# Safety Assessment of Inorganic Sulfates as Used in Cosmetics

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*All interested persons are provided 60 days from the above date to comment on this Scientific Literature Review and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Lillian J. Gill D.P.A.*

The 2013 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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## 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 stating that these ingredients 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.[1]

## 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).[2] 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).[3]

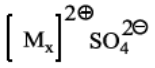

**Figure 1**. Inorganic Sulfates – wherein "M" is an ammonium or metal cation

The formulas of the inorganic sulfates reviewed in this safety assessment are included in Figure 2.[4] 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.[5]

### 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.[2] 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 $BaSO_4$.[6] 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.[7]

**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.[8]

## 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 are being used in cosmetic products: aluminum sulfate, ammonium sulfate, barium sulfate, calcium sulfate, copper sulfate, magnesium sulfate, manganese sulfate, potassium sulfate, sodium bisulfate, sodium sulfate, and zinc sulfate.[9] 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 96.8% (sodium sulfate, in bath products).[10]

Summarized 2013 data on frequency and concentration of use in cosmetics for these ingredients are presented in Table 3.

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%), magnesium sulfate (up to 11%), and sodium sulfate (up to 2%). Additionally, the following ingredients are being used in powders (highest maximum use concentration = 15.8% [barium sulfate]): aluminum sulfate (up to 0.2%), barium sulfate (15.8%), magnesium sulfate (up to 1%), and sodium sulfate (up to 0.005%). 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.[11,12,13,14] 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.[11,12]

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.[15] 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.[16] 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.[17] 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.[18]

**Barium Sulfate**

Barium sulfate has been used as a gastrointestinal contrast agent in roentgenographic procedures.[19]

Barytes (the naturally occurring rock form of $BaSO_4$) has been referred to as the standard densification agent used in drilling fluids worldwide.[6]

**Magnesium Sulfate**

Magnesium sulfate is an anticonvulsant that is used frequently to prevent or treat seizures in obstetric patients with preeclampsia or eclampsia, and as tocolytic agent in patients with premature labor.[20] 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.[21,22]

**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.[23] 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).[24] 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, more significant, 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).[25] The animals received either 0.1 or 1.0 mg/kg of aluminum sulfate. Following administration of either dose, the blood and plasma aluminum profiles were monoexponential in most cases. Increasing the administered dose also caused an increase in the elimination half-life (mean ± standard deviation) from 1.20 ± 0.25 h to 2.41 ± 0.26 h ($p < 0.05$). A corresponding decrease in systemic clearance was observed (49.6 ± 11.0 to 18.4 ± 4.6 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. This difference in volume distribution was also statistically significant. At both doses, blood:plasma 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.[26] 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 systemic clearances were 48.9 ± 10.6 and 35.1 ± 3.64 ml/h·kg ($p < 0.050$). Following portal administration, the systemic availability was 0.66, indicating a significant "first-pass" effect. Biliary aluminum recovery (% dose) was negligibile both following portal administration (0.83 ± 0.062%) and systemic administration (1.3 ± 0.22%); the difference between these values was statistically significant ($p < 0.05$). A decrease in bile flow (~ 40%; $p < 0.05$) was observed immediately after portal administration of aluminum sulfate only; 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**

In a study involving rats, the animals inhaled barium sulfate (40 mg/m$^3$) for 2 months and were then observed for 4 weeks.[27] The rats were killed at 2-week intervals, and the barium content of the lungs, lymph nodes, jaw, and femur was determined. It was noted that the extent of lymph transport was not worth mentioning. The barium content of bone increased initially, but gradually decreased during treratment. After 2 weeks, the barium content of the lungs was described as high, but decreased rapidly and then increased considerably over the next 4 weeks.

**Copper Sulfate and Zinc Sulfate**

The percutaneous absorption and cutaneous bioavailability of zinc and copper from zinc sulfate and copper sulfate was studied using formulations that were applied topically to human skin *in vitro* (Franz diffusion cell).[28] The formulations used caused an increase in zinc and copper concentrations in whole skin and the epidermis.

**Ferrous Ammonium Sulfate**

The absorption of ferrous ammonium sulfate was studied using male Sprague-Dawley rats (number not stated).[29] The rats designated as normal received a rat chow diet. Other rats were made iron-deficient by feeding with a diet that contained 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). At doses > 20,000 µg, ferrous ammonium sulfate was often lethal (mortalities not stated).

**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 certain maternal, placental, and fetal tissues was evaluated at 8, 12, 15, 17, 19, 20, and 21 days of gestation.[30] Large amounts of $^{59}$Fe were transferred from maternal plasma to the fetuses late in gestation, when fetal development is proceeding at a rapid rate. $^{59}$Fe was rapidly transported across the placenta, and there was no indication of a large iron pool within it. Both the allantoic and yolk sac placentae contributed significantly to placental iron uptake during the period of maximum iron transfer. Because very little iron is deposited in the uterus during gestation, secretions from uterine glands entering the yolk sac cavity are a minor source for fetal iron. The authors also 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.

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 body weight/day), 70 mg Fe/kg (5.69 mg/kg body weight/day), and 140 mg Fe/kg (11.54 mg/kg body weight/day), respectively.[31] 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. The feeding of iron derived from ferrous sulfate at doses up to 11.5 mg/kg body weight/day did not result in tissue iron excess.

**Hydroxylamine Sulfate**

Ten male Sprague-Dawley rats (ages not stated) were given hydroxylamine sulfate i.v. (40 mg/kg or 16.6 mg of hydroxylamine base/kg) and then killed at various intervals ranging from 2 minutes to 3 h.[32] 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. At 2 h, the blood of 1 rat contained 0.9 µg/ml; at 3 h, the blood of another rat contained 0.7 µg/ml. 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, along with standards containing hydroxylamine sulfate, 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.[33]

**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.[34] The target nominal particle size (mass mean aerodynamic diameter [MMAD]) was approximately 1.5 to 2 µm, with a geometric standard deviation ($\delta_g$) of < 2. The control group was exposed to filtered air only. End-of-exposure olfactory bulb, striatum, cerebellum, bile, lung, liver, femur, serum, and testes (n = 6 rats/concentration) manganese concentrations and whole body $^{54}Mn$ elimination were determined. 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 concentrations of $\geq$ 0.3 mg $Mn/m^3$. Elevated manganese concentrations in the striatum, testes, liver, and bile were observed at a concentration of 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.[35] 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 0.03 or 0.3 mg $MnSO_4/m^3$. The target nominal particle size (MMAD) was approximately 1.5 to 2 µm, with a geometric standard deviation ($\delta_g$) of < 2. Control groups were exposed to filtered air only. End-of-exposure tissue manganese concentrations and whole body $^{54}Mn$ elimination rates were determined. Male rats exposed to 0.03 mg $MnSO_4/m^3$ had elevated lung manganese concentrations when compared to the control male rats. Male rats exposed to 0.3 mg $MnSO_4/m^3$ developed 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 noted 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 CD rats and fetuses after inhalation of a $MnSO_4$ aerosol during pregnancy.[36] The animals were evaluated following pre-breeding (2 weeks), mating (up to 14 days), and gestational (from gestation day (GD) 0 through 20) exposure to air or $MnSO_4$ (0.05, 0.5, or 1 mg $Mn/m^3$, 7 days/week [6 h/day]). The following samples (maternal) were collected and analyzed for manganese content: whole blood, lung, liver, brain, and skull cap. Manganese concentrations in the lung (maternal) were increased at exposure concentrations $\geq$ 0.05 mg $Mn/m^3$. Manganese concentrations in the maternal brain and placenta were increased at exposure concentrations $\geq$ 0.5 mg $Mn/m^3$. Increased fetal liver manganese concentrations were noted after *in utero* exposure to $\geq$ 0.5 mg $Mn/m^3$. Concentrations of manganese in all other fetal tissues were not different from air-exposed controls. The authors noted that the results of this study demonstrated that the placenta partially sequesters inhaled manganese, thereby limiting exposure to the fetus.

