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NTP Technical Report
on Toxicity Studies of

Cupric Sulfate

(CAS No. 7758-99-8)

Administered in Drinking Water and Feed
to F344/N Rats and B6C3F$_1$ Mice

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This NTP report on the toxicity studies of cupric sulfate is based primarily on 2-week drinking water studies conducted in January 1988, on 2-week feed studies conducted in February and March 1989, and on 13-week feed studies that began in August 1989 and ended in November 1989 at Battelle Columbus Laboratories, Columbus, OH.

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ABSTRACT

Cupric Sulfate

$CuSO_4 \cdot 5H_2O$

Molecular Formula	$CuSO_4 \cdot 5H_2O$
CAS Number	7758-99-8
Molecular Weight	249.68
Synonyms	Chalcanthite
	Copper sulfate
	Bluestone
	Blue vitriol
	Roman vitriol
	Salzburg vitriol

Cupric sulfate is an inorganic salt which is widely used in industry, agriculture, and veterinary medicine. Its applications include use as an algicide in potable waters and as a feed additive and therapeutic agent in swine, sheep, and cattle. Because copper salts are found in human water supplies, toxicity studies of cupric sulfate pentahydrate were conducted in male and female F344/N rats and B6C3F$_1$ mice by the drinking water (2-week studies only) and dosed feed routes (2-week and 13-week studies). Animals were evaluated for hematology, clinical chemistry, urinalysis, reproductive toxicity, tissue metal accumulation, and histopathology.

In the 2-week drinking water studies, groups of five rats and five mice per sex received cupric sulfate at concentrations of 300 to 30,000 ppm for 15 days. One female rat, one male mouse, and three female mice in the 3000 ppm groups and all rats and mice in the 10,000 and 30,000 ppm groups died before the end of the studies. The remaining mice and rats in the 3000 ppm groups gained little or lost weight. Water consumption in the three highest dose groups of both species was reduced by more than 65%. Clinical signs observed in these groups were typical of those seen in moribund animals and were attributed to dehydration. The only gross or microscopic change specifically related to cupric sulfate toxicity was an increase in the size and number of cytoplasmic protein droplets in the epithelium of the renal proximal convoluted tubule in male rats from the 300 and 1000 ppm groups.

In the 2-week feed studies, groups of five rats and five mice per sex were fed diets containing 1000 to 16,000 ppm cupric sulfate. No chemical-related deaths occurred in any dose group. Compared to the controls, rats and mice in the two highest dose groups had reduced body weight gains which were attributed to decreased feed consumption. Hyperplasia with hyperkeratosis of the squamous epithelium on the limiting ridge of the forestomach was seen in rats and mice of each sex; this lesion was more severe in rats than in mice. Inflammation of the liver, periportal to midzonal in distribution, occurred in rats in the 8000 and 16,000 ppm groups. Depletion of hematopoietic cells was evident in rats of each sex in the bone marrow (8000 and 16,000 ppm) and spleen (16,000 ppm). Kidneys of male and female rats in the 4000, 8000, and 16,000 ppm groups had an increased number and size of protein droplets in the epithelia of the renal cortical tubules.

In the 13-week feed studies, groups of 10 rats per sex received diets containing 500 to 8000 ppm cupric sulfate, and groups of 10 mice per sex received diets containing 1000 to 16,000 ppm cupric sulfate for 92 days; estimates of cupric sulfate consumption ranged from 32 to 551 mg/kg per day for rats and 173 to 4157 mg/kg per day for mice. There were no chemical-related deaths in rats or mice, and no clinical signs of cupric sulfate toxicity were recorded. Final mean body weights were lower than those of the controls for animals of both species receiving doses of 4000 ppm cupric sulfate and greater. In mice in the 13-week studies, there was a dose-related decrease in liver weights.

Hematologic, clinical chemistry, and urinalysis evaluations of rats in the 13-week study revealed variable chemical-related changes that were, for the most part, restricted to the 4000 and 8000 ppm groups. Increases in serum alanine aminotransferase and sorbitol dehydrogenase activities in both sexes were indicative of hepatocellular damage, as were increases in 5′-nucleotidase and bile salts in males. Decreases in mean cell volume, hematocrit, and hemoglobin indicated the development of a microcytic anemia, while increases in reticulocyte numbers at the same time points suggested a compensatory response to the anemia by the bone marrow. Increases in urinary glucose and *N*-acetyl-β-D-glucosaminidase (a lysosomal enzyme) and aspartate aminotransferase (a cytosolic enzyme) were suggestive of renal tubule epithelial damage.

Dose-related increases in copper occurred in all male rat tissues examined (liver, kidney, plasma, and testis). These increases were accompanied by increases in zinc in the liver and kidney. Plasma calcium was significantly reduced in the 4000 and 8000 ppm groups,

and there was a trend toward reductions in calcium in the kidney and testis as well. In the 8000 ppm group, plasma magnesium was significantly increased relative to the controls.

Rats in the three highest dose groups had hyperplasia and hyperkeratosis of the forestomach, inflammation of the liver, and increases in the number and size of protein droplets in the epithelial cytoplasm and the lumina of the proximal convoluted tubules. These effects were similar to those seen in the 2-week feed study, and the incidence and severity of these lesions were dose related. Many of the droplets in male rat kidneys were large and had irregular crystalline shapes. These droplets stained strongly positive for protein but were negative by iron, PAS, and acid-fast (lipofuscin) staining methods. α-2-Microglobulin was present in the droplets of male rats, but there was no dose-related, qualitative difference in the content of this protein. In the 4000 and 8000 ppm groups, copper was distributed in a periportal to midzonal pattern in the liver and was restricted to the cytoplasm of the proximal convoluted tubule epithelium in the kidney. Copper was present in some, but not all, of the protein droplets. Transmission electron microscopy of the livers of rats of each sex revealed increases in the number of secondary lysosomes in hepatocytes in the periportal area.

In mice of each sex receiving 4000 ppm cupric sulfate and higher in the 13-week study, there was a dose-related increase in hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge of the forestomach. Minimal positive staining for copper was present in the liver and was limited to high-dose (16,000 ppm) male and female mice.

Cupric sulfate produced no adverse effects on any of the reproductive parameters measured in rats or mice of either sex.

