenged with either 100 or 2500 ppm Sodium Persulfate for 24 or 48 hours. Two of the subjects had grade 4 reactions at the site treated with 2500 ppm Sodium Persulfate, and one of these also had reactions at the 100 ppm site.

Van Joost et al. (1984) analyzed data from 242 patients who had one or more positive reactions to a routine battery of test antigens and who were also tested with 2% aqueous Ammonium and Potassium Persulfate. Six patients had delayed-type responses to Ammonium Persulfate and 11 had positive responses to Potassium Persulfate. The incidences of delayed-type response at 48 and 72 hours were subjected to studies of shifts (48 vs. 72 hours) and statistically analyzed. Neither of the ingredients had a negative shift over the time interval studied, increasing from the low value of 0.12 at 48 hours to 1.82 at 72 hours. In general, the persulfates had a significantly higher confidence limit for the mean value of shifts as compared to that of the routine battery as a whole. The investigators speculated that this may indicate that the persulfates behave differently in early delayed-type responses.

Ammonium Persulfate proved to fulfill classification criteria for a contact allergen and a sensitizer by inhalation in a project of the Nordic Council of Ministers. The conclusion on criteria documents from national research in Norway also stated that Ammonium Persulfate may cause allergy by skin contact (Nordic Council of Ministers, 1991).

Guerra, Bardazzi, and Tosti (1992a) reported that of 49 clients of hair dressers, only 7 (2.7%) had a positive patch test to 2.5% Ammonium Persulfate in petrolatum. One of these subjects, who had complained of generalized urticaria after exposure to a hair bleach, had a positive reaction to an open patch that confirmed the diagnosis of an immediate contact reaction caused by Ammonium Persulfate.

Ammonium Persulfate was used in a comparison of test results using Duhring and Finn chambers (Frosch and Kligman 1979). (Details of the testing were not provided.) A 1% aqueous Ammonium Persulfate solution produced a reaction of 2+ using the Duhring chamber and a reaction of 0 using the Finn chamber. A 10% solution produced reactions of 3+ and 1+ using the Duhring and Finn chambers, respectively.

A number of case studies of dermal and respiratory problems associated with persulfates have been reported in the literature. All of the cases were associated with the use of hair bleaches containing these ingredients. See Table 2 for a further description of these cases.

Urticarial Reactions

In the study performed by Jordan (1998) described earlier, the incidence of contact urticaria was examined by removing the challenge patches 1 hour after application and evaluating the test site. Application of Ammonium, Potassium, and Sodium Persulfate did not result in urticarial reactions.

Calnan and Shuster (1963) studied reactions to Ammonium Persulfate in five women with hand dermatitis. Saturated solutions of Ammonium Persulfate were applied topically or scratched into the skin. Wheals were produced in all of the women after 15 minutes; the wheals were larger when the solution was scratched into the skin. A 1:10 solution of Ammonium Persulfate was the lowest concentration at which these reactions were observed after the solution was scratched into the skin. Intradermal injections (0.05 ml) of a 1:100 solution of Ammonium Persulfate caused wheals greater than 15 mm in diameter. The investigators noted that skin responses were delayed by 15 to 30 minutes after topical exposure and by 10 to 15 minutes following intradermal exposure. The subsequent wheal and flare were indicative of a histamine response.

In order to investigate this further, four of the patients were tested using antihistamines. Ammonium Persulfate was applied to the skin both before and after antihistamines were injected. One patient's response was unchanged, two patients had reduced wheals, and one patient had no response. The investigators concluded that the characteristic cutaneous reactions caused by Ammonium Persulfate were due to histamine being slowly released from the skin.

This conclusion was also supported by results of a study in which Ammonium Persulfate had no effect upon skin that had been depleted of histamine. Four patients were injected with compound 48/80 in the forearm at each corner of a 2-cm^2 area of skin on the forearm. Twenty-four hours later a saturated solution of Ammonium Persulfate was scratched into the center of the square, as well as sites both proximal and distal to the square. No reactions occurred at the center of the square, but distal sites treated with Ammonium Persulfate had reactions.

The investigators were unable to conclude whether Ammonium Persulfate works directly on mast cells or whether histamine release is due to immediate-type immune hypersensitivity. Seven of 57 subjects developed wheals after being scratch tested with Ammonium Persulfate. This number was considered low and was not consistent with the idea that Ammonium Persulfate initiated histamine release. Slow absorption did not appear to be a factor because no reactions were observed when normal subjects were injected with Ammonium Persulfate. Additionally, the cutaneous responses could not be attributed to increased sensitivity to histamine because wheals induced by histamine acid phosphate were of similar size in normal individuals. Thus, the investigators surmised that the reactions observed in the five patients were due to increased sensitivity to Ammonium Persulfate (Calnan and Shuster 1963).

TABLE 2
Case studies of dermal reactions to Persulfates in hair bleaches

Case studies	Reference
A 54-year-old woman developed itchiness of the face and became red and swollen on the upper part of her body within .5 hour of having a hair bleach applied. She went into shock and had generalized erythema and urticaria. Patch tests with 2% Ammonium Persulfate were negative. However, direct application of a supersaturated solution of Ammonium Persulfate caused a 1+ response, and when the solution was rubbed in the skin, a 4+ response. A freshly prepared hair bleach caused a response of 1+ when applied to the skin and a 2+ urticarial wheal when rubbed into the skin.	Brubaker (1972)
The face of a 49-year-old woman became red and edematous immediately following exposure to a hair bleach containing a persulfate-peroxide mixture. This condition lasted for several hours. Generalized urticaria persisted for 24 hours. Patch tests with 2% and 5% aqueous Ammonium Persulfate were negative, but tests with 5% aqueous Ammonium Sulfate were positive.	Fisher and Dooms-Goossens (1976)
A 46-year-old woman developed redness and slight crustiness on the anterior portion of the scalp and forehead one day after treatment with a hair bleach containing Ammonium Persulfate. Erythema and crusting were apparent on day 3. Patch tests with 2% and 5% Ammonium Persulfate were negative. The authors believed that the reaction was due to "... excessive concentrations of Ammonium Persulfate producing a strongly irritating alkaline effect."	Fisher and Dooms-Goossens (1976)
The face of a 49-year-old woman became red and edematous, her eyelids could not be opened, and generalized urticaria developed immediately upon her first-time application of a persulfate-peroxide hair bleach. Edema lasted for several hours and generalized urticaria persisted for 24 hours. An open patch test with 2% aqueous Ammonium Persulfate applied to the forearm produced a large urticarial wheal within 7 minutes for the woman but not in three controls. The author believed this was a severe histamine reaction because it was a first time exposure and that Ammonium Persulfate is not primarily urticariogenic because the controls did not have a reaction.	Fisher 1977
A 45-year-old woman stated that on several occasions immediately upon application of Ammonium Persulfate hair bleach, a burning sensation and diffuse erythema developed on the forehead, back of the neck, and upper back, followed by a mild crusted dermatitis of the scalp and back of the neck the next day. The use of prednisone and Chlor-Trimeton prior to bleaching resulted in minimal symptoms.	Fisher 1977
A 72-year-old woman developed erythema and edema of the face 1 hour following exposure to hair bleach containing 5% aqueous Ammonium Persulfate. The following day, her cheeks and forehead were sharply demarcated and she had marked edematous urticaria on her face and forehead. When she was tested with 5% Ammonium Persulfate, an immediate wheal was produced. However, a 48-hour patch test with 2% aqueous Ammonium Persulfate was negative.	Fisher (1985a)
A 70-year-old woman developed pruritic edema on her cheeks and forehead 3 hours after the application of a hair bleach containing Ammonium Persulfate.	Fisher (1985a)
A 69-year-old woman experienced facial flushing following exposure to a hair bleaching formulation containing 2% Ammonium Persulfate. She reported a stinging and burning sensation of the scalp and her forehead and face were erythematous with no itching. This condition persisted for 48 hours. Patch tests with 2% Ammonium Persulfate were negative.	Fisher (1993)