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 concentrations of 30.1 ± 6.4, 294.8 ± 66.0, and 3,220 ± 578 µg $Mn/m^3$, respectively, 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). Blood and the following tissues were collected and analyzed for manganese content via neutron activation analysis: olfactory bulb, globus pallidus, caudate/putamen, cerebellum, frontal cortex, liver, lung, testis, and kidney. When compared to the control group, manganese concentrations in the 3,000 $mg/m^3$ exposure group were increased significantly in blood, kidney, lung, testis, and in all regions of the brain. Significant differences were also observed in the 300 $\mu g/m^3$ 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 $\geq$ 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. For urine elimination and tissue and organ samples, the results were expressed as the percentage of the total quantity administered. The pharmacokinetic data indicated that the distribution half-life of zinc sulfate was 0.134 $h^{-1}$. Urinary elimination was very low and did not exceed 1% of the administered dose. Distribution in the kidneys, small intestine, skin, and fur was reported, the liver having accounted for 17% of the total radioactivity, highest value 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 excreted 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 excreted 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 study day and on the study day 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; plot of 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 of 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 magnesium sulfate i.v. (3 g) and intramuscularly (10 g) as the initial therapeutic dose.[42] The highest single level in plasma, observed at 60 minutes, was 6.0 mEq per liter (7.2 mg/100 ml) 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 described as 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. Improvement was defined by 2 or more of the following: decreased redness, infiltration or scaling; fewer new lesions; and central clearing. No significant improvement of psoriasis was detected in the 19 patients. In zinc therapy and distribution studies (4 patients), the majority of the zinc sulfate (dietary and therapeutic) ingested was excreted in the feces during the baseline period and the period of zinc sulfate therapy. 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). This difference was statistically significant ($0.05 > P > 0.01$). Biopsies of involved skin contained more zinc than did uninvolved skin, but the difference was not statistically significant.

## 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 generator used to produce the aerosols produced a heterogeneous aerosol in the submicrometer size range. 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 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] Groups of mice (same strain) were exposed to the following concentrations of aluminum sulfate (as mg $SO_4/m^3$; [calculated mg metal/$m^3$ in brackets]): 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. The animals were exposed for 3h. Exposure to any of the test concentrations significantly increased the mortality rate (males and females). The magnitude of the response appeared directly related to the exposure concentration. Differences in mortality between experimental and control mice increased linearly with increasing exposure concentration. There was a significant ($p < 0.056$) between-sex difference in overall mortality.

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. However, again, there was no sex difference in the concentration-response. 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 negative controls. The animals were exposed to the test substance (49.6 mg/$m^3$) for 1 h.[47] None of the animals developed labored breathing, and there were no significant differences in respiratory frequency, total respiratory system resistance, or pseudo-flow rates when test and control animals were compared. There were no statistically significant differences in lung weight (g) or histamine content of the lung (µg/lung) between test and control groups. However, a significant reduction in histamine content of the trachea (µg/g) was observed in animals exposed to ammonium sulfate.

#### 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, and 12 h and 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 the lung tissue after copper sulfate instillation, and the amount of MT increased with the dose of copper sulfate. The pulmonary toxicity of copper sulfate was evaluated by examining time-course and dose-effect profiles of cytological and biochemical inflammatory indices that were retrieved in bronchoalveolar lavage fluids. Activities of lactate dehydrogenase (an index of cell lysis) and β-glucuronidase (an index of

lysosomal secretion) were measured, and the same was true for protein concentration (an index of exudation of blood plasma protein into the alveolar space). LDH and β-glucuronidase activities had maximum values at 12 h to 2 days post-instillation, and returned to control levels after 7 days. LDH activity and protein content increased almost linearly with dose; β-glucuronidase activity increased more sharply with dose, that is, below 20 µg copper sulfate/rat. 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, and the count median diameter was approximately 0.31 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 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 to zinc sulfate only 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. Pulmonary function studies were performed before, during, and after 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 SGaw at a concentration of 1 mg/m$^3$, when compared to the NaCl control. Also, in normal subjects, 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 significant variation in forced expiratory measures, but the changes were small in magnitude.

**Ferric Sulfate**

The effect of human 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; geometric standard deviation of 3) 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-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. Five subjects had small, but significant, decremental trends in pulmonary function. However, 9 subjects tended to improve after 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. Pathological changes such as hemorrhage, degeneration, or tissue necrosis were not observed microscopically in the following tissues: brain, heart, lung, spleen, liver, and stomach. When compared to test animals, there were no positive findings (microscopic examination included) in the 2 control rats.

**Barium Sulfate**

Six groups of 16 to 26 young male CBL-Wistar albino rats (ages not stated) received the following 6 total oral 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% of the total dose was given initially, followed by 35% of the total dose 3 h later, and 25% of the total dose 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. Mean $LD_{50}$ values were not reported for female mice of the Swiss-Webster, BDF-1, and C-5 strains. However, 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 that 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 noted that these results suggest 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 for ferrous sulfate. 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 dose levels 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 level 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 authors considered that the lethal dose would be 1,200 mg/kg (200 mg/kg/h). 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 and ages 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**

Following endotracheal administration of barium sulfate (Barosperse™) into 220 Sprague-Dawley rats and 3 dogs (ages not stated), 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. Lung specimens were obtained for microscopic examination. After outlining the trachea and stem bronchi, Barosperse™ was virtually cleared from these areas 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. The authors noted that, after a few hours, 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 Sulfate

Groups of 10 adult male rats (ages not stated) were exposed to ~300 mg/m$^3$ ammonium sulfate 8 h per day for 1, 3, 7, and 14 days.[70] 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 (specific pathogen free, pre-treated intratracheally with saline) 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).[71] 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 specific-pathogen-free male Wistar rats (12 weeks old; number not stated) were exposed 5 days per week (7h/day) to barium sulfate dust.[72] Aerosols of the barium sulfate test dust were produced using a Wright dust feed, and human respirable dust concentrations were measured daily using a gravimetric dust sampling instrument. The procedure was described as whole-body inhalation, and involved 6 time points. At each time point, 12 rats were drawn, 6 for bronchoalveolar lavage (BAL) and 6 for dust burden measurements. Each exposure condition was performed using 2 chambers. At each time point, the animals for each assay were drawn in approximately equal numbers from each chamber. 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 after exposure to barium sulfate dust 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; a negligible effect or no effect was noted at the lower (37.5 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.[72]