In summary, administration of cupric sulfate to rats in feed or drinking water resulted in significant gastric changes and hepatic and renal damage. The primary lesion in rats was an increase in the size and number of proteinaceous droplets in the epithelial cytoplasm and lumen of the proximal convoluted tubule. For rats in the 13-week study, the no-observed-adverse-effect level (NOAEL) for evidence of histologic injury to the kidney was 1000 ppm for males and 500 ppm for females, while the NOAEL for liver inflammation was 1000 ppm for males and 2000 ppm for females. Hyperplasia with hyperkeratosis of the epithelium on the limiting ridge separating the forestomach from the glandular stomach

was also seen in rats of each sex, and the NOAEL for this change was 1000 ppm cupric sulfate in the feed. Additionally, clinical pathology alterations noted in the 13-week study, along with histologic changes in bone marrow noted in the 2-week feed study, were indicative of a microcytic anemia with a compensatory bone marrow response. Mice appeared to be much more resistant to the toxic effects of cupric sulfate than rats. The primary target tissue in mice was the epithelium of the limiting ridge of the forestomach. The NOAEL for the hyperplasia and hyperkeratosis seen at this site in mice was 2000 ppm cupric sulfate in the feed.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of cupric sulfate on December 2, 1992, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members determine if the design and conditions of these NTP studies are appropriate and ensure that this toxicity study report presents the experimental results and conclusions fully and clearly.

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SUMMARY OF PEER REVIEW COMMENTS

On December 2, 1992, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of cupric sulfate.

Dr. Charles D. Hébert, NIEHS, introduced the short-term toxicity studies of cupric sulfate by reviewing the study rationale, experimental design, and results.

Dr. Davis, a principal reviewer, questioned the range of clinical signs attributed to dehydration in the drinking water studies and said that he thought data on feed consumption would help clarify the lack of weight gains noted in the studies. Dr. Davis also requested further discussion of statements that attributed atrophy and cellular depletion of several organs to dehydration resulting from poor palatability of the more concentrated cupric sulfate solutions. He also questioned the usage of the term chronic active hepatitis for the liver lesion depicted in Plate 3 of the report. Finally, Dr. Davis suggested that assertions that much higher internal doses were achieved in the dosed feed studies than in the drinking water studies would be bolstered by actual plasma copper concentration data.

Dr. Bailey, a second principal reviewer, said that he thought the report was thorough and well prepared. He noted that the changes in copper and calcium in the testis could possibly affect reproduction, although none of the endpoints evaluated appeared affected in the study. He questioned the no-observed-adverse-effect level (NOAEL) given for the rat kidney and also wondered if the cytoplasmic alteration seen at the 300 ppm exposure level in the 2-week drinking water study in rats should be listed among the reported NOAELs. Dr. Bailey stated that the copper content of the diet would be useful information to add to the report.

Dr. Hébert responded to Dr. Davis' comments by stating that he would clarify the relationship between the clinical signs, organ atrophy, and dehydration. He agreed that feed consumption data would be helpful in interpreting the drinking water studies but pointed out that this information was not collected. Dr. Michael Elwell, NIEHS, commented on the liver lesion in Plate 3 and stated that the terminology used, inflammation, chronic active, is an accurate description of the lesion and is not the same as chronic active hepatitis in the human. Dr. Hébert concurred with Dr. Bailey's

suggestion that reproductive effects might be expected with the high copper and low calcium levels in the testis. He stated that the typical dietary level of copper in the NIH-07 diet is between 5 and 15 ppm. With regard to the NOAEL observed in the 2-week drinking water study in rats, Dr. Hébert agreed with the statements of Dr. Bailey, but he said that NOAELs were only given for the 13-week studies.

Dr. Carlson, a member of the panel, also had comments about some of the specific values listed as NOAELs; Dr. Hébert responded that the NTP would revisit the issue and change them if corrections were needed.

Dr. Klaassen, the panel chairman, suggested the addition of more information concerning human subpopulations that are susceptible to copper, such as individuals with Wilson's disease; he also suggested that more information be added concerning the peculiarity of sheep with regard to their sensitivity to excess copper. Dr. Hébert agreed to these suggestions, and Dr. Klaassen then accepted the report on behalf of the peer review panel.

INTRODUCTION

Physical Properties, Production, Use, and Exposure

Soluble salts of copper, including cupric sulfate, are used in a wide variety of industrial and agricultural applications. They are used as pigments in varnishes and paints, in electroplating solutions, as battery electrolytes, in metal and laundry marking inks, in petroleum refining, in pest control, and in human and veterinary medicine. Cupric sulfate in particular finds wide application, especially in agriculture and veterinary therapeutics. It is used as an aquatic algicide in impounded potable waters, and in an injectable form it is effective in the prevention and control of Dutch elm disease. Cupric sulfate pentahydrate mixed with lime forms Bordeaux mixture, which is used as a general fungicide and seed treatment for control of downy mildew, blights, leaf spots, and other fungal diseases of plants, especially grapes (Hayes, 1988). In veterinary medicine, cupric sulfate is used as a food additive for swine feed to promote weight gain and efficiency of food utilization, and it is also used as a treatment for foot rot and parasitic gastritis in sheep. Cupric sulfate has been used therapeutically in humans and animals as an astringent, an anthelminthic, and a treatment for trachoma. Because cupric sulfate is a gastrointestinal irritant, with ingestion usually resulting in violent vomiting, it has been used as an emetic (Gosselin *et al.*, 1984). Such use is no longer permitted in the United States but continues in parts of Europe and Asia (Arena and Drew, 1986). Cupric sulfate is also commonly used as a suicide agent, especially in India and Japan (Chuttani *et al.*, 1965; Singh and Singh, 1968; Cohen, 1974; Takahashi *et al.*, 1987).

Cupric sulfate is produced by the reaction of copper or copper oxide with dilute sulfuric acid, followed by evaporation and crystallization. In the pentahydrate form, it exists as blue, odorless crystals. These crystals have a negligible vapor pressure but are slowly efflorescent in air, losing water with increasing temperature to eventually form the anhydride by 250° C (*Merck Index*, 1983). Cupric sulfate pentahydrate has a specific gravity of 2.285 at 15.6° C. It is soluble in water, glycerin, and methanol but is practically insoluble in most organic solvents (Hartley and Kidd, 1987). In aqueous solution, it reacts with ammonia and amines, leading to its use as a laboratory reagent for the quantitation of proteins. While copper can exist in either the monovalent (cuprous, Cu^{+}) or divalent (cupric, Cu^{2+}) forms in aqueous solution, the more highly oxidized cupric form is the more stable of the two (Venugopal and Luckey, 1978).

Exposure to copper in the environment may occur through drinking water, food, soil, or ambient air. The daily intake of copper in the diet is estimated to be 15 to 45 µg/kg body weight in adult humans (Aaseth and Norseth, 1986). Copper levels in water usually range from 1 to 5 µg/L, but levels may be higher than 1 mg/L in some areas. In the United States, copper levels in the air range from 10 ng/m^3 in rural areas to as high as 570 ng/m^3 in urban areas (Aaseth and Norseth, 1986). Occupational exposure to copper and copper salts can occur during a number of operations, including mining and smelting; exposure can also occur during the treatment of vineyards with Bordeaux mixture sprays.