It was reported that a wheal developed after a patient was scratch tested with Ammonium Persulfate powder "as is" (Fisher and Dooms-Goossens 1976). A different patient who was tested in the same manner developed a large wheal, asthma, and erythema of the face. Other patients tested with 5% Ammonium Persulfate developed large pruritic wheals without any systemic reaction. The investigators noted that although patch tests with Ammonium Persulfate in dermatitic patients indicated that reactions were allergic in nature and were of the delayed variety, the results of the scratch tests and the fact that a few control subjects also had positive responses indicated that Ammonium Persulfate may also be a primary urticariogenic agent and that some immediate reactions could be due to a nonallergic release of histamine.

Patients with urticarial reactions or asthma after exposure to Ammonium Persulfate may have immediate reactions to patch

tests (Fisher and Dooms-Goossens 1976). One subject patch tested with 5% aqueous Ammonium Persulfate developed a large urticarial wheal within 10 minutes of exposure, which was followed by an urticarial reaction of the head and neck that persisted for 16 hours.

Adverse Reaction Reporting

The FDA Consumer Experience Reporting System aggregates all consumer complaints received by cosmetic companies that participate in the FDA voluntary program by number of complaints received (not by complaint type). Information is submitted for both retail and professional use. The 1990 to 1993 annual rate for all adverse experiences reported for hair lighteners was 8.79 complaints/million units sold, with a mean of 8.46 complaints reported for 1.07 million units sold on average per year (CTFA 1995a). The mean complaint rate for hair lighteners with dyes was 2.60 complaints/million units sold, with a mean of 167 complaints reported for 130,000 units sold on average per year. (These complaint rates are lower than those reported for shampoos, baby shampoos, bath soaps, and permanent waves.)

Occupational Studies

A number of occupational studies regarding dermal problems associated with exposure to persulfates have also been reported. Fisher (1985a) reports that "The persulfates are unique chemicals that can produce not only irritant dermatitis and allergic eczematous dermatitis of the delayed 'Type IV' variety but also 'immediate' reactions including localized contact urticaria, generalized urticaria, rhinitis, asthma and syncope." In general, reactions such as severe, immediate localized, and generalized urticaria and possibly syncope are associated with formulations containing 10% to 20% Ammonium Persulfate, whereas delayed localized urticaria is associated with preparations containing 2% to 5% Ammonium Persulfate. However, the ACGIH (1986) reported that, based on 20 years experience with persulfates in one industry, even when the threshold value of 15 mg/m^3 for nuisance dust was employed for control purposes, no cases of occupational illness occurred. They also stated no "significant cases of dermatitis have occurred from skin contact when good personal hygiene practices were being followed."

Adverse effects are most commonly reported in the hairdressing industry. Reports of dermatitis in the manufacturing of persulfates exist, but are limited due to the preventive measures taken to limit exposure. In the past, dermatitis was also associated with the baking industry in Europe, which used persulfates in the making of bread. Several countries banned the use of persulfates in baking, and in general, potassium bromate has replaced persulfates in the baking industry (Fisher 1985b).

See Table 3 for the details of these occupational studies.

Threshold Limit Value

The persulfates are assigned a time-weighted average threshold limit value (TLV) of 5 mg/m^3, measured as persulfate (ACGIH 1986). However, the ACGIH recommends a TLV of 2 mg/m^3 for Potassium Persulfate (Sullivan 1992).

SUMMARY

Ammonium, Potassium, and Sodium Persulfate are inorganic salts used as oxidizing agents in hair bleaches and hair-coloring preparations. In 1998, it was reported to the FDA that Ammonium, Potassium, and Sodium Persulfate used in 30, 36, and 26 formulations, respectively. Data submitted to CIR state that Persulfates are contained in hair lighteners at concentrations up to 60%, in bleaches and lighteners at up to 22% (use concentration up to 8%) and up to 16% (use concentration up to 6%), respectively, and in off-the-scalp products used to highlight hair strands at up to 25% (on-head); they are used in professional product bleaches and lighteners at similar concentrations.

The dermal LD_{50} of Ammonium Persulfate was 2 and 10 g/kg for rats and rabbits, respectively. For rats, the reported oral LD_{50} of Ammonium Persulfate ranged from 600 to 820 mg/kg and for Potassium Persulfate was 802 mg/kg. The inhalation LC_{50} of Ammonium Persulfate for rats was 2.95 mg/l after a 4-hour exposure, and for a 25% water suspension and a 1-hour exposure, it was 520 mg/l. The intravenous minimal lethal dose and the intraperitoneal LD_{50} of Sodium Persulfate were 176 and 226 mg/kg, respectively. In a short-term feeding study of Ammonium Persulfate using rats, the LOAEL was 600 ppm. In a subchronic feeding studies, no signs of toxicity were observed in rats or dogs fed Ammonium Persulfate–treated flour or bread. Local damage to the mucous membrane in the gastrointestinal tract, but no other systemic effects, was observed in one subchronic feeding study with Sodium Persulfate, but no lesions were observed in another study. Inhalation toxicity was observed when rats were exposed to aerosolized Ammonium Persulfate at concentrations of 4 mg/m^3 and greater. Ammonium Persulfate was not an irritant to intact rabbit skin, but was sensitizing to the guinea pig. It was slightly irritating to rabbit eyes.