Histological sections from animals killed at timepoint 6, the end of dust exposure, were examined. The inhalation of barium sulfate elicited an accumulation of pulmonary macrophages around the dust deposition sites. Most of the macrophages had phagocytosed many dust particles. The highest concentrations of macrophages with phagocytosed dust were at the bifurcations of the terminal airways and bronchioles. The macrophage aggregations around these bifurcations remained mainly in the air spaces. However, 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.[72]

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 concentrations of 30.1 ± 6.4, 294.8 ± 66.0, and 3,220 ± 578 µg Mn/$m^3$, respectively, 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 (36 rats; ages not stated) were exposed, nose-only, to 100 mg/$m^3$ calcium sulfate 5 days per week (6 h/day) for 3 weeks. An additional group of 36 rats served as the control group.[73] 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]), and the lavage fluid centrifuged. The supernatant was subjected to biochemical analysis to determine protein and non-protein sulfhydryl (NPSH) concentrations and γ-glutamyl transpeptidase (γ-GT) activity. Based on the number of alveolar macrophages, the authors stated that there was no inflammatory response, but acknowledged that inflammation may have been localized to the acini region. There were no exposure-related changes in the protein concentration or γ-GT activity in animals killed immediately after the end of exposure. The authors noted that the observation that protein levels in the BAL remained unchanged after exposure suggested that the lung was not severely damaged after exposure to calcium sulfate. The absence of a difference in γ-GT activity between test and control groups was indicative of the absence of lung epithelial cell damage. Following 3 weeks of non-exposure after the end of 3 weeks, NPSH levels were increased. This finding was proposed to have been 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.[74] The remaining group served as the untreated control. Following inhalation exposure, the levels of copper in the lung, liver, kidney, and plasma were analyzed using atomic absorption spectrophotometry. When compared to the control group, higher levels of copper were detected in all tissues examined; however, only liver and plasma levels were found to be statistically significant. 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 was indicative of the accumulation of copper in a low-molecular-weight protein, which was probably metallothionein. It was noted that copper toxicity has been correlated with an increased metallothionein level in the liver and kidneys. 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.[75] 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/$m^3$. The following groups were used in the 4-week study: 42 rats (exposed to short whisker), 42 rats (exposed to long whisker) and 40 control rats. The groups used in the 1-year study included: 27 rats (exposed to short whisker), 27 rats (exposed to long whisker), and 26 control rats. 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 a frequent occurrence of adenoma and carcinoma during the year after the 1-year chronic exposure. However, the 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/$m^3$.[76] 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/$m^3$, respectively. The corresponding geometric MMAD 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.[77] One cohort of monkeys (n = 4 to 6 animals/exposure concentration) was exposed to air or manganese sulfate at a concentration of 0.06, 0.3, or 15 mg $Mn/m^3$ for 65 days. Another 8 animals were exposed to manganese sulfate at a concentration of 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 a concentration of 1.5 mg $Mn/m^3$ and then evaluated after 15 or 33 days of exposure. Evaluations included measurement of lung manganese concentrations and evaluation of respiratory histologic changes. Histopathological examinations were performed. It was concluded that the highest exposure concentraioin was associated with increased lung manganese concentrations and small airway inflammatory changes in the absence of observable clinical signs. Exposure concentrations of $\leq 0.3$ 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 concentrations of $30.1 \pm 6.4$, $294.8 \pm 66.0$, and $3{,}220 \pm 578$ µg $Mn/m^3$, respectively, for 13 weeks.[37] A fourth group (control) was exposed to $0.3 \pm 0.02$ 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 doses of 10, 30, or 100 µg/$m^3$.[78] 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). These findings were indicative of a minimal pulmonary effect. 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. The authors concluded that these data indicated that the inhalation of zinc sulfate at environmentally relevant levels induces cardiac effects. They also noted that, though these changes were considered small in healthy rats, they may be especially relevant in individuals with pre-existent cardiovascular disease.

**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.[79] The mean exposure concentration of ammonium sulfate aerosol (mass median diameter = $0.97 \pm 0.05$ µm) was $1.0 \pm 0.05$ ppm. Each subject acted as his or her control. Pulmonary function tests (body plethysmography and spirometry) and bronchial reactivity to methacholine were performed. There were no significant changes in pulmonary function or bronchial reactivity either during the exposure period or at 24 h post-exposure.

**Oral**

**Animal**

**Aluminum Sulfate**

A group of 8 male Sprague-Dawley rats received aluminum sulfate (2.5% in drinking water) daily for 3 months.[80] A second group of 8 rats received the same concentration in drinking water daily for 6 months. Animals of the 2 groups were 2 months old. Treated animals consumed $17 \pm 6$ ml of water supplemented with 2.5% aluminum sulfate, corresponding to 33 mg of aluminum per rat per day. Control rats (2 months old) received tap water *ad libitum*. Additionally, 6 age-matched Sprague-Dawley male rats (3 years old) received tap water *ad libitum*. At the end of treatment, the animals were anesthetized with halothane and the kidneys and liver were excised and prepared for histopathologic examination. 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, 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.[81] 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 number of red blood cells, 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%.[82] 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 NOEL was determined to be 1.5% ammonium sulfate in the diet (886 mg/kg/day) in male rats and 3% ammonium sulfate in the diet (1,975 mg/kg/day) in female rats.

The chronic toxicity of ammonium sulfate was studied using groups of 10 F344/DuCrj rats (5 weeks old; 5 males, 5 females/group).[83] 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**

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.[84] The control group received feed without the test material. Complete necropsy was performed on all animals that died early and at the end of the study (all test and control animals included). Complete histopathologic examination involved the following groups: all control animals, all animals that died early, all animals in the highest dose group with at least 60% survivors, and all animals in higher dose groups. In a second experiment, groups of 10 $B6C3F_1$ mice (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 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. The control group received feed without the test material. Complete necropsies and histopathologic examinations were performed as stated for rats in the preceding experiment.