Copper has been detected in water samples taken near chemical waste sites and is the eighth most commonly occurring inorganic material in such sites. Estimated annual U.S. exports and consumption of cupric sulfate pentahydrate for the years 1980 to 1987 were between 32,000 and 37,000 metric tons, and apparent consumption in 1988 was 42,000 metric tons (SRI International, 1992). Approximately 65% to 70% of the product was used in animal feeds; the remaining product was used in other agricultural applications (8%) and in industrial applications (12%), water treatment (8% to 10%), and wood treatment (2%). The National Institute for Occupational Safety and Health (NIOSH) estimates that during the years 1981 to 1983, 10,821 workers were exposed to cupric sulfate in industrial settings (NIOSH, 1990). The United States Environmental Protection Agency (USEPA) recommends a maximum safe level of 1 mg $CuSO_4$/L for drinking water (Sittig, 1991). There is no Occupational Safety and Health Administration (OSHA) standard or American Conference of Governmental Industrial Hygienists (ACGIH)-recommended threshold limit value-time weighted average (TLV-TWA) specifically for cupric sulfate; however, the OSHA standard and the ACGIH-recommended TLV-TWA for copper dusts and mists, averaged over an 8-hour work period, is 1 mg/m^3 (ACGIH, 1986).

Biochemistry

Copper is an essential element. The World Health Organization (WHO) has estimated the daily requirements for humans to be 30 µg/kg for adults, 40 µg/kg for older children, and 80 µg/kg for infants (WHO, 1973). When conditions for copper utilization are optimal, 4 to 5 ppm copper in swine and poultry feed and 8 to 10 ppm in ruminant rations appear to be adequate (NAS, 1980). Copper is a constituent of a number of enzymes, including tyrosinase (pigment production), dopamine-β-hydroxylase (catecholamine production), Cu-Zn-superoxide dismutase (SOD) and Cu-SOD (free radical detoxification), and cytochrome oxidase. In addition, copper binding to ceruloplasmin is essential to the ability

of this enzyme to oxidize ferrous iron to the ferric (transferrin-binding) state. Copper deficiency results in an iron-deficiency type of anemia, abnormal bone growth, abnormal connective tissue formation, cardiovascular collapse, and death (Venugopal and Luckey, 1978; Aaseth and Norseth, 1986).

Absorption, Disposition, Metabolism, and Excretion

The pharmacokinetics of copper are intimately interrelated with those of other metals, including iron, zinc, molybdenum, nickel, and manganese, as well as with the pharmacokinetics of sulfate (Venugopal and Luckey, 1978). Elevated levels of nickel, manganese, and zinc have been shown to reduce copper storage in the liver while molybdenum and sulfate reduce copper absorption and enhance excretion. In the agricultural setting, copper supplementation in the diet is used to inhibit the harmful effects of excess dietary molybdenum and vice versa (Buck *et al.*, 1976). Multiple studies have demonstrated that copper and its salts are well absorbed from the intestinal tract in mammals and birds. Absorption generally occurs from the stomach and the proximal small intestine and is mediated by metallothionein in the mucosal cells. Absorbed copper in the form of the cupric ion is transported to the liver bound to albumin (Cohen, 1974). In the liver, copper is initially bound to metallothionein or a metallothionein-like low-molecular weight protein. Later, copper appears in the plasma bound primarily (approximately 95%) to the liver-produced protein ceruloplasmin. The highest levels of copper occur in the liver, heart, muscle, kidney, and brain (Aaseth and Norseth, 1986), but most copper in the body is stored bound to metallothionein in the liver and bone marrow. Haywood *et al.* (1985) found that in rats fed diets supplemented with copper, the metal accumulated fastest in the liver cytosol, becoming localized in hepatocyte lysosomes. Subsequent accumulation of copper in liver and kidney nuclear fractions corresponded with the development of hepatic and renal tubule necroses.

Excretion of copper occurs primarily via the bile; biliary copper is protein-bound and is not normally reabsorbed. Studies in humans have shown that urinary excretion of copper is very low, with only 1% of an intravenous dose being excreted in the urine within 72 hours, while over the same period, biliary excretion amounted to 9% of the dose (Tauxe *et al.*, 1966).

Interactions

Copper compounds have been shown to modulate the pharmacokinetics and toxicity of a number of other metals, drugs, and chemicals. The interactions of copper with iron, zinc, and molybdenum metabolism have been extensively investigated (reviewed in Buck *et al.*, 1976; Venugopal and Luckey, 1978). In general, the presence of copper reduces absorption and storage of these metals. Gipp *et al.* (1973) found that liver and plasma iron levels and iron utilization were reduced in pigs fed diets containing 2 or 250 ppm copper. Conversely, Theil and Calvert (1978) found that treatment of sheep with excess copper resulted in increases in plasma iron levels but no change in liver or bone marrow iron stores. These authors also reported that copper interfered with the utilization of iron by reticuloendothelial cells of the spleen.

Toxicity

HUMAN TOXICITY

Acute poisoning and death in humans following accidental or intentional ingestion of cupric sulfate have been reported (Chuttani *et al.*, 1965; Fairbanks, 1967; Singh and Singh, 1968; Chugh *et al.*, 1975; Stein *et al.*, 1976; Chugh *et al.*, 1977; Takahashi *et al.*, 1987; Akintonwa *et al.*, 1989). Common symptoms of overdose include nausea, vomiting, epigastric pain, diarrhea, jaundice, hemoglobinuria, and anuria. A blue discoloration of the tongue and gums is sometimes seen, and urine and stool are frequently greenish-black or black. Cupric sulfate can cause widespread vascular injury and hemolytic anemia resulting in severe kidney and liver damage. In the most severe cases, patients become comatose and somnolent, and death from circulatory failure results.

Chuttani *et al.* (1965) reported on the clinical course of 48 patients admitted to hospitals in New Delhi, India, after attempted suicide by ingestion of cupric sulfate, and Akintonwa *et al.* (1989) described four cases of acute cupric sulfate poisoning that occurred in Nigeria after church members taking part in a purification ritual were administered "spiritual water," a traditional concoction containing high levels of cupric sulfate. All patients suffered severe nausea, vomiting, and epigastric pain, which were presumed to have resulted from copper-induced injury to the gastrointestinal mucosa. Superficial to deep ulceration of the gastric and intestinal mucosae was present in patients that died. Coma occurred in several patients and was attributed to uremia resulting from damage to the kidney. Hemoglobinuria, hematuria, and increases in blood urea nitrogen

(BUN) were observed and were believed to have resulted from intravascular hemolysis with subsequent renal tubule necrosis. Several patients developed acute renal failure and died.