Ammonium Persulfate was negative in the Ames test and the chromosomal aberration test. No significant evidence of tumor promotion or carcinogenicity were observed in studies of rats receiving topical applications of Ammonium Persulfate. In a study examining the sensitization potential of and the incidence of urticarial reactions to 17.5% Ammonium, Potassium, and Sodium Persulfate in a lightener/developer mixture, the Persulfate mixture was not a sensitizer and none of the Persulfates caused an urticarial reaction; signficant irritation to the vehicle was observed during induction. In a clinical patch test, 5 of 26 subjects had positive sensitization reactions to 5000 ppm Sodium Persulfate. These reactions were confirmed in two subjects when rechallenged. In another study, it was noted that reactions to Ammonium Persulfate were more severe when the ingredient was scratched into the skin. Noting a characteristic wheal and flare response, the investigators concluded that histamine release was involved. This is supported by results of in vitro and in vivo animal studies. However, it could not be determined

TABLE 3
Occupational exposure to Persulfate Salts

Reaction	Study description	Reference
	Hairdressers	
Contact dermatitis	A 32-year-old hairdresser developed acute eczematous dermatitis on both hands following exposure to hair bleaches containing Ammonium Persulfate. Patch tests with 2% Ammonium Persulfate were positive.	Fisher and Dooms-Goossens (1976)
Contact dermatitis	Twelve of 49 hairdressers patch tested with 2.5% Ammonium Persulfate in petrolatum had positive reactions, compared to 1 of 118 nonhairdressers tested.	Kellett and Beck (1985)
Contact dermatitis	Over a 5-year period, 2320 patients with reactions to one or more allergens in a standard series were also tested with 2.5% pet. Ammonium Persulfate and 2% aqueous Potassium Persulfate. A total of 22 individuals had positive reactions to these persulfates. Retrospectively, 14 of these patients were hairdressers, of which 11 reacted to both persulfates and 3 reacted to only Ammonium Persulfate. Of the remaining eight nonhairdressers, five reacted to both persulfates and three reacted to only Ammonium Persulfate. The investigators noted that the hand dermatitis of four of these nonhairdressers was exacerbated by their personal use of hair bleaches.	Van Joost and Roesyanto (1991)
Contact dermatitis	A multicenter study was performed in Italy in order to evaluate the frequency and source of contact sensitization in hairdressers. Of the 302 hairdressers studied, 11.3% tested positively to 2.5% Ammonium Persulfate in petrolatum.	Guerra, Tosti, and Bardazzi (1992b)
Contact dermatitis	Patch test results from nine European centers were evaluated in order to determine the frequency of sensitization among European hairdressers. Of the 809 hairdressers tested, 8% had positive patch test results with 2.5% petrolatum Ammonium Persulfate. Of 104 clients who were patch tested because of suspected contact sensitization, none reacted to Ammonium Persulfate.	Frosch et al. (1993)
Contact dermatitis	Over a 5-year period, 143 atopic and nonatopic hairdressers with hand eczema were patch-tested with a hairdressers and standard series of allergens. The subjects were divided into three groups: 45 were eczematous atopics, 32 were mucous membrane atopics, and 66 were nonatopic. Seven (16%), 4 (13%), and 10 (15%) of the subjects of each group, respectively, were sensitized to Ammonium Persulfate.	Sutthipisal, McFadden, Cronin 1993
Contact dermatitis	One hundred three hairdressers were patch-tested with a number of allergens over a 4-year period. Thirty-seven hairdressers reacted to 2.5% Ammonium Persulfate in petrolatum. One patient had a type 1 reaction, with airways obstruction, in addition to allergic contact dermatitis.	van der Walle and Brunsveld 1994
Contact dermatitis	Over a 9-year period, 106 hairdressers were patch-tested with a hairdressers and standard series of allergens. Nineteen subjects (17.9%) had a positive reaction to 2.5% Ammonium Persulfate in petrolatum.	Katsarou et al. 1995
Asthma	A 29-year-old woman acquired rhinitis and asthma while working in a beauty salon. A scratch test performed using 1% aqueous Ammonium Persulfate immediately produced a wheal, followed by a mild asthma attack.	Fisher and Dooms-Goossens (1976)

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TABLE 3
Occupational exposure to Persulfate Salts *(Continued)*

Reaction	Study description	Reference
Asthma	A 21-year-old hairdresser had a nonimmediate asthmatic reaction to hair bleach containing persulfates. This type of reaction was reproduced by exposure to the bleach and was blocked by inhalation of beclomethasone dipropionate but not by sodium cromoglycate. Patch tests with Potassium Persulfate and the bleach were negative. The investigators noted that at the time of these tests, the subject had changed jobs and was no longer being exposed to the bleach.	Pepys, Hutchcroft, and Breslin (1976)
Asthma	Eleven of 23 employees of a hair salon complained of upper or lower respiratory tract symptoms. Four of six with asthma had cases that were occupationally related. These subjects developed late type asthmatic reactions after exposure to bleach powder. Bronchial provocation tests with the components of the bleach indicated that Potassium Persulfate was the cause.	Davies and Blainey (1983)
Asthma	Four of 23 employees of one hairdressing salon had occupational asthma due to inhalation of bleach powders containing persulfate salts. One of the four was positive in a skin prick test to persulfate salts. When specific bronchial provocation tests were conducted on 14 of the employees, as well as 8 other individuals, the investigators reported that only those with a history of work related asthma and bronchial hyperreactivity had positive reactions. They concluded that the response to the bleach powder was specific. Further studies indicated that the response was caused by changes in airway caliber rather than lung volumes and that mast cells may play a part in the pathogenesis of persulfate induced asthma.	Blainey et al. (1986)
Asthma	A 21-year-old hairdresser suffered from rhinitis and wheezing dyspnea during 5.5 years of employment when she was exposed to hair bleaches and hair dyes containing bleaches. She had elevated total IgE in allergy tests and a provocation test with 10 mg/ml histamine was positive. Exposure tests with a hair bleaching product and 1% Ammonium Persulfate caused wheezing and dyspnea 3 to 4 hours following exposure. These responses were partially inhibited when disodium cromoglycate was inhaled 15 minutes prior to exposure, and completely inhibited when betamethasone was administered. The investigators concluded that the patient suffered from late onset bronchial asthma due to sensitivity to Ammonium Persulfate.	Gamboa et al. (1989)
Contact dermatitis and asthma	A 21-year-old hairdresser developed rhinitis from exposure to commercial bleaches, had urticarial reactions when she applied the bleach to her own hair, and eventually developed conjunctivitis and edema of the eyelids. Patch tests were positive for Potassium and Sodium Persulfate, and inhalation tests with the hair bleach produced an immediate asthmatic reaction within 1 minute.	Pepys, Hutchcroft, and Breslin (1976)
Contact dermatitis and asthma	A 23-year-old hairdresser developed acute pruritus and rashes on her hands and forearms after using hair bleach containing Ammonium Persulfate. An open test with 5% aqueous Ammonium Persulfate caused slight reddening and pruritus after 20 minutes. A scratch test with 1% aqueous Ammonium Persulfate caused erythema and wheal information after 5 minutes. A closed patch test with 2% aqueous Ammonium Persulfate was positive at 72 hours.	Widstrom(1977)