The procedures for the 3rd and 4th experiments in this study were: 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. The control group received drinking water without the test material. An additional group of 20 rats (10 males, 10 females/group) was involved in special studies (intermediate time points for clinical pathology determinations). Complete necropsy was performed on all animals that died early and at the end of the study (all test and control animals included). Complete histopathologic examination involved the following groups: all control animals, all animals that died early, all animals in the highest dose group with at least 60% survivors, and all animals in higher dose groups. Groups of 20 $B6C3F_1$ mice (10 males, 10 females/group; 6 weeks old) received copper sulfate at the following concentrations in dosed 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. Results for mice and rats in the 15-day drinking water and feeding experiments and the 92-day feeding experiments are included below.[84]

When administered in drinking water, copper sulfate concentrations of 3,000 to 30,000 ppm were lethal to rats and mice within 2 weeks. In feeding studies, copper sulfate concentrations of 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 dosed feed studies had 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 the lumina of the proximnal convoluted tubules. Droplets

were larger and more numerous in males than in females. Urinalysis results were suggestive of 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 dosed feed study, were indicative of microcytic anemia. Copper sulfate produced no adverse effects on any of the reproductive parameters measured in rats or mice of either sex. The authors stated that the results of this study indicate that copper sulfate, at high exposure levels, is a hepatic and renal toxicant. They also stated that the results indicate that copper sulfate is an inducer of anemia in rodents, with rats being more sensitive than mice after subchronic exposure.[84]

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.[85] 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. The evidence of these changes was based on light and electron microscopy and enhancement in Küpffer cell-dependent respiratory burst that is paralleled by an activation of NF-κB (key marker in copper-induced cellular response to oxidative damage) DNA binding activity. These effects peaked at 9 weeks and diminished after 12 to 16 weeks of copper sulfate exposure. Ultrastructural assessment showed results consistent with early hepatocellular changes due to copper loading, including an increase in lysosomal number and diversity, appearance of heterochromatin and pycnotic nuclei, and varied mitochondrial alterations. 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 body weight/day), 70 mg Fe/kg (5.69 mg/kg body weight/day), and 140 mg Fe/kg (11.54 mg/kg body weight/day), respectively.[31] 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. 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, or 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. It was concluded that the feeding of iron derived from ferrous sulfate at doses up to 11.5 mg/kg body weight/day did not result in tissue iron excess or any other toxicologically significant effects.

A group of 12 (6 males, 6 females; ages not stated) Sprague-Dawley rats received ferrous sulfate (25 mg/kg/day) in drinking water for 4 weeks.[62] The control group (6 males, 6 females) received regular tap water. 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 (6 males, 6 females) received regular tap water. Both groups of animals dosed with ferrous sulfate had gastric mucosal erosions, but no lesions of the esophagus. Macroscopic damage was not observed in the upper GI tract (small intestine) in either group. However, intestinal mucosal irritation ranging from simple mucosal edema and congestion to submucosal hemorrhages was observed in the lower GI tract (colon and rectum) in animals of both groups. 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.[86] 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 different points in time and the white blood cell count was greatly elevated.

**Zinc Sulfate**

Groups of specific pathogen-free mice of the ICR strain (groups of 8 [4 males, 4 females/group]; 4 weeks old) and rats of the Wistar strain (groups of 8 [4 males, 4 females/group]; 4 weeks old) were fed a diet containing $ZnSO_4$ at

concentrations of 300, 3,000, and 30,000 ppm in the diet for 13 weeks.[87] 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 in these animals included 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 females (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).[87]

**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.[88] 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%). The finding of constipation was slightly more frequently reported ($P = 0.03$) in placebo-treated infants when compared to iron-treated infants (1%). 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 (ages not stated) were each injected i.p. with aluminum sulfate (1 mg/200 g body weight) 3 times per week over a period of 2 weeks.[89] The animals were killed and kidney tissues prepared for routine histology and electron microscopy. The control rats were dosed i.p. with saline according to the same procedure. Aluminum sulfate 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 (ages not stated) were injected i.p. with 6 mg/kg manganese sulfate ($MnSO_4 \cdot 4\ H_2O$), in physiological saline, daily for 30 days.[90] Another group of 10 rats served as the untreated control group. The animals were killed at the end of the experiment and liver tissues were obtained for histopathological examination. 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. The authors noted that dosing with manganese sulfate did not produce damage in hepatocytes.

Six male albino rats (control group; ages not stated) were injected i.p. with manganese sulfate in saline (6 mg Mn/kg body weight) daily for 25 days.[91] 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. The animals were killed at the end of the dosing period, and the brain, liver, and testis were removed and prepared for gross and histopathological examination. 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. Results for gross and microscopic examination of the testis are included in the section on Reproductive and Developmental Toxicity.

**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 ($p < 0.050$) in the supernatant fraction. The hepatic changes observed were thought to have been caused by dosing with zinc sulfate.

**Intravenous**

**Copper Sulfate**

Four male Clun Forest, Suffolk cross sheep (~ 9 months old) were given copper sulfate (20 mg $CuSO_4$ $5H_2O$, dissolved in 40 ml of sterile saline) as a slow i.v. infusion daily.[92] 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 toxicity study, groups of female beagle dogs received the following i.v. doses of magnesium sulfate: 12.56, 50, 100, and 200 mg/kg/h.[93] 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 killed in a moribund state 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 ≥ 50 mg/kg/h, and was considered toxicologically insignificant. These treatment-related changes were not observed at the end of dosing. The authors noted that 50 mg/kg/h was considered the nontoxic dose of magnesium sulfate 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.[94] 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 authors concluded that a dose of ≤ 50 mg/kg/h was considered the nontoxic dose level in this study.

**Intratracheal**

**Zinc Sulfate**

Male Sprague-Dawley rats (4 animals; ages not stated) received a single intratracheal injection of zinc sulfate in distilled water (1 mM; volume = 0.1 ml).[95] The four vehicle control rats were dosed with distilled water. Both gross and histologic examinations of the lungs were performed. Histological examination of the lungs occurred at days 1, 7, 14, and 28 after dosing. At gross or histological examination, lung abnormalities were not observed in test animals at any time point; the same was true for the vehicle control group.

**Cytotoxicity**

**Ferric Sulfate**

Balb/c 3T3 fibroblasts were incubated with different dilutions of ferric sulfate.[96] The number of cells corresponding to each concentration of the test substance was calculated relative to the control (untreated cells), considered as 100%. The plot of the relative number of cells as a function of test substance concentration allowed the graphical estimate of dose (mg/mL) that kills 50% of the cells ($LD_{50}$ = 7.74 mg/mL) in relation to the control group. Cell viability was evaluated using the MTT assay and the neutral red uptake (NRU) assay, whereby the relative number of viable cells at the $LD_{50}$ for ferric sulfate was determined. Relative cell viability (%) relative to control wells containing cell culture medium without the test

substance was calculated. Dose-related inhibition (~ 0.001 to 30 µg/ml) of cell proliferation after 24 h of exposure to all dilutions of the test substance was noted. Ferric sulfate was classified as cytotoxic.

**Hydroxylamine Sulfate**

Femoral bone marrow smears (from male Sprague-Dawley rats, groups of 4 or 5) were prepared at 4 h after i.p. injection of hydroxylamine sulfate; 1,000 nucleated cells were counted.[32] A series of hydroxylamine sulfate doses, increased 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%.[97] The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a colorimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye MTT to its insoluble formazan. This assay measures cellular metabolic activity via NAD(P)H-dependent cellular oxidoreductase enzymes, and may reflect the number of viable cells present. 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. Parameters of histopathological changes were eosinophilic staining, cellular and nuclear morphology, and separation between the dermis and epidermis. 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**

There has been substantial discussion in the literature about speculations that exposure to elemental aluminum or aluminum compounds could play a role in the etiology of Alzheimer's disease, and other health problems. Overall, the available studies have not substantiated a causal link between aluminum exposure and Alzheimer's disease or other neurodegenerative disorders.