Biochemical analyses performed on blood from a patient with acute cupric sulfate poisoning revealed that copper was able to penetrate the erythrocytes and inhibit glycolysis, enhancing NADPH oxidation and promoting denaturation of hemoglobin (Fairbanks, 1967). The inhibition of glycolysis was thought to be due to the inhibition of erythrocyte glucose-6-phosphate dehydrogenase activity. Singh and Singh (1968) studied blood from 40 patients with acute cupric sulfate poisoning and found evidence of liver damage, including elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Hemolysis occurred and was attributed to the accumulation of copper in erythrocytes, resulting in the precipitation of hemoglobin. Jaundice in some patients was believed to be partly due to hemolysis and partly due to direct liver damage, inasmuch as jaundice appeared in several cases where AST and ALT were elevated in the absence of hemolysis.

Cupric sulfate, as well as other copper salts and copper metal itself, can result in greenish-black discoloration of the skin, itching eczema, and contact dermatitis after dermal exposure (Cohen, 1974; Stokinger, 1981-82 ACGIH, 1986). Contact with the eyes has been reported to cause conjunctivitis, edema of the eyelids, and turbidity and ulceration of the cornea (Cohen, 1974; Grant, 1986). Holtzman *et al.* (1966) reported a case of acute hemolytic anemia in a child who was treated with cupric sulfate crystals applied therapeutically to severely burned skin.

Inhalation of copper or cupric sulfate results in irritation of the nasal mucosa and pharynx, as well as occasional ulceration of the nasal septum. Pathologic changes in vineyard workers using Bordeaux mixture sprays included pulmonary inflammation, granuloma formation, fibro-hyaline nodules, desquamation of macrophages, and progressive diffuse fibrosis (Pimentel and Marques, 1969; Pimentel and Menezes, 1975; Reynolds and Prasad, 1982). Clinical signs of toxicity included shortness of breath, weakness, weight loss, and a productive cough. Recovery occurred after removal of the patients from cupric sulfate exposure; however, symptoms recurred immediately when exposure resumed. Elevated levels of copper have been detected in milk and placental tissue from female vineyard workers exposed to Bordeaux mixture (Hayes, 1988).

Human populations exist which may be unusually susceptible to poisoning by copper. For example, individuals with Wilson's disease, an inborn defect in copper transport and excretion, excrete copper at about half the rate of unaffected individuals and accumulate excessive amounts of copper in the liver. Similarly, persons suffering from various diseases of the liver, such as primary biliary cirrhosis, can have impaired copper excretion. In patients with Wilson's disease, hemolytic anemia has been attributed to the release of copper from the liver into the circulation following necrosis of copper-laden hepatocytes (Aaseth and Norseth, 1986).

ANIMAL TOXICITY

Poisoning by copper and copper salts has been investigated in many mammalian and nonmammalian species, including cattle, swine, sheep, guinea pigs, rats, mice, ducks, geese, chickens, turkeys, fish, snails, and clams. Table 1 summarizes the toxicity data for some of these species. In general, mammals are more susceptible to copper toxicity than birds but are more resistant than aquatic species. Nonruminants are more resistant to the effects of copper than are ruminants (Venugopal and Luckey, 1978), and mature animals are more resistant than younger animals, in which the excretory mechanisms for copper removal may be less well developed (Todd, 1962). The signs and lesions of copper toxicity in mammals and birds are similar to those observed in humans and include vomiting (in those species where vomiting is possible), jaundice, green or dark discoloration of urine and excreta, diarrhea, intravascular hemolytic anemia, liver damage, and kidney damage leading to renal failure. Because the literature on copper poisoning in animals is voluminous, only an overview is provided below.

Copper supplementation in the diets of farm animals, particularly swine, has been shown repeatedly to promote weight gain and efficiency of feed utilization (Bowler *et al.*, 1955; Gipp *et al.*, 1973; Buck *et al.*, 1976). However, the efficiency of the copper for this purpose is highly dependent on the animal species, the levels of zinc and iron in the diet, and the protein composition of the feed. Pigs fed experimental diets containing 250 ppm copper were found to have significantly reduced feed consumption and body weight gain as well as reduced hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, and plasma and liver iron levels (Gipp *et al.*, 1973).

TABLE 1 Summary of Selected Animal Toxicity Data for Cupric Sulfate

Route of Exposure	Species	Dose[1]	Reference
Oral	Rat	LD_{50} = 300	Hayes, 1988
Oral	Rat	LD_{50} = 960	Venugopal and Luckey, 1978
Oral	Mouse	LD_{100} = 50	Venugopal and Luckey, 1978
Oral	Rabbit	LD_{100} = 50	Venugopal and Luckey, 1978
Oral	Mammal	LD_{50} = 470	RTECS
Oral	Human	TDLo = 272	RTECS, Wahal *et al.*, 1965
Oral	Human	LDLo = 1088	RTECS, Wahal *et al.*, 1965
Oral	Dog	LDLo = 60	Flury and Zernik, 1935
Oral	Farm animals	LDLo = 5	RTECS, Ishmael and Gopinath, 1972
Oral, powder	Chicken	LDLo = 300 to 500	Pullar, 1940a,b
Oral, aqueous	Chicken	LDLo = 1000 to 1500	Pullar, 1940a,b
Oral	Pigeon	LDLo = 1000 to 1500	Pullar, 1940a,b
Oral	Duck	LDLo = 400 to 600	Pullar, 1940a,b
Intraperitoneal	Mouse	LD_{50} = 33	Curzon and Schnieden, 1965
Intraperitoneal	Mouse	LD_{0} = 8.9	Curzon and Schnieden, 1965
Intraperitoneal	Mammal	LD_{50} = 7.5	RTECS
Subcutaneous	Guinea pig	LDLo = 8.3	Moore *et al.*, 1913
Subcutaneous	Mouse	LDLo = 20.8	Moore *et al.*, 1913
Intravenous	Guinea pig	LDLo = 2	Venugopal and Luckey, 1978
Intravenous	Rabbit	LD_{100} = 4.5	Venugopal and Luckey, 1978
96-hour solution	Striped bass	LC_{50} = 0.25 to 0.5 ppm	Hughes, 1970
96-hour solution	Striped bass	LC_{100} = 0.1 to 0.15 ppm	Hughes, 1970
96-hour solution	Freshwater snail	LC_{50} = 0.06 to 0.39 ppm	Gupta, 1981
48-hour solution	Brine shrimp	LC_{50} = 3.2 ppm	Tanaka *et al.*, 1982

[1] Doses are in mg/kg body weight except where noted.