(Continued on next page)

TABLE 3
Occupational exposure to Persulfate Salts *(Continued)*

Reaction	Study description	Reference
Contact dermatitis and asthma	A hairdresser who developed cutaneous and respiratory symptoms after 1 year of employment was tested in clinical and immunological studies. Skin prick tests with 1:5 *w/v* Potassium and Sodium Persulfate were positive, but were negative with 10 control subjects. The hairdresser had no reaction to a 2% concentration of either of the persulfates in an open patch test. Hyperreactivity was observed in a methacholine inhalation test. A bronchial provocation test with 1:50 *w/v* Potassium Persulfate elicited a nonimmediate asthmatic response, which was followed by a recurrent nocturnal fall in airflow that was resolved after 3 days. Plethysmography indicated air trapping due to increased airway resistance. Histamine release tests were not conclusive and determinations of specific immunoglobins against persulfate salts were negative.	Parra et al. (1992)
Rhinoconjunctivitis and asthma	A hairdresser developed rhinoconjunctivitis and bronchial asthma associated with hair bleach containing persulfate after 2 years. A prick test was positive for the persulfate.	Pankow et al. (1989)
	Bakers	
Contact dermatitis	Forty-two of 400 bakers examined had positive patch test reactions to Ammonium Persulfate. However, only one of 150 individuals not in the baking industry reacted to this ingredient.	Grosfeld (1951)
Contact dermatitis	Five bakers with occupational eczematous dermatitis were tested with a variety of baking ingredients using on-off and patch tests to determine the cause of their dermatitis. Two of the workers were sensitive to persulfates.	Nava et al. (1983)
	Industrial workers	
Contact dermatitis	Over a 5-year period, the incidence of rashes among persulfate workers in one factory was determined. Although 15 workers comprised the production staff, the turnover rate was such that up to 25 new workers were involved in 1 year. Over the 5-year period, 20% to 70% of the new employees developed rashes within 1 month of employment. The rashes were characterized by itchy red papules and eczematous patches on the wrists and forearms, hands, neck, and face. It was predominantly the workers involved in the manufacture of Potassium Persulfate that were affected rather than those working with Ammonium Persulfate. The affected workers fell into two classes: those who after removal from the persulfate did not relapse after reexposure and those who rapidly relapsed after reexposure.	White, Catchpole, and Rycroft (1982)
Asthma	A cross-sectional study of 52 employees of a plant that produced Persulfates was performed; 12 subjects were directly involved in Persulfate production, the remaining 40 subjects had indirect contact. Thirteen persons from the medical profession were used as controls. Questionnaires were administered, skin prick tests were performed with 1% and 5% (*w/v*) Ammonium and Potassium Persulfate, atopy screening was done, and lung function was assessed. Three, two, and three test subjects reacted to Ammonium, Potassium, and both Ammonium and Potassium Persulfate, respectively; of these eight reactors, only three had direct contact with Persulfates. Six of the	Wrbitzky, Drexler, and Letzel 1995

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TABLE 3
Occupational exposure to Persulfate Salts *(Continued)*

Reaction	Study description	Reference
	eight reactors reported workplace-related breathing difficulties; 9 of the 44 nonreactor test subjects also reported breathing difficulties. None of the controls reacted to the prick test. The mean total IgE was increased in 16 subjects; a Phadiatop test reported positive results in 12 test subjects. Test subjects that had positive results to the prick test had decreased lung function values compared to those subjects that had negative results.	
Asthma	A cross-sectional study of 32 employees of a chemical plant that produced Persulfates was performed. Eighteen workers at the plant who were not exposed to Persulfates were used as the controls. Questionnaires were used, skin prick tests were performed with 80 mg/ml buffered Ammonium (pH 3.1) and Sodium Persulfate (pH 3.9), total IgE and specific IgE were measured, and lung function and bronchial responsiveness to histamine were assessed. Work-related rhinitis was reported by one test subject, and work-related conjunctivitis and bronchitis were reported by two control subjects. Early and/or late skin reactions to Persulfates were not observed for test or control subjects. Lung function, total IgE, and response to histamine were similar for test and control subjects. Bronchial hyperresponsiveness was present in four nonatopic test subjects and in one nonatopic and one atopic control worker. It was noted that 7 of 36 exworkers left because of medical reasons; 6 had work-related contact dermatitis and 1 reported asthma.	Merget et al. (1996)
Contact dermatitis and asthma	Of 106 workers in a hydrogen-peroxide factory, 34% had eczematous dermatitis and 15% had asthmatic bronchitis thought to be occupational in nature. Patch tests with Ammonium Persulfate were positive in 32 of 46 workers. None of the workers had positive responses to Potassium Persulfate, sulfuric acid, or hydrogen peroxide. It was noted that inhalation tests with aerosolized Ammonium Persulfate exacerbated the symptoms. The investigators concluded that the observed reactions were allergic in nature.	Barsotti, Parmeggiani, and Sassi (1951)
Contact dermatitis and asthma	Two industrial workers developed dermatitis, rhinitis, bronchitis, and asthma following occupational exposure to the dust of persulfate salts. Patch tests induced late cutaneous reactions and occupational exposure to the workplace for 8 hours induced a pathological increase in airway resistance.	Baur, Fruhmann, and Leibe (1979)

whether Ammonium Persulfate works directly on mast cells or whether histamine release is due to immediate-type immune hypersensitivity.

The persulfates caused both delayed-type and immediate skin reactions. These reactions include irritant dermatitis, allergic eczematous dermatitis, localized contact urticaria, generalized urticaria, rhinitis, asthma, and syncope. The most common causes of allergic dermatitis in hairdressers are the active ingredients in hair dyes, and Ammonium Persulfate has been identified as a frequent allergen. A number of occupational case studies document these types of reactions, but no incidence data were available.

DISCUSSION

The Expert Panel was concerned with the sensitization and urticaria potential of Persulfates. A sensitization study that also examined the incidence of urticarial reactions was performed with 17.5% Ammonium, Potassium, and Sodium Persulfate. At this concentration, a mixture of these Persulfates was not sensitizing, and application of Ammonium, Potassium, and Sodium Persulfate did not result in an urticarial reaction.