**Aluminum Sulfate**

Groups of 5 male C57BL/6 mice (20 days old) were treated with 2.5% aluminum sulfate in tap water for 2, 4, 6, and 12 months, respectively.[98] 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. The authors noted that the major pathohistological findings in brains with Alzheimer's disease are the presence of neuritic plaques containing β-amyloid (Aβ), which may interfere with neuronal communication. They also noted that GRP78 is reduced in the brains of patients with Alzheimer's disease. GRP78 is a stress-response protein induced by conditions that adversely affect endoplasmic reticulum function. In the present 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. The latter finding was similar to what has previously been observed in Alzheimer's disease. The authors hypothesized that chronic aluminum administration is responsible for oxidative cell damage that interferes with endoplasmic reticulum functions, inducing Aβ accumulation and neurodegenerative damage.

**Manganese Sulfate**

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 concentrations of $30.1 \pm 6.4$, $294.8 \pm 66.0$, and $3{,}220 \pm 578$ µg $Mn/m^3$, respectively, for 13 weeks.[37] A fourth group (control) was exposed to $0.3 \pm 0.02$ µg $Mn/m^3$. Based on the cascade impactor test results, 95% of the particulate MMAD was below 6 µm (respirable fraction). Blood and the following tissues were collected and analyzed for manganese content via neutron activation analysis: olfactory bulb, globus pallidus, caudate/putamen, cerebellum, frontal cortex, liver, lung, testis, and kidney. At the 2 highest concentrations of exposure, neuronal cell counts for 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.

Three cynomolgus macaque monkeys were trained to perform variable delayed response (VDR), visual discrimination (VD), and object retrieval tasks. One control animal performed the VDR, VD, and object retrieval tasks.[99] The animals were then exposed to manganese sulfate (10 to 15 mg/kg/week) for 272 ± 17 days. By the end of the exposure period, the 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). The author noted that these study results suggest that chronic exposure to levels of manganese achieved in this study may have detrimental effects on behavior, cognition, and motor functioning.

Groups of 4 juvenile rhesus monkeys (between 20 and 24 months old) were exposed to airborne-manganese sulfate. A $MnSO_4$ aerosol concentration of 4.62 $MnSO_4/m^3$, corresponding to 1.5 mg $Mn/m^3$, was generated in this study.[100] The animals were exposed to the test substance 5 days per week (6 h/day). Two groups were exposed for 15 ad 33 days, respectively. 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. Biochemical endpoints indicative of oxidative stress and excitotoxicity were assessed using the following tissues: cerebellum, frontal cortex, caudate, globus pallidus, olfactory cortex, and putamen. Additionally, levels of the following antioxidants, enzymes, and proteins (all biomarkers of neurotoxicity) were determined in all regions of the brain: glutamine synthetase (GS), glutamate transporters (GLT-1 and GLAST) and tyrosine hydroxylase (TH) protein levels, metallothionein (MT), GLT-1, GLAST, TH, and GS mRNA levels, and total glutathione (GSH). When 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, overall, the nonhuman primate brain responds to airborne manganese in a heterogenous manner, and most alterations in these biomarkers of neurotoxicity are 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.[101] These injections caused uveitis, which is characterized by prolonged ocular hypotony, increased protein concentrations, 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).[102] 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 (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. 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 and 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.[103] Applications were made, under 1" x 1" gauze squares, to intact and abraded skin. The trunk was then wrapped with rubber sheeting, secured with adhesive tape. 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. It was concluded that sodium bisulfate was highly corrosive to abraded skin of rabbits.

## 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).[102] Copper sulfate was applied (brush application) to 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 of tissue excised from the application site was performed. A reaction was classified as an irritant reaction when tissue changes (surface necrosis and/or inflammatory cells) could be clearly identified 6 h after oral provocation. The test material was classified as a non-irritant.

## Skin Sensitization

**Animal**

**Copper Sulfate**

Three series of maximization tests involving groups of 19 guinea pigs were performed.[104] 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 (2 to 3 months old). Test concentrations ranged from 0.05% to 25% in water.[102] The test material was applied to different sites on the neck daily for 5 days. Oral elicitation was initiated 7 days later. The buccal mucosa was excised and examined 24 h after elicitation. At 24 h after oral provocation with a predetermined non-irritant preparation of the test material, the investigators defined oral reactions as evident of sensitivity in a skin presensitized animal whenever mononuclear cell infiltrates could clearly be identified in the mucosal tissue. 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.

**Human**

**Provocative Testing**

**Copper Sulfate**

A group of 354 eczema patients (ages not stated) was patch tested with 5% copper sulfate in petrolatum.[105] 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. Reactions were not observed at control sites tested with 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.[104] 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.[106] The patch test procedure was not stated. 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).[107] 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. Of the 26 patients reevaluated, only 10 (38%) were judged positive. Nine of 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.[108] 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 6 patients. The reactions in these 2 patients (7 and 38 years old) were described as moderate, i.e., mostly erythema and slight infiltration.

**In Vitro**

**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), or adult Hartley guinea pigs.[109] 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; draining auricular lymph nodes were excised and a single suspension of lymph node cells (LNC) was prepared. A stimulation index (SI), the increase in $^{3}$HTdR incorporation relative to vehicle-treated controls, was derived for each experimental group. 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 ($ZnSO_4 \cdot 7H_2O$).[110] The procedure in the preceding study was used, and 25 µl of each chemical was applied to the ears. Control mice received an equal volume of DMSO. Copper(II) sulfate increased LNC proliferation, whereas, iron(II) sulfate and zinc sulfate did not induce LNC proliferation in this assay.

**Case Reports**

**Aluminum Sulfate**

A 23-year-old man developed dermatitis after application of a cream for acne and hyperpigmentation.[111] 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.[112] 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 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 as having acute appendicitis, and barium sulfate was used in diagnostic studies, i.e., upper gastrointestinal series and barium enema.[112] 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.[113] 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 caused barium granuloma of the rectum in 2 patients (males 75 and 78 years old).[114] 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 and 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.[115] The ingredients of the barium enema mixture were as follows: 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 an upper GI series.[116] 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 was associated with prior upper GI series that involved drinking barium sulfate. 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 the same volume of barium sulfate, she developed urticaria on her face, trunk, and lower extremities.

Cases of patients with a "magenta colon" from radiologic barium, occurring every 3 to 4 years, have been noted.[117] 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, they are sufficiently severe to the point where one has trouble determining whether there is true colitis. The severe reaction is characterized by edema, loss of all vascular markings, and redness to the point of 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.[118] 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, strong skin irritant, was 37.5.