Sheep appear to be among the most sensitive of mammalian species to copper toxicity. Booth and McDonald (1982) reported that as little as 10 to 15 ppm copper in the diet could poison sheep. The course of poisoning in this species is unusual in that sheep are able to consume fairly high levels of copper for extended periods without apparent ill effect. During this time, copper accumulates in liver lysosomes. When the lysosomal capacity for storage has been exceeded, the copper is released suddenly, precipitating a hemolytic crisis. Gopinath *et al.* (1974) observed that in sheep given 20 mg copper/kg body weight daily for 9 weeks, kidney function was not impaired until the onset of the hemolytic crisis, suggesting that damage to the kidney occurred as a result of intravascular hemolysis. Sheep in which hemolysis had occurred were anemic and jaundiced, and they had pale, friable livers and dark, swollen kidneys with extensive renal tubule necrosis. BUN increased in these sheep, and urine was dark and contained granular casts and elevated

levels of protein. Todd and Thompson (1963) described loss of hemoglobin, decreases in packed cell volume, and increases in plasma bilirubin, ALT, AST, and lactate dehydrogenase, indicating that extensive hepatic damage had occurred following the hemolytic crisis.

Early studies of cupric sulfate toxicity in rodents demonstrated that albino rats experienced retarded weight gain when fed cupric sulfate at 500 ppm in the diet and suffered damage to the liver, kidneys, and other organs when given feed containing 1000 ppm (Boyden *et al.*, 1938). Poisoned rats either died rapidly (within 1 hour) or survived several days before dying. Rana and Kumar (1981, 1983) dosed adult male albino rats by gavage with 0 or 0.1 g cupric sulfate/kg body weight for 20 days and found that copper-treated rats had lower body weight gains than those of the controls as well as delayed skeletal development. Copper-induced tubule necrosis in the renal cortex but had no effect on the glomeruli. In a similar series of experiments, rats were administered the same doses of cupric sulfate for a period of 30 days, and no overt signs of toxicity were seen, although weight gains were somewhat reduced (Kumar and Sharma, 1987). The occurrence of hemolytic anemia was suggested by increases in bilirubin concentrations in the blood and by decreases in erythrocyte numbers, hemoglobin concentrations, mean cell volume, and percent hematocrit. Evidence of liver damage included increases in blood cholesterol, AST, ALT, and serum alkaline phosphatase. Likewise, an increase in BUN was indicative of renal injury.

The irritant potency of inhaled cupric sulfate to guinea pigs was examined by Amdur *et al.* (1978). Guinea pigs were exposed to cupric sulfate (0.4 to 2.4 mg sulfate/m^3) for 1 hour. The 0.4 mg/m^3 concentration produced a slight decrease in lung compliance, while the 2.0 and 2.4 mg/m^3 concentrations produced both an increase in resistance and a decrease in compliance. These changes were attributed to the sulfate moiety rather than to the copper, since ammonium sulfate and ammonium bisulfate produced similar results.

Poultry appear to be less susceptible to copper poisoning than most mammals, with the exception of rats (Buck *et al.*, 1976). Chicks fed moderate levels of copper in the diet (100 to 200 ppm) gained weight faster than those fed unsupplemented diets; however, at high copper levels (350 to 800 ppm), feed intake and weight gain were significantly reduced (Smith, 1969; Funk and Baker, 1991). As in swine, the efficiency of weight gain promotion at low concentrations and toxicity at higher concentrations were highly dependent on the

protein composition of the diet. Goldberg *et al.* (1965) showed that concentrations of 50 and 100 mg copper/kg body weight per day were toxic to adult chickens, producing anemia and death. Similar effects have been reported in turkeys, ducks, geese, and pigeons after consumption of excess copper (Pullar, 1940a,b; Vohra and Kratzer, 1968; Henderson and Winterfield, 1975).

Cupric sulfate has also been shown to be toxic to a number of freshwater and marine species, including fish (Mount, 1968; Hughes, 1970), shrimp (Tanaka *et al.*, 1982), and mollusks (Eisler, 1977; Gupta *et al.*, 1981) (Table 1). The greater sensitivity of aquatic animals to copper toxicity compared to mammalian or avian species is thought to be due to the lack of an efficient means of excretion of copper. The LC_{50} values for the freshwater snail *Viviparus bengalensis* were in the range of 0.06 to 0.39 ppm (Gupta *et al.*, 1981). Recommended use levels for cupric sulfate as an algicide in impounded waters are 0.1 to 1.0 ppm. It is clear that these recommended levels are not safe for all species of aquatic life.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Elemental copper has been shown to produce adverse effects on the female reproductive system. Copper interferes with a variety of functions, including oocyte maturation and implantation (Mattison, 1983). However, such effects are local and occur when copper is instilled directly into the uterus. For this reason, copper has been used to enhance the contraceptive effectiveness of intrauterine devices.

Cupric sulfate fed to cattle at levels of up to 500 ppm in the diet for as long as 16 months produced no adverse effects on reproduction, although hepatic copper levels were elevated significantly (NAS, 1980). Copper is teratogenic in hamsters, producing malformations of the right ventricle and the interventricular septum (Ferm and Hanlon, 1974). In these experiments, cupric citrate was found to be more teratogenic than cupric sulfate. No other information on the reproductive or developmental toxicity of cupric sulfate was found in the literature.

CARCINOGENICITY

Epidemiological evidence of high rates of cancer in coppersmiths and a high incidence of stomach cancer in areas with elevated Zn:Cu soil ratios suggests a role for copper in carcinogenesis. In addition, many malignant tumors are found to contain elevated levels of copper (Venugopal and Luckey, 1978). However, experimental evidence from rodent studies does not support such a carcinogenic role for copper (Gilman, 1962; USEPA, 1984; TDB, 1986). Most studies that showed a lack of carcinogenic effect for copper were of insufficient duration to adequately assess carcinogenic potential.

GENETIC TOXICITY

Cupric sulfate, tested as either the anhydrous salt or the pentahydrate, induced the formation of auxotrophic mutants in both streptomycin-sensitive and resistant strains of *Bacillus subtilis* (Berek and Kiss, 1974), but it did not induce the SOS DNA repair response in *Escherichia coli* strain PQ35 or PQ37 (Olivier and Marzin, 1987). It was not mutagenic in *Salmonella* mutation tests (Lemma and Ames, 1975; Marzin and Phi, 1985). Cupric sulfate was reported to induce an increase in the frequency of respiratory deficient mutants, but not auxotrophic mutants, in *Saccharomyces cerevisiae* (Takahashi, 1972).