Also, the Expert Panel was concerned that the greatest concentration of Persulfates tested was 17.5%, yet data submitted to CIR reported that Persulfates are used in hair lighteners at concentrations of 60%. Because the test materials were applied

under occlusive patches, it was assumed that, in normal use (i.e., not occluded and rinsed off), a concentration greater than 17.5% would also be safe. Given the clinical reports of urticarial reactions, the Expert Panel concluded that manufacturers and formulators should be aware of the potential for urticarial reactions at concentrations of Persulfates greater than 17.5%.

CONCLUSION

The CIR Expert Panel concludes that Ammonium, Potassium, and Sodium Persulfate are safe as used as oxidizing agents in hair colorants and lighteners designed for brief discontinuous use followed by thorough rinsing from the hair and skin.

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Personal Care Products Council
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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: June 5, 2013

SUBJECT: Concentration of Use by FDA Product Category: Magnesium Sulfate and other Sulfate Ingredients

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Concentration of Use by FDA Product Category

Magnesium Sulfate
Aluminum Sulfate
Ammonium Persulfate
Ammonium Sulfate
Barium Sulfate
Calcium Sulfate
Calcium Sulfate Hydrate
Copper Sulfate
Ferric Sulfate
Ferrous Ammonium Sulfate
Ferrous Sulfate
Hydroxylamine Sulfate
Manganese Sulfate
Potassium Persulfate
Potassium Sulfate
Silver Sulfate
Sodium Bisulfate
Sodium Persulfate
Sodium Sulfate
Zinc Sulfate

Ingredient	FDA Code†	Product Category	Maximum Concentration of Use
Magnesium Sulfate	01C	Other baby products	0.7%
Magnesium Sulfate	02A	Bath oils, tablets and salts	0.1%
Magnesium Sulfate	02B	Bubble baths	0.5%
Magnesium Sulfate	02C	Bath capsules	49%
Magnesium Sulfate	02D	Other bath preparations	10-25%
Magnesium Sulfate	03C	Eye shadow	2%
Magnesium Sulfate	03D	Eye lotion	0.5-2.1%
Magnesium Sulfate	03G	Other eye makeup preparations	0.4%
Magnesium Sulfate	05A	Hair conditioners	1-15%
Magnesium Sulfate	05B	Hair sprays pump sprays	 0.5-11%
Magnesium Sulfate	05F	Shampoos (noncoloring)	0.1-1%
Magnesium Sulfate	05G	Tonics, dressings and other hair grooming aids	0.01-5%
Magnesium Sulfate	05I	Other hair preparations (noncoloring) spray	 11%
Magnesium Sulfate	07C	Foundations	0.05-2%
Magnesium Sulfate	07D	Leg and body paints	1%
Magnesium Sulfate	07F	Makeup bases	0.05-1%
Magnesium Sulfate	07H	Makeup fixatives	0.6%

Magnesium Sulfate	07I	Other makeup preparations	0.7-1%
Magnesium Sulfate	10A	Bath soaps and detergents	0.00001%
Magnesium Sulfate	11G	Other shaving preparations	22%
Magnesium Sulfate	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.01-20%
Magnesium Sulfate	12C	Face and neck products not spray	0.002-2%
Magnesium Sulfate	12D	Body and hand products not spray	0.34-1.5%
Magnesium Sulfate	12E	Foot powders and sprays	1%
Magnesium Sulfate	12F	Moisturizing products not spray	0.01-0.8%
Magnesium Sulfate	12G	Night products not spray	0.15-0.4%
Magnesium Sulfate	12H	Paste masks and mud packs	0.7-25%
Magnesium Sulfate	12J	Other skin care preparations	0.5-1.8%
Magnesium Sulfate	13A	Suntan products not spray	1-2%
Aluminum Sulfate	06A	Hair dyes and colors (all types requiring caution statement and patch test)	2%
Aluminum Sulfate	07B	Face powders	0.2%
Aluminum Sulfate	07C	Foundations	0.2%
Aluminum Sulfate	07E	Lipstick	0.07%
Aluminum Sulfate	07I	Other makeup preparations	0.35%
Ammonium Persulfate	06A	Hair dyes and colors (all types requiring caution statement and patch test)	17.2-22.1%
Ammonium Persulfate	06F	Hair lighteners with color	21.6%
Ammonium Persulfate	06G	Hair bleaches	4.2-21.1%
Ammonium Sulfate	05A	Hair conditioners	0.035%

Ammonium Sulfate	05F	Shampoos (noncoloring)	0.04-0.19%
Ammonium Sulfate	06A	Hair dyes and colors (all types requiring caution statement and patch test)	0.5%
Ammonium Sulfate	06B	Hair tints	2%
Ammonium Sulfate	06G	Hair bleaches	3.5%
Ammonium Sulfate	10A	Bath soaps and detergents hand soap	 0.06%
Ammonium Sulfate	10E	Other personal cleanliness products	0.04%
Barium Sulfate	02D	Other bath preparations	0.94%
Barium Sulfate	03A	Eyebrow pencil	0.095-6%
Barium Sulfate	03B	Eyeliner	1-18.6%
Barium Sulfate	03C	Eye shadow	0.095-10%
Barium Sulfate	03D	Eye lotion	6.6%
Barium Sulfate	03F	Mascara	0.095-0.1%
Barium Sulfate	03G	Other eye makeup preparations	0.01-2.3%
Barium Sulfate	04B	Perfumes	15%
Barium Sulfate	04C	Powders (dusting and talcum)	15.8%
Barium Sulfate	04E	Other fragrance preparations	15%
Barium Sulfate	05F	Shampoos (noncoloring)	0.55%
Barium Sulfate	06B	Hair tints	0.62%
Barium Sulfate	07A	Blushers (all types)	0.095-17%
Barium Sulfate	07B	Face powders	0.034-15%
Barium Sulfate	07C	Foundations	0.027-20%
Barium Sulfate	07E	Lipstick	0.04-37%
Barium Sulfate	07F	Makeup bases	0.095-17%
Barium Sulfate	07G	Rouges	0.04-5.2%
Barium Sulfate	07I	Other makeup preparations	8%
Barium Sulfate	08A	Basecoats and undercoats (manicuring preparations)	0.0015%

Barium Sulfate	08E	Nail polish and enamel	0.0074-3%
Barium Sulfate	08G	Other manicuring preparations	0.001%
Barium Sulfate	10A	Bath soaps and detergents	0.072%
Barium Sulfate	10E	Other personal cleanliness products	0.04-0.5%
Barium Sulfate	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0035%-0.99%
Barium Sulfate	12C	Face and neck products not spray	1.2-15%
Barium Sulfate	12D	Body and hand products not spray	0.3-11%
Barium Sulfate	13A	Suntan products not spray	1.5-1.9%
Calcium Sulfate	03C	Eye shadow	5.8-14%
Calcium Sulfate	07A	Blushers (all types)	2-20%
Calcium Sulfate	07E	Lipstick	1.8%
Calcium Sulfate	08A	Basecoats and undercoats (manicuring preparations)	0.0001%
Calcium Sulfate	09A	Dentifrices paste	3%
Calcium Sulfate	09C	Other oral hygiene products	3%
Calcium Sulfate	12B	Depilatories	2%
Calcium Sulfate	12C	Face and neck products not spray	1-8%
Calcium Sulfate	12D	Body and hand products not spray	9%
Calcium Sulfate	12H	Paste masks and mud packs	8-9%
Copper Sulfate	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.042%
Copper Sulfate	12C	Face and neck products not spray	0.042%
Manganese Sulfate	03C	Eye shadow	1.5%
Manganese Sulfate	03D	Eye lotion	0.25%