**Copper Sulfate**

A 36-yaer-old male employee of a nursery regularly came in contact with pesticides, one of which was copper sulfate.[119] Patch testing with 2.5% aqueous copper sulfate yielded positive reactions at 48 h (++ reaction) and 72 h (+++ reaction). Re-testing with 1% aqueous copper sulfate yielded a negative reaction, and, based on this result, the positive reaction to 2.5% aqueous copper sulfate was regarded as a false positive.

**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.[120] 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.[121] 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.[122] 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.[123] 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.[124] 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.[125]

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 one that consisted of hydroxylamine sulfate and lithium chloride.[126] 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 due to preterm labor.[127] 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.[128] 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 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), loss of tendon reflexes (at 5 mmol/l), respiratory arrest (at 7.5 mmol/l), and cardiac arrest (at 12.5 mmol/l).[129]

**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.[130] 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.[131] 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 for 6 minutes. Results indicated an immediate reaction, which was a maximum decrease in forced expiratory volume in one second ($FEV_1$) of 23%. It was concluded that zinc can cause occupational asthma.

Twenty-two patients with Wilson's disease (10 boys; mean age = 6.2 years) were treated orally with zinc sulfate.[132] 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. Height z-scores at the time of diagnosis (mean $-0.24 \pm 1.53$) did not differ significantly ($P = 0.6$) from scores at the end of treatment (mean of $-0.31 \pm 1.29$). 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 to the beneficial effects of zinc sulfate on the skin.[133] 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. Treatment with Clobetasol™ + 2.5% zinc sulfate was more effective than treatment with Clobetasol™ cream alone ($P < 0.05$). Furthermore, the recurrence rate of eczema was significantly lower in the group treated with Clobetasol + 2.5% zinc sulfate. No significant side-effect was reported or observed by patients in both groups, and treatment was said to have been generally well-tolerated.

Oral zinc sulfate therapy was studied using 31 patients with multiple, non-genital viral warts (11 men, 20 women; mean age = 26).[134] 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 actually completed the study, and 13 experienced complete resolution of their warts after 2 months of treatment. 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.[135] Dosing was associated with a reduction in the lymphocyte stimulation response to phytohemagglutinin as well as 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 (ages not stated).[136] 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 animals were killed and the testis examined histologically. 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).[137] 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 body weight/day.

**Calcium Sulfate**

The teratogenicity of calcium sulfate was evaluated in experiments involving 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).[138] 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 (ages not stated) 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.[138]

**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).[139] A stock solution of 0.125 M copper sulfate (0.80 mg $Cu^{+2}$/ml) in water was used. 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.[140] 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 $5 \times 10^{-6}$ M, $2.5 \times 10^{-5}$ M, and $5 \times 10^{-5}$ 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*. The authors noted that the results of this study indicated that copper sulfate had a toxic effect on embryonic development.

Five pregnant, random-bred female Wistar rats (ages not stated) were dosed i.p. with aqueous copper sulfate (2 mg Cu/kg; dose volume = 0.1 ml/100g) on gestation day 8.[141] 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 also 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; 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.[84] The control group received drinking water without the test material. Complete necropsy was performed on all animals that died early and at the end of the study (all test and control animals included). Complete histopathologic examination involved the following groups: all control animals, all animals that died early, all animals in the highest dose group with at least 60% survivors, and all animals in higher dose groups. Groups of 20 B6C3F$_1$ mice (10 males, 10 females/group; 6 weeks old) received copper sulfate at the following concentrations in dosed 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. Sperm morphology and vaginal cytology evaluations were performed on rats and mice. The control and 3 highest exposure groups for mice and rats were evaluated. 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. Other study results are included in the Repeated Dose Toxicity – Oral section of this report.

The structural integrity of rabbit spermatozoa after exposure to copper sulfate was evaluated in an *in vitro* study.[142] 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.[143] 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*.[144] 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.[145] 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. The testis in each control rat was analyzed prior to the initiation of treatment. Testis morphology was studied using light and electron microscopy, and the number of spermatids was counted. 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.[146] 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 non-toxic dose level for general toxicological effects on the dams was 250 mg/kg/day (3 times per day), and that the non-toxic dose levels 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; ages not stated) were injected i.p. with manganese sulfate in saline (6 mg Mn/kg body weight) daily for 25 days.[91] 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. The animals were killed at the end of the dosing period, and the testis was removed and prepared for gross and histopathological examination. 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.[147] The six control rats received distilled water. The animals were killed on day 21. Maternal and fetal livers were prepared for electron microscopy. 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).[148] 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 the same doses of saline. The animals were killed on day 18 of gestation and the fetuses were examined for malformations. 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 (ages not stated). Ten rats received manganese sulfate in tap water (1,000 ppm/liter) for 12 weeks.[149] Control rats received tap water for the same time period. 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 numbers of pregnant females, implantation sites, viable fetuses, and resorptions were recorded. 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 (ages not stated) were used to evaluate the placental transfer of zinc during early embryogenesis.[150] 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 (ages not stated) were given feed containing 4,000 ppm zinc sulfate (anhydrous).[151] 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 reseorption 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, as expressed per mated female, in experimental rats was also reduced; however, this finding was not 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 reseorption 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.[151]

Ten male Sprague-Dawley rats (ages not stated) were injected i.p. with zinc sulfate (in saline, 3 mg/kg/day) for 4 weeks.[152] 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.[153] 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.[154] 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.[155] 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 wthat 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.[156] 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 had a higher incidence of hypotonia and lower median Apgar scores when 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).[157] Magnesium sulfate was administered as a 4-g bolus, followed by infusion of 2 to 3 g of magnesium sulfate per hour. At the time of delivery, umbilical cord blood was collected for later determination of serum ionized magnesium levels. Neonatal cranial ultrasound scans were obtained periodically for the diagnosis of intraventricular hemorrhage (IVH) and periventricular leucomalacia (PVL). The diagnosis of cerebral palsy was made at the age of 18 months. 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 resulted in 11 composite adverse pediatric outcomes, which included IVH, PVL, and cerebral palsy. 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).

During the time period between January of 2000 and February of 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).[158] 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.[159] 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.[160] 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. The authors noted the absence of data on the neurodevelopmental status of the infants of mothers involved in the study. 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,[161] 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.[162]

#### 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.[163] 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.[164]

#### 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. Two image analysis parameters were evaluated, tail intensity (% migrated DNA) and tail moment. The tail moment was calculated as the product of the tail length (DNA migration) and the fraction of DNA in the comet tail (% DNA in the tail). 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.[165] 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.[166]

#### 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*.[167] At a concentration of 240 µg/ml, the % tail DNA of cells exposed to gypsum for 24 h was $3.07 \pm 0.29$. Values for native and active bentonite particles tested at this concentration in cultures exposed for 24 h were $4.29 \pm 0.43$ and $4.40 \pm 0.43$, 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.[168] The doses 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) performed at a geometric ratio of 2, 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*.[168] 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.[169] 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 was expressed as % of the control,

and 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.[170] The reversion frequency (x $10^8$) 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-charomtid exchanges (SCE) assay using Chinese hamster cells.[171] 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.[172] 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 positive without metabolic activation.