When administered to pregnant rats as a single intraperitoneal injection (8 mg/kg), cupric sulfate did not induce micronuclei in maternal bone marrow cells or in preimplantation embryos analyzed 30 hours after treatment (Ornaghi and Giavini, 1989). However, significant increases in chromosomal aberrations were detected in bone marrow cells of Swiss Albino mice sampled 6, 12, and 24 hours after a single intraperitoneal injection of 1.1 to 6.6 mg/kg cupric sulfate pentahydrate (Agarwal *et al.*, 1990).

Cupric sulfate at concentrations of 0.08 mM and above was found to enhance virally induced Syrian hamster embryo cell transformation (Casto *et al.*, 1979). Two other copper salts, cupric acetate and cupric chloride, were also found to increase the incidence of errors in DNA synthesis *in vitro* (Sirover and Loeb, 1976).

Study Rationale and Design

Copper and copper compounds were found in 210 of 1177 National Priorities List chemical waste sites (ATSDR, 1990), and dissolved copper has been detected in water samples taken from the ground around and under selected hazardous waste sites. Due to the extensive use of copper and its salts in industry, agriculture, and plumbing, continuing contamination of water supplies with copper is anticipated. The National Institute of Environmental Health Sciences (NIEHS) selected copper for study from a preliminary list of 58 chemicals compiled by the USEPA under the Comprehensive Environmental Response, Compensation, and Liability Act (Superfund). The toxicologic data available were not considered adequate by the USEPA for assessing the risk of cupric sulfate toxicity by the oral route, for deriving acceptable intake levels for chronic copper exposure, or for determining the carcinogenic potential of copper. Cupric sulfate pentahydrate was selected for study because it is the most common form of copper found in chemical waste sites.

Two-week drinking water studies were performed in male and female rats and mice using cupric sulfate pentahydrate. Because of high mortality in the upper dose groups and the reduced water consumption in these groups due to the poor palatability of the cupric sulfate solutions, 13-week drinking water studies were not conducted. Two-week and 13-week dosed feed studies of cupric sulfate toxicity were carried out with male and female F344/N rats and $B6C3F_1$ mice. In the 13-week studies, gross and histopathologic examinations and sperm morphology and vaginal cytology evaluations were performed on rats and mice, and clinical pathology analyses were conducted for rats.

MATERIALS AND METHODS

Procurement and Characterization of Cupric Sulfate

Cupric sulfate (CAS Number 7758-99-8) was obtained in one lot (Lot 533344) from J.T. Baker (Phillipsburg, NJ). Initial analyses were performed by Midwest Research Institute (MRI, Kansas City, MO) on two batches of Lot 533344. For both batches, the chemical, a blue, crystalline solid, was identified as cupric sulfate pentahydrate, and infrared and ultraviolet/visible spectra were consistent with the structure of cupric sulfate pentahydrate and the available literature references (*Sadtler Standard Spectra*). The results of elemental analyses of both batches for copper, sulfur, and hydrogen agreed with theoretical values. Spark source mass spectroscopy indicated 140 ppm silicon present in Batch 1 and 80 ppm lead present in Batch 2 as impurities. All other impurities detected by spark source mass spectrometry totaled less than 270 ppm for Batch 1 and 212 ppm for Batch 2. Karl Fischer analysis indicated a water content of 32.7% ± 0.4% for Batch 1, which was low compared to the theoretical value of 36.1%; however, weight loss on drying at 250° C indicated a water content of 35.74% ± 0.03% for Batch 1 and 35.9% ± 0.1% for Batch 2. For both batches, chelometric titration with 0.01 M EDTA showed a purity of 100% ± 2%, and cumulative analytical data indicated a purity of approximately 99%.

Subsequent identity and purity analyses of Batch 1 were performed by MRI after particle size reduction of the chemical to 180 µm using a FitzMill®. Concomitant analyses of a reference standard without particle size reduction indicated that particle size reduction did not significantly alter the purity of the bulk chemical.

Because literature references indicate that cupric sulfate is stable at normal storage temperatures when kept dry, no accelerated stability studies were performed on the bulk chemical. At the study laboratory, the bulk chemical was stored at room temperature. Reference samples (approximately 1 g each) were stored protected from light at -20° C in glass vials with Teflon®-lined caps.

Bulk chemical reanalyses performed by the study laboratory using ultraviolet spectroscopy and chelometric titration showed consistent purity levels throughout the studies.

Dose Formulations

DRINKING WATER STUDIES

Drinking water solutions of cupric sulfate were prepared in deionized water. A premix was formulated by placing the appropriate amount of cupric sulfate pentahydrate into a graduated cylinder and adding deionized water to a volume of 1000 mL. This solution was mixed by inverting the cylinder until the chemical was dissolved and then transferred into a polypropylene carboy for bulk mixing; the appropriate amount of deionized water was added to the carboy to produce the desired final volume and then mixed to ensure homogeneity.

Stability studies were performed at MRI using ultraviolet absorption spectroscopy (435 nm). Results indicated that a 0.03% concentration of cupric sulfate was stable after 3 weeks of storage in polypropylene containers in the dark at room temperature. Solutions stored for 96 hours under animal room conditions were also stable.

Dose formulations of cupric sulfate were stored at room temperature in 10 L polypropylene carboys. Dose formulations and animal room samples were analyzed by ultraviolet absorption spectroscopy; all results were within 10% of the theoretical concentrations.

FEED STUDIES

For the 2-week and 13-week feed studies, cupric sulfate was mixed with NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in meal form. A premix was prepared for each dose concentration by mixing the appropriate amount of cupric sulfate pentahydrate (milled to a fine powder and sifted through a USS No. 70 sieve) in a large beaker with an equal weight of feed flour obtained by sifting the meal through a USS No. 80 sieve. Additional feed was added to the mix until the desired premix weight was reached. This premix was blended with a weighed amount of feed in a Patterson-Kelley twin-shell blender (East Stroudsburg, PA) for 15 minutes, with the intensifier bar on for the first 5 minutes, and then stored at room temperature in sealed plastic buckets.

Homogeneity analyses were conducted by MRI on a cupric sulfate feed mixture (1000 ppm) using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Evaluations of ashed samples indicated a maximum variation in concentration of 8.4% among three

sampling points. Analyses conducted by the study laboratory on feed mixtures confirmed the homogeneity of the mixtures.

No stability studies were conducted on cupric sulfate in feed for the following reasons: cupric sulfate is a relatively stable inorganic compound, although the pentahydrate does lose 2 moles of water on exposure to air at 30° C; cupric ion remains biologically available in feed when stored at 25° C; and in feed mixtures, cupric ions will not convert to cuprous ions when stored at ambient conditions (with low moisture and no significant acidity).