Manganese Sulfate	05A	Hair conditioners	0.1%
Manganese Sulfate	05F	Shampoos (noncoloring)	1%
Manganese Sulfate	05G	Tonics, dressings and other hair grooming aid	13.2%
Manganese Sulfate	06A	Hair dyes and colors (all types requiring caution statement and patch test)	0.5%
Manganese Sulfate	07C	Foundations	1.5%
Manganese Sulfate	11A	Aftershave lotions	2%
Manganese Sulfate	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	26.7%
Manganese Sulfate	12C	Face and neck products not spray	36.6%
Manganese Sulfate	12D	Body and hand products not spray	0.58%
Manganese Sulfate	13A	Suntan products not spray	1.5%
Potassium Persulfate	06A	Hair dyes and colors (all types requiring caution statement and patch test)	43.1%
Potassium Persulfate	06F	Hair lighteners with color	62.4%
Potassium Persulfate	06G	Hair bleaches	10.3-72%
Potassium Sulfate	03D	Eye lotion	0.02%
Potassium Sulfate	04E	Other fragrance preparations	0.01%
Potassium Sulfate	05A	Hair conditioners	0.001%
Potassium Sulfate	05G	Tonics, dressings and other hair grooming aids	0.02%
Potassium Sulfate	07C	Foundations	0.01%
Potassium Sulfate	07E	Lipstick	0.02%
Potassium Sulfate	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.016%
Potassium Sulfate	12C	Face and neck products not spray	0.04%

Potassium Sulfate	12D	Body and hand products not spray	0.02%
Potassium Sulfate	12J	Other skin care preparations	0.00015-0.016%
Potassium Sulfate	13A	Suntan products not spray	0.01%
Potassium Sulfate	13B	Indoor tanning products	0.005%
Sodium Bisulfate	10A	Bath soaps and detergents	0.0013%
Sodium Persulfate	06A	Hair dyes and colors (all types requiring caution statement and patch test)	5.5%
Sodium Persulfate	06F	Hair lighteners with color	5%
Sodium Persulfate	06G	Hair bleaches	5-8%
Sodium Sulfate	01A	Baby shampoos	0.29%
Sodium Sulfate	02A	Bath oils, tables and salts	69-96.8%
Sodium Sulfate	02B	Bubble baths	0.14-3.5%
Sodium Sulfate	03B	Eye liner	0.0012%
Sodium Sulfate	03D	Eye lotion	0.000046%
Sodium Sulfate	03E	Eye makeup remover	0.0064%
Sodium Sulfate	03F	Mascara	0.0005%
Sodium Sulfate	04C	Powders (dusting and talcum)	0.005%
Sodium Sulfate	05A	Hair conditioners	0.00095-0.15%
Sodium Sulfate	05D	Permanent waves	0.6%
Sodium Sulfate	05F	Shampoos (noncoloring)	0.081-1.1%
Sodium Sulfate	05G	Tonics, dressings and other hair grooming aid	0.013-2%
Sodium Sulfate	06A	Hair dyes and colors (all types requiring caution statement and patch test)	1-2.1%
Sodium Sulfate	06B	Hair tints	1%
Sodium Sulfate	06G	Hair bleaches	2.7%
Sodium Sulfate	07C	Foundations	0.00001-0.005%

Sodium Sulfate	08E	Nail polish and enamel	0.001%
Sodium Sulfate	08G	Other manicuring preparations	9.1%
Sodium Sulfate	09A	Dentifrices (aerosol, liquid, pastes and powders)	0.44-0.83%
Sodium Sulfate	09B	Mouthwashes and breath fresheners (liquids and sprays)	0.00015-0.0063%
Sodium Sulfate	09C	Other oral hygiene products	0.037%
Sodium Sulfate	10A	Bath soaps and detergents	0.1-8%
Sodium Sulfate	10B	Deodorants (underarm) not spray	 0.0001-0.0027%
Sodium Sulfate	10E	Other personal cleanliness products	0.077-0.2%
Sodium Sulfate	11E	Shaving cream (aerosol, brushless and lather)	0.012%
Sodium Sulfate	12A	Skin cleansing (cold creams, cleansing lotions liquids and pads)	0.003-0.48%
Sodium Sulfate	12C	Face and neck products not spray spray	 0.5% 0.0088%
Sodium Sulfate	12D	Body and hand products not spray	 0.0038-0.08%
Sodium Sulfate	12F	Moisturizing products not spray	 0.0015%
Sodium Sulfate	12I	Skin fresheners	0.4%
Sodium Sulfate	12J	Other skin care preparations	0.063%
Sodium Sulfate	13A	Suntan products not spray	 0.000095%
Zinc Sulfate	05G	Tonics, dressings and other hair grooming aids	0.44%
Zinc Sulfate	10A	Bath soaps and detergents	0.057%
Zinc Sulfate	12C	Face and neck products not spray	 0.12-1%
Zinc Sulfate	12D	Body and hand products not spray	 0.07%

Zinc Sulfate	12F	Moisturizing products not spray	0.2%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.
†Product category codes used by FDA

Information collected in 2013
Table prepared: June 5, 2013

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Personal Care Products Council
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Memorandum

TO: Lillian Gill D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: January 27, 2014

SUBJECT: Comments on the Scientific Literature Review on the Safety of Inorganic Sulfates as Used in Cosmetics

The Council has no suppliers listed for Ferric Sulfate.

Key Issues

As previously suggested by the CIR SSC and some members of the CIR Expert Panel, it is not appropriate to review various metal compounds in one report. Rather than organizing the report by the sulfate group, some members of the CIR Expert Panel suggested that a report on inorganic magnesium compounds be created as the lead ingredient for this report is Magnesium Sulfate.

p.1, 2, 44 - In the Introduction, Use section and Summary it is inappropriate to say that these ingredients "function mostly as...". This language suggests that a majority of the ingredients have the specified functions. There are 17 ingredients in this report. Only 3 have astringent reported as a function, 3 have opacifying agent, 3 have skin conditioning agent and 3 have viscosity increasing agent reported as a function. There is not one function that is common to a majority of the ingredients included in this report.