Ferrous sulfate was evaluated for genotoxicity in the L5178Y TK+/- mouse lymphoma assay.[172] Mouse lymphoma cells were tested in the presence and absence of metabolic activation at concentrations up to 1.2 µg Fe/ml and up to 201 µg Fe/ml, respectively. Ferrous sulfate was genotoxic in the presence and absence of metabolic activation.

The comet assay[173] and chromosome aberrations analysis were used to determine the DNA-damaging and clastogenic effects of ferrous sulfate ($FeSO_4 \cdot 7H_2O$).[174] 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, respectively, 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.[173] The genotoxicity of ferrous sulfate (250 to 1,000 µM) did not differ significantly from that of $H_2O_2$ and HNE.[175]

**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.[176] 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 involving Chinese hamster ovary cells,[177] the genotoxicity of ferrous sulfate or magnesium sulfate (in physiological saline) was evaluated at concentrations up to 4 mg/ml. Ferrous sulfate induced chromosomal aberrations in this assay, but magnesium sulfate did not.[176]

**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.[178] 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,[179] 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.[180] 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 (8 to 10 weeks old).[181] In this assay, male rodents are dosed singly with subtoxic concentrations of the test substance. Each male mouse was dosed with the test substance or solvent control and subsequently caged with 3 untreated female virgin mice. The female mice were replaced weekly for 8 consecutive weeks. 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. Concurrent control groups usually consisted of 10 males. Matings during weeks 1-3, 4-5, and 6-8 after treatment of male mice and during weeks 1-5, 6-8, and 8-12 after treatment of male rats represent samplings of post-meiotic, meiotic, and premeiotic stages of spermatogenesis, respectively. Female mice were inspected daily for vaginal plugs, dissected on day 13 of pregnancy, and scored for corpora lutea and for total implants, comprising early and late fetal deaths and living fetuses. 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. The authors noted that these negative results do not preclude the possibility of an effect beyond the dose range selected or an effect in another strain, species, or test system.

**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.[182] 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 ($6.20 \pm 0.43$ 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.[183] 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,[163] 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.[184]

The genotoxicity of manganese (II) sulfate monohydrate was evaluated in the sex-linked recessive lethal test using *Drosophila melanogaster*.[185] 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 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.[186] The test substance was evaluated without metabolic activation, and results were negative.

The genotoxicity of manganese sulfate was evaluated in the fluctuation test[187] 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.[188]

**Zinc Sulfate**

The genotoxicity of zinc sulfate (in Hank's balanced salt solution) was evaluated in the micronucleus test.[189] 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 administered 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),[190] zinc sulfate (test concentration not stated) was considered a diagnostic negative. In order 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.[191]

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.[192] Negative and positive control groups received distilled water and cyclophosphamide (in distilled water), respectively. Blood was collected from the retroorbitral plexus of each mouse at 24 h, 48 h, 72 h, and 96 h and at one week post-dosing. The comet assay was used to evaluate the effect of various doses of zinc sulfate on the DNA (i.e., single strand DNA breaks, as represented by comet tail-lengths) of blood samples. Cell viability was determined using the trypan blue exclusion method. 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.[193] 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 human myelogenous leukemia K562 cells, in the presence of zinc and hydrogen peroxide.[194] 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.[195] DNA damage was assessed using the comet assay.[173] 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 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. In the comet assay, 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.[194]

The effect of zinc sulfate on cell proliferation was investigated using the WIL2-NS human lymphoblastoid cell line.[196] 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.[197] 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.[198] 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.[199] 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. The mitotic index was decreased significantly ($p < 0.05$) only in males of the oldest age group, the only significant finding regarding this parameter. The cell cycle for mitogenic stimulated lymphocytes was delayed in all treated cultures as a function of donor age; however, this finding was not statistically significant. The frequency of micronuclei was enhanced in all treatment sets. However, this increase was significant only for cultures established from the middle age group of males and from the oldest group of females. Intergroup comparisons indicated a significantly higher frequency of micronuclei among cultures established with blood obtained from the middle age group, when compared to cultures established using blood from the youngest group of males.

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 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 effect of treatment alone was significant, but the effects of age and sex 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, but the effects of age and sex were not significant.[199]

**Copper Sulfate**

An analytical grade of copper sulfate was injected intraperitoneally into male, inbred Swiss mice (10 to 12 weeks old; 3 per group).[200] 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, consisting of a chain of 3 association and single univalent, were also induced. However, none of the individual results in the treated series differed significantly form the corresponding control value, though the total aberrations frequency was statistically significant. The authors noted that, though the occurrence of translocation (1%) in the treated series was insignificant, this finding indicates that copper sulfate induced translocations in spermatogonial stem cells that were transmitted to the spermatocytes. 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:[201] 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 (10 to 12 weeks old) 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. The number of sperms examined per animal was 500. Finally, 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. Polychromatic erythrocytes (PCEs), normochromatic erythrocytes (NCEs), and immature white cells (1,000 of each cell type) were examined.

Treatment with copper sulfate ($CuSO_4 \cdot 5H_2O$) produced a dose response relationship for the yield of micronuclei, bone marrow chromosomal aberrations, and sperm abnormalities. The chromosomal aberrations included chromatid gaps, isochromatid gaps, chromatid breaks, fragments, double minutes, exchanges, and rings. Gaps were more frequent than breaks. Of the 3 cell types, the highest percentage of micronuclei was reported for polychromatic erythrocytes and the lowest percentage was reported 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 sperms.[201]

The genotoxicity of copper sulfate was evaluated in the micronucleus assay using groups of 6 Swiss albino mice (8 to 10 weeks old) per dose administered.[202] 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. Bone marrow was removed from both femurs and slides prepared. Data were evaluated as % aberrant metaphase cells (excluding gaps) and as the number of aberrations per cell (excluding gaps). 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.[203] In each assay, male CBA mice (8 to 11 weeks old) 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). The second assay involved new groups of 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, even if 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.[204] Groups of 5 Swiss male albino mice (4 weeks old) 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 body weight. Samples of whole blood were collected at 24 h, 48 h, and 72 h during week 1 and week 2 post-dosing. A total of 150 individual cells (leukocytes) was screened per sample. Samples were also used to study repair efficiency. Single-strand DNA breaks, as represented by comet tail-length, were determined. Study results indicated significant DNA damage at all doses, when compared to untreated controls, i.e., a clear dose-dependent response ($p < 0.05$) was observed. Further evaluation of samples identified a decrease in mean comet tail-length, indicative of repair efficiency capacity, which was less when compared to controls.

**Ferrous Sulfate**

The genotoxicity of ferrous sulfate was evaluated in the micronucleus test using groups of 6 ddY mice (8 weeks old).[205] The test material (in water) was injected i.p. at single doses 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. Slides containing femoral bone marrow cells were prepared for microscopic examination. One-thousand polychromatic erythrocytes per mouse were scored, and the number of micronucleated polychromatic erythrocytes (MNPCEs) and proportion of polychromatic erythrocytes (PCEs) were determined. 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; 3 weeks old).[206] 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 of the bone marrow micronucleus test indicated that the high ferrous sulfate diet caused an increased frequency of micronucleated polychromatic erythrocytes (MnPCEs), when compared to the low ferrous sulfate diet. Ascorbic acid supplementation in the low iron diet did not cause an effect on the incidence of MnPCEs and protected against the increased frequency of MnPCEs induced by the high ferrous sulfate diet. The authors noted that the results of this study indicated that ascorbic acid had a protective effect against the clastogenic effects of ferrous sulfate.