For the 2-week studies, feed mixtures were analyzed prior to the study by ICP-AES; all results were within 10% of the theoretical concentrations. For the 13-week studies, feed mixtures were analyzed prior to the study and twice during the study by ICP-AES. All mixtures analyzed were within 10% of the theoretical concentrations. Results of referee analyses performed by MRI on dose formulations used in the 13-week studies were in agreement with study laboratory results.

Toxicity Study Designs

BASE STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for the 2-week drinking water studies and from Simonsen Laboratories (Gilroy, CA) for the 2-week and 13-week feed studies. Rats and mice were shipped to the study laboratory (Battelle, Columbus Division) at approximately 4 weeks of age, quarantined for 12 to 16 days, and then placed on study at about 6 weeks of age. Blood samples were collected from three males and two females per species in the 2-week drinking water studies, from two animals per sex per species in the 2-week feed studies, and from five animals per sex per species in the 13-week feed studies. The sera were analyzed for viral antibody titers; data showed no positive antibody titers (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). Additional details concerning study design and performance are listed in Table 2.

In the 2-week drinking water studies, five animals per sex per species were administered 0, 300, 1000, 3000, 10,000, or 30,000 ppm cupric sulfate pentahydrate in drinking water that was available *ad libitum* for 15 days. In the 2-week feed studies, five animals per sex

per species were administered 0, 1000, 2000, 4000, 8000, or 16,000 ppm cupric sulfate pentahydrate in feed that was available *ad libitum* for 15 days.

Doses for the 13-week feed studies were based on the results of the 2-week feed studies. In the 13-week base study, groups of 10 rats per sex were administered 0, 500, 1000, 2000, 4000, or 8000 ppm cupric sulfate pentahydrate in feed; additional rats were used in a supplemental clinical pathology study. Groups of 10 mice per sex were administered 0, 1000, 2000, 4000, 8000, or 16,000 ppm cupric sulfate pentahydrate in feed. For rats and mice, the feed mixture was available *ad libitum* for 92 days.

For all studies, rats were housed five animals per cage, and mice were housed individually. Animal rooms were maintained at 69° to 79° F and 31% to 73% relative humidity, with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day. During the 2-week drinking water studies, feed in pellet form (NIH-07 Open Formula Diet, Zeigler Brothers, Inc., Gardners, PA) and dosed water were available *ad libitum*. During the 2-week and 13-week feed studies, dosed feed and deionized water were available *ad libitum*. The copper content of NIH-07 feed is typically about 10 ppm.

Complete necropsies were performed on all base-study animals in the 2-week and 13-week studies. For each animal, body weight was determined, and the liver, thymus, right kidney, right testis, heart, lungs, and brain were weighed prior to fixation. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded, sectioned, and stained with hematoxylin and eosin.

In the 2-week drinking water studies, complete histopathologic examinations were performed on all rats and mice in the 0, 3000, 10,000, and 30,000 ppm groups and on all animals, excluding male mice, in the 1000 ppm group. For the 2-week and 13-week feed studies, complete histopathologic examinations were performed on all rats and mice in the control and high-dose groups as well as on animals that died early. Gross lesions and selected tissues were examined in the lower dose groups to a no-observed-effect level. Tissues examined microscopically are listed in Table 2.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block

match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

Supplemental Histologic Examinations

To characterize the distribution of copper in the liver and kidney, sections of both organs from selected male and female rats and mice in the 13-week feed studies were stained for copper using the rhodanine method (Sheehan and Hrapchak, 1980). In order to determine the nature of the proteinaceous droplets seen in the kidneys of rats in the 13-week feed study, sections from selected animals were stained for carbohydrate (PAS method), protein (Mallory-Heidenhain method), lipofuscin (AFIP method), and α-2-microglobulin (immunohistochemistry). Liver sections from the same rats were stained for lipofuscin, and kidney and liver sections from rats of both sexes were examined by transmission electron microscopy. Perl's stain for iron was used to stain sections of spleen from rats and mice in the control and dosed groups.

SUPPLEMENTAL EVALUATIONS

Clinical Pathology

In the 13-week feed study, hematology and clinical chemistry evaluations were performed on 10 supplemental male and female rats per sex per dose group (0, 500, 1000, 2000, 4000, or 8000 ppm) on Days 5 and 21 and on surviving base-study rats at study termination (Day 92). Urine samples were collected from supplemental male and female rats for evaluation on Day 19 and from base-study rats on Day 90. For the hematology and clinical chemistry evaluations, rats were anesthetized with CO_2/O_2, and blood samples were collected from the retroorbital sinus. Samples for hematology analysis were collected in Microvette® tubes (Sarstedt, Nuembrecht, West Germany) containing sodium EDTA as an anticoagulant; samples for clinical chemistry evaluations were collected in Microvette® serum separator tubes and centrifuged to obtain serum. For the urinalysis studies, rats were placed individually into polycarbonate metabolism cages (Lab Products, Inc., Maywood, NJ) for overnight urine collection in chilled collection cups. During this period, animals had access to water but were not fed.

Hematologic determinations were performed with an Ortho ELT-8 Hematology Counter (Ortho Instruments, Westwood, MA). The parameters that were evaluated are listed in Table 2. Differential leukocyte and nucleated erythrocyte counts were determined from blood smears stained with a modified Wright-Giemsa stain. Reticulocyte counts were determined from blood smears stained with new methylene blue.

Clinical chemistry variables were measured with an Hitachi 704® chemistry analyzer (Boehringer Mannheim, Indianapolis, IN). Reagent for assays of sorbitol dehydrogenase was obtained from Sigma Chemical Company (St. Louis, MO). Urinalysis parameters were measured with an American Optical Refractometer/Total Solids Meter (American Optical, Buffalo, NY) and an Hitachi 704® chemistry analyzer. Parameters evaluated are listed in Table 2.

Tissue Metal Level Analyses

In the 2-week feed study, three groups of three male rats designated for a special method validation study were administered cupric sulfate in feed at concentrations of 0, 1000, or 16,000 ppm for 15 days. At the end of the study, plasma and tissue samples (liver, kidney, and testis) were collected from the surviving animals and analyzed for copper, manganese, zinc, calcium, and magnesium. In the 13-week feed study, plasma and tissue samples (liver, kidney, and testis) were collected from all surviving male base-study rats at the end of the study (Day 92) and analyzed for copper, zinc, magnesium, and calcium.