The additional Key Issues only if the grouping of ingredients in this report is not changed

If the common sulfate group is important for function and for limiting the safety of these ingredients, this report should include information about sulfate and sulfur metabolism. As Sulfuric Acid is in the *Dictionary* (as a pH adjustor), and if this report is about "salts of sulfuric acid", perhaps Sulfuric Acid should be included in this report.

The report should justify why grouping all the sulfate compounds together in one report is warranted. The report should also make it clear that it is not appropriate to read across from one metal to another metal compound. Rather than organizing the report by endpoint, the report should be reorganized to present information by compound.

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Information on the normal dietary intake and the normal levels of the various metals that are found in the body should be added to the report. Without this information, the report shows a lack of understanding that some of these metals, e.g., zinc, iron, are essential.

Basic toxicology textbooks such as *Casarett & Doull's Toxicology: The Basic Science of Poisons,* include separate sections summarizing the toxicity of each metal. For those metals that are also essential, such as iron, copper, magnesium and manganese, the functions and normal levels of the metals are also described. None of this type of information is included in the SLR.

When available, the pH values of the dosing solutions should be added to this report.

Additional Comments

p.1, 2 - It is misleading to state that "ferrous ammonium sulfate functions only as a pesticide in cosmetics" as there are no uses or use concentrations reported for this ingredient. Although pesticide is listed as a function for Ferrous Ammonium Sulfate in the *Dictionary*, there is no evidence that Ferrous Ammonium Sulfate is being used in cosmetic products.

p.1 - The Chemistry section should also state that Copper Sulfate is practically insoluble in water (see Table 2).

p.2 - Please indicate the source of the gypsum that was reported to contain radium.

p.2, 44 - It is misleading to state that "these ingredients are being used at concentrations up to 96.8%". This implies that all of the ingredients in the report are being used at 96.8%, when only one ingredient, Sodium Sulfate, is being used at 96.8% in bath products. The next highest use concentration is 49% for Magnesium Sulfate in bath capsules.

p.2 - It should be made clear that the CIR report author is only speculating that the perfume product containing Barium Sulfate and the hair grooming product containing Sodium Sulfate are sprays. It should also be made clear that the CIR report author is speculating that the foot product containing Magnesium Sulfate is a powder.

p.2 - Please indicate the type of OTC products for which these ingredients have been approved (use in OTC skin products would be relevant to the consideration of their safety for use in cosmetic products).

p.4 - What was the particle size of the Barium Sulfate used in reference 27? How many hours/day, days/week were the rats exposed to Barium Sulfate?

p.4 - As dermal penetration varies by formulation, please indicate the types of formulations used in the dermal penetration study of Copper and Zinc Sulfate.

p.4 - As the summary of the results suggests that the absorption of iron was studied, it is misleading to state "The absorption of ferrous ammonium sulfate was studied..." (reference 29).

p.4 - What dose of Magnesium Sulfate was used in reference 33? When during gestation were the rats treated? Did they actually measure the transfer of Magnesium Sulfate, or just magnesium?

p.7 - What concentrations of sulfate salts were studied in reference 45?

p.7 - Which sex of mice was found to be more sensitive to inhalation exposure to Copper Sulfate?

p.7 - "Intrathecally" is not the same as "intratracheally" and needs to be corrected in several places in the description of reference 48.

p.8 - If available, please provide information about the pH of the particles used in the human exposure study (reference 50). The description of the study indicates that the effect occurred "in relation

to their acidity."

p.8 - What health effects were studied in reference 51?

p.8 - The structure of the following phrase needs to be revised as it does not make sense:"The effect of human exposure on pulmonary function...".

p.9 - What was the pH of the Ammonium Sulfate given to three Japanese white rabbits?

p.9 - It is not clear how the Barium Sulfate dosing solution used in reference 57 could be "150% w/v suspension".

p.9-13 - As reference 58 completed acute studies of several compounds by different routes of exposure, it would be helpful to present all the information from that study in a table so that the LD_{50} values by route and compound could be easily compared.

p.12 - Were the dogs used in the study described in reference 69 really "radiographed for a total of 9 months"? Or were they radiographed periodically during the 9 month period?

p.13 - How were "toxicological effects" assessed in the inhalation study of Ammonium Sulfate (reference 70)?

p.13 - What was the duration of exposure used in reference 72? It says there were 6 time points, but the duration of the time points is not stated.

p.14 - Delete the first paragraph of this page as it is a study of Manganese Sulfate in the Barium Sulfate subsection. This information also is correctly presented in the Manganese Sulfate section.

p.14 - The protocol of the 1 year study (reference 75) is not clear? When were the rats examined, directly after the one year exposure, or 1 year after the 1 year exposure (or were there 2 groups examined at different time points)?

p.15 - In the first paragraph, it is not necessary to state: "evaluation of respiratory histologic changes" and "Histopathological examinations were performed". If the examinations included tissues other than those of the respiratory tract, it should state the additional tissues that were examined. Please correct: "concentraioion".

p.15 - Units of $\mu g/m^3$ represent a concentration and should not be called dose (reference 78).

p.15 - The age of the rats used in reference 80 is not clear. It says the rats were 2 months old, and then it says 6-age matched rats (3 years old) were used. This does not make sense.

p.16 - Please correct "kidney or liver exposed to aluminum" as rats not the organs were exposed.

p.16, 28 - In the Copper Sulfate feed study (reference 84), it does not make sense to state that "the control group received drinking water without the test material". Copper Sulfate was added to the feed - all of the groups received drinking water without the test material. How much copper was in the control diet?

p.17 - What "reproductive parameters" were measured in rats and mice in the Copper Sulfate drinking water study?

p.19 - How many i.v. doses did each dog receive (reference 93)?

p.19 - The dosing of the dogs in reference 94 is not clear. It says they got 24 hour i.v. infusions for 4 weeks. It is unlikely they were infused continuously for 4 weeks.

p.19 - Please move the intratracheal study after the inhalation exposure studies.

p.20 - Please provide a reference for the lack of causality between aluminum exposure and Alzheimer's disease.

p.21, 46 - The intravitreal exposure study is not relevant and should be deleted from the report.