**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.[207] Negative control animals received distilled water. Bone marrow cytogenetic preparations were made, and 60 well-scattered metaphase plates were scanned for chromosome analysis. The chromosomal aberrations screened were chromatid gaps, breaks, fragments, isochromatid gaps, chromatid exchanges, and double minutes. For the micronucleus test, groups of 5 mice received the same 3 oral doses (2 of same dose per animal). One-thousand polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) were examined per animal, and the endpoint was percentage of micronuclei in PCEs/NCEs. 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. When compared to untreated controls, the 3 doses also increased the frequencies of micronucleated PCEs and NCEs. The 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. Following dosing with the test substance, the frequencies of chromosomal aberrations in bone marrow cells and micronuclei increased significantly when compared to controls. Significant enhancement of sperm-head abnormalities was noted as well. The authors noted that it has been suggested that these effects were mediated by $Mn^{2+}$ ions produced directly from manganese sulfate, following conversion at acidic pH of the 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).[208] 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 disturbing, 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.[186] 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). Any 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.[209] 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).[83] 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 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).[210] 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).[211] 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).[212] 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).[86] 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).[213] 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 the the 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).[214] 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.[214]

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 reltaed to the ingestion of manganese (II) sulfate monohydrate.[214]

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.[214]

**Magnesium Sulfate**

The tumorigenicity of magnesium sulfate fibers was evaluated using 20 female Syrian hamsters (ages not stated).[215] 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.[216] 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.[217] 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.

## Effect on Tumor Growth

### Ferrous Ammonium Sulfate

The inhibitory effect of ferrous ammonium sulfate ($FeNH_4(SO4)_2 \cdot 12H_2O$) on the MTK-sarcoma III, a rat ascites tumor, was studied using groups of 10 rats (males and females) of the Wistar, Long-Evans, or Gifu-agouti strain.[218] After tumor implantation, the test substance was administered i.p., at a dose of 0.6 g g/100 g, beginning on day 3 or day 4 of tumor transfer; dosing was continued for a total of 7 consecutive days. All of the animals in which the tumor was transplanted died within 9 days. Control animals were injected with physiological saline. The test substance exerted a marked damaging effect on both the cytoplasm and nucleus of tumor cells. Most cells generally underwent metaphase block, meaning that the cells were damaged at metaphase. A marked decrease in the number of dividing cells was observed in the ascites tumor as a result of repeated chemical application.

### Zinc Sulfate

The effect of zinc sulfate on mouse melanoma growth was evaluated *in vivo*.[219] Cloudmamn S91 mouse melanomas were transplanted in the DBA2 inbred strain of mice and, B16 mouse melanomas, in inbred C57B1/10 mice. After subcutaneous tumor inoculation, the mice were divided into 2 groups, one allowed to drink distilled water and the other allowed to drink a 0.05% zinc sulfate (in distilled water) solution constantly. There were no significant differences in tumor growth between the 2 groups.

## 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.[220] The incidence of colon tumors (adenocarcinomas) was not significantly different from that observed in the 14 animals fed control diet. The same was true for tumors of the small intestine (adenocarcinomas). However, it should be noted that 6 and 15 tumors (small intestine) were observed in the test and control groups, respectively. This was the greatest difference in tumor incidence (small or large intestine) that was observed.

## 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:[221] 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). Increased tumor incidence, tumor size, and number of aberrant crypt foci (ACF) were accompanied by a decrease in lipid peroxidation, glutathione-*S*-transferase, superoxide dismutase (SOD) and catalase. However, 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. Well-differentiated signs of dysplasia were evident in colonic tissue sections (after DMH administration alone). However,

the administration of zinc sulfate to DMH-treated rats greatly restored normalcy in the colonic histoarchitecture, with no apparent signs of neoplasia. Using the energy dispersive X-ray fluorescence technique (EDXRF), 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.[222] This response was indicated by the number of antibody forming cells (AFC) per million nucleated cells. Ferrous sulfate (0.1 mM) in Mischell-Dutton culture significantly decreased ($p \leq 0.01$) the SRBC 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).[223] 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 inhibit, enhance, or alter 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.[224] 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. Hair pigmentation was examined macroscopically, by routine histology, and by electron paramagnetic resonance (EPR). Oral dosing with zinc sulfate induced a bright brown lightening of new hair shafts produced during anagen, but did not induce an 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*.[225] 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).[226] 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 increase in alkaline phosphatase activity and increase in 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.[227] 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, sodium sulfate, 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 96.8% (sodium sulfate, in bath products).

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 (≥ 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 $\mu g/m^3$) 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 (75$\mu g/m^3$) 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.[4]

| | |
|---|---|
| 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.[4]
(The italicized text below represents additions made by CIR staff.)

| Ingredient/CAS No. | Definition | Function |
|---|---|---|
| Aluminum Sulfate<br>10043-01-3<br>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<br>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<br>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<br>10034-76-1<br>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<br>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<br>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<br>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<br>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<br>7720-78-7<br>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<br>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<br>18939-43-0<br>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<br>10034-96-5<br>10124-55-7<br>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<br>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<br>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<br>7681-38-1 | is the inorganic salt that conforms to the formula. *Sodium Bisulfate is the sodium salt of hydrogen sulfate.* | pH Adjusters |
| Sodium Sulfate<br>7727-73-3 (decahydrate)<br>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 |

**Table 1.** Definitions and functions of the ingredients in this safety assessment.[4]
(The italicized text below represents additions made by CIR staff.)

| Ingredient/CAS No. | Definition | Function |
|---|---|---|
| Zinc Sulfate<br>7446-19-7 (monohydrate)<br>7446-20-0 (heptahydrate)<br>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; C osmetic Biocides; Oral Care Agents |

**Table 2.** Properties of Inorganic Sulfates.[5]

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

**Table 3.** Current Frequency and Concentration of Use According to Duration and Type of Exposure.[9,10]

| | Aluminum Sulfate | | Ammonium Sulfate | | Barium Sulfate | |
|---|---|---|---|---|---|---|
| | # of Uses | Conc. (%) | # of Uses | Conc. (%) | # of Uses | Conc. (%) |
| **Exposure Type** | | | | | | |
| *Eye Area* | 29 | NR | NR | NR | 84 | 0.01-18.6 |
| *Incidental Ingestion*[10] | 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 | 0.01 -11 |
| *Incidental Inhalation- Powders* | 13 | NR | 9 | NR | 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 | NR | 28 | NR | NR | NR |
| *Incidental Inhalation- Powders* | NR | NR | 27 | NR | 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.[9,10]

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

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

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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