p.22 - What is the meaning of "grade I allergen"?
p.22 - As Copper Sulfate was not sensitizing, "Skin sensitized..." needs to be changed to "Dermally exposed..." What happened to the rats exposed to 25% Copper Sulfate?
p.23 - Animals (usually mice) are treated in LLNAs. Therefore, LLNAs should not be in the *in vitro* subsection.
p.24 - The first paragraph does not describe specific case reports, and the information (mining exposure; use as radiocontrast agent) is not relevant to the cosmetic use of Barium Sulfate. This paragraph should be deleted from the report.
p.24 - Reference 118 is an experimental exposure study. It should not be presented in the Case Reports section.
p.26 - The information about zinc being tolerated by subjects with Wilson's disease is not relevant to the use of Zinc Sulfate in cosmetic products. If you are going to mention Wilson's disease, you should describe it.
p.26 - References 133 and 134 describe clinical studies. These studies should not be presented in the Case Reports section.
p.26 - Reference 26 on the effect of zinc ingestion on immune response is not relevant to the cosmetic use of Zinc Sulfate and should be deleted from the report. If it is left in the report, it should not be presented in the Case Reports section as it is a clinical study.
p.27 - On what gestation days were the rabbits treated (reference138)? Please clarify the species used as a negative control. In the description of the rat study, it says that mice served as the negative controls.
p.27 - *Cricetus auratus* is not a strain, it is a species. What dose was used in the hamster study? The method description says 2.13 mg Cu/kg, the results says 2.3 mg Cu/kg.
p.27 - What dose of Copper Sulfate was used in the i.v. mouse study (reference 140)?
p.29 - What doses of Manganese Sulfate were used in mice treated on gestation day 10 (2 protocols are given for gestation day 9 mice) (reference 148)?
p.29 - It is not clear how many female mice were used in the rat fertility study (reference 149). Was it a total of 2 per male, or was it 2/male/day (which is generally how these studies are completed)?
p.29-30 - The study on the placental transfer of zinc should be presented in the absorption, distribution, metabolism and excretion section. How did the zinc levels found in embryonic tissue compare with normal levels of zinc?
p.31 - Were the 20 women in the study described in reference 159 zinc deficient at the start of the study? Zinc deficiency is known to adversely affect development. What are the current daily recommendations for zinc intake in pregnant women?
p.32-38 - Rather than a detailed summary in the text, the genotoxicity data should be summarized in a table.
p.34 - The i.p. study of Hydroxylamine Sulfate is not an *in vitro* study. It should not be included in the *in vitro* subsection.
p.35 - The micronucleus test and the comet assay of Zinc Sulfate are not *in vitro* studies. These studies should not be included in the *in vitro* subsection.
p.36 - The study of the effects of Aluminum Sulfate on human leukocytes is an *in vitro* study. It should

not be presented in the *in vivo* subsection.

p.37 - The description of reference 201 is not clear. What is meant by "repeatedly 5 times"? If the mice were injected with several series of 5 injections, please indicate the number of series of 5 injections that were used. If the mice were injected 5 times, the word "repeatedly" is not needed.

p.38 - *Mus musculus* is the genus species name for house mouse. It should not be called "strain".

p.42 - Please check the dose units used in the description of reference 218. It currently says "g g/100g".

p.44 - Please delete the studies under the headings Modulation of Hormonal Effect and Lipid Peroxidation as they are not relevant to the cosmetic use of Zinc Sulfate or Ferrous Ammonium Sulfate.

p.44- 46 - As it is inappropriate to read across from data on one ingredient to other ingredients in this report, it would be helpful if the Summary was organized by ingredient rather than endpoint.

p.45 - It is unlikely that the LD_{50} of Barium Sulfate was 307 g/kg in rats. It would be helpful if the same units were used throughout the paragraph describing LD_{50} values.

p.46 - The CIR Expert Panel has indicated that they do not like the description "results were mixed." This is used several times in the Summary. The results should be described. As these are completely different compounds, of course the results are not the same. Which compounds are reproductive and developmental toxicants, which are not developmental toxicants? It also should be noted that zinc is required for normal development. Which compounds are genotoxic which are not genotoxic?

p.49, Table 2 - As the formulas are known for each of the ingredients in this report, please include a molecular weight for each ingredient in Table 2 (even if it is calculated).

p.50-51, Table 3 - What is the meaning of the asterisk? Why is there a footnote (10) with "Incidental Ingestion" in the first table? Use information should be presented under the same protocol under which is was collected. For example, the 0.01% Magnesium Sulfate concentration and the 2% Sodium Sulfate in the "spray" row was reported for hair grooming products which was not considered a spray product under the protocol the information was collected. The 0.00015% Sodium Sulfate concentration in the "spray" is from the mouthwash product category and was not considered a spray under the protocol the information was collected.

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Personal Care Products Council
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Memorandum

TO: Lillian Gill D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)
CIR Expert Panel Members
Liaisons to the CIR Expert Panel

FROM: CIR Science and Support Committee of the Personal Care Products Council

DATE: January 27, 2014

SUBJECT: Comments on Ingredient Grouping as Presented in the Scientific Literature Review: Safety Assessment of Inorganic Sulfates as Used in Cosmetics

The CIR Science and Support Committee of the Personal Care Products Council appreciates the opportunity to provide comments on the Scientific Literature Review (SLR): Safety Assessment of Inorganic Sulfates as Used in Cosmetics.[1]

Although we acknowledge that CIR staff did not include the previously reviewed oxidizing agents, Ammonium Persulfate, Potassium Persulfate and Sodium Persulfate in this SLR, the inclusion of other inorganic sulfates is inappropriate, and not scientifically supportable. It is not appropriate to group ingredients for safety review based solely on one component of the compound when other components of the compound are functional in cosmetics and affect the safety profile. For the sulfate ingredients, it is the metal ion (or ammonium or hydroxylamine) that determines both the function in cosmetic products and how it interacts with the body.

There is no information in the SLR that justifies grouping all the sulfate ingredients in one report. Chemical properties, such as water solubility vary among the ingredients. The reported functions of these ingredients in cosmetic products are different, and the safety profiles reflect the non-sulfate component of the ingredient. The safety studies included in the report are predominantly studies on the metal ions for which the sulfate compound was found to be an appropriate delivery system.

By reviewing these ingredients in the same report that is organized by endpoint, the report implies that it is appropriate to apply data on one metal compound, such as Ferrous Sulfate to another completely unrelated metal compound such as Magnesium Sulfate. As Magnesium Sulfate is the lead ingredient in this report, a report on inorganic magnesium compounds would be a better approach to reviewing the safety of Magnesium Sulfate. This alternative approach was previously suggested by some members of the CIR Expert Panel (September 2012 CIR Expert Panel meeting).

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[1]Magnesium Sulfate is the lead ingredient in this SLR. The SLR also includes Aluminum Sulfate, Ammonium Sulfate, Barium Sulfate, Calcium Sulfate, Calcium Sulfate Hydrate, Copper Sulfate, Ferric Sulfate, Ferrous Ammonium Sulfate, Ferrous Sulfate, Hydroxylamine Sulfate, Manganese Sulfate, Potassium Sulfate, Silver Sulfate, Sodium Bisulfate, Sodium Sulfate and Zinc Sulfate.