For both the 2-week and 13-week tissue metal level analyses, blood samples (approximately 2 mL) were collected from the retroorbital sinus and placed into 3 mL Vacutainer® tubes (Becton-Dickinson and Co., Rutherford, NJ) containing EDTA. The samples were centrifuged, and the separated plasma was frozen and stored at approximately -20° C. Tissue samples were collected at termination, quickly frozen in a dry ice-ethanol bath, and stored at approximately -20° C. To prepare for analyses, samples were thawed at room temperature, patted dry (tissue samples only), weighed to the nearest 0.1 mg, digested in a nitric acid-perchloric acid mixture, and heated until evolution of nitric acid was complete. The residue was then dissolved in a 10% perchloric acid solution, and an aliquot was removed for analysis by ICP-AES. Metal concentrations were determined by comparing the instrument response to the digested tissues to the instrument response to spiked tissue standards.

Sperm Morphology and Vaginal Cytology in Rats and Mice

Sperm morphology and vaginal cytology evaluations (SMVCE) were performed on rats and mice from the 13-week base studies. Rats from the 0, 500, 2000, and 4000 ppm groups (10 animals per sex and dose group) and mice from the 0, 1000, 4000, and 8000 ppm groups (10 animals per sex and dose group) were evaluated. Methods were those outlined in the National Toxicology Program's SMVCE protocol (modified December, 1987). Briefly, beginning 12 days prior to sacrifice, the vaginal vaults of 10 females of each species and dose group were lavaged, and the aspirated lavage fluid and cells were stained with Toluidine Blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body and weighed. Test yolk (rats) or Tyrode's buffer (mice) was applied to slides, and a small incision was made in the cauda. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five microscopic fields per slide.

Following completion of sperm motility estimates, each left cauda was placed in phosphate buffered saline solution. Cauda were finely minced and swirled, and the tissue was incubated and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacytometer.

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Studies of Cupric Sulfate

EXPERIMENTAL DESIGN	
Study Laboratory	Battelle Columbus Laboratories, Columbus OH
Size of Study Group	2-Week Drinking Water Studies: Five males and five females of each species per dose group 2-Week Feed Studies: Base studies: Five males and five females of each species per dose group Tissue metal level analyses (method validation study): Three male rats per dose group 13-Week Feed Studies: Base studies: 10 males and 10 females of each species per dose group Clinical pathology study: 10 rats per sex per dose group
Route of Administration	Drinking water or feed
Doses/Duration of Dosing	2-Week Drinking Water Studies: 0, 300, 1000, 3000, 10,000, or 30,000 ppm, *ad libitum* for 15 days 2-Week Feed Studies: Base studies: 0, 1000, 2000, 4000, 8000, or 16,000 ppm, *ad libitum* for 15 days Tissue metal level analyses (method validation study, rats only): 0, 1000, or 16,000 ppm, *ad libitum* for 15 days 13-Week Feed Studies: Base studies: Rats: 0, 500, 1000, 2000, 4000, or 8000 ppm, *ad libitum* for 92 days Mice: 0, 1000, 2000, 4000, 8000, or 16,000 ppm, *ad libitum* for 92 days Clinical pathology study: 0, 500, 1000, 2000, 4000, or 8000 ppm, *ad libitum* for 21 days
Date of First Dose	2-Week Drinking Water Studies: Rats: 8 January 1988 Mice: 13 January 1988 2-Week Feed Studies: Rats: 14 February 1989 Mice: 15 February 1989 13-Week Feed Studies: Base studies: Rats: 8 August 1989 (male), 9 August 1989 (female) Mice: 15 August 1989 (male), 16 August 1989 (female) Clinical pathology study: 8 August 1989 (male), 9 August 1989 (female)

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Studies of Cupric Sulfate (continued)

EXPERIMENTAL DESIGN (continued)

Date of Last Dose	2-Week Drinking Water Studies: Rats: 22 January 1988 Mice: 27 January 1988 2-Week Feed Studies: Rats: 28 February 1989 Mice: 1 March 1989 13-Week Feed Studies: Base studies: Rats: 7 November 1989 (male), 8 November 1989 (female) Mice: 14 November 1989 (male), 15 November 1989 (female) Clinical pathology study: 28 August 1989 (male), 29 August 1989 (female)
Necropsy Dates	2-Week Drinking Water Studies: Rats: 22 January 1988 Mice: 27 January 1988 2-Week Feed Studies: Base studies: Rats: 28 February 1989 Mice: 1 March 1989 13-Week Feed Studies: Base studies: Rats: 7 November 1989 (male), 8 November 1989 (female) Mice: 14 November 1989 (male), 15 November 1989 (female)
Type and Frequency of Observation	2-Week Drinking Water Studies: Observed twice daily for mortality/morbidity. Clinical observations were recorded twice daily. Individual body weights were recorded 2 days prior to the start of the study, on Day 8, and at necropsy. Water consumption was recorded weekly. 2-Week Feed Studies: Base studies: Observed twice daily for mortality/morbidity. Clinical observations were recorded twice daily. Individual body weights were recorded 1 (rats) or 2 (mice) days prior to the start of the study, on Days 1 (mice only) and 8, and at necropsy. Food consumption was recorded up to twice weekly, and water consumption was recorded two times per week. Tissue metal level analyses (method validation study): Observed two times per day for mortality/morbidity. 13-Week Feed Studies: Base studies: Observed twice daily for mortality/morbidity. Individual body weights were recorded prior to the start of the study, on Day 1, and weekly thereafter. Clinical observations and food consumption were recorded weekly. Clinical pathology study: Observed two times per day for mortality/morbidity. Body weights were recorded on Day 1.

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Studies of Cupric Sulfate (continued)

EXPERIMENTAL DESIGN (continued)

Necropsy and Histologic Examinations	Complete necropsies were performed on all animals in the 2-week and 13-week base studies. In the 2-week drinking water studies, histopathologic examinations were performed on all rats and mice in the 0, 3000, 10,000, and 30,000 ppm groups and on all animals, excluding male mice, in the 1000 ppm group. For the 2-week and 13-week feed studies, histopathologic examinations were performed on all rats and mice in the control and high-dose groups and on animals that died early. Tissues to be examined included: adrenal glands, brain (three sections), esophagus, eyes (if grossly abnormal), femur with marrow, gallbladder (mice only), gross lesions, heart, intestines (large: cecum, colon, rectum; small: duodenum, jejunum, ileum), kidneys, liver, lung/mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial or clitoral glands (rats, all studies, and 2-week drinking water and 13-week feed study mice), prostate gland, salivary glands, spinal cord/sciatic nerve (if neurologic signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus. Tissues examined in the lower dose groups in the 2-week drinking water studies were the lung (males), liver (males), and salivary gland for rats, and the liver, lymph nodes, pancreas, preputial gland, prostate gland, salivary gland, seminal vesicle, spleen, stomach, testis, thymus, and bone marrow for male mice. In the 2-week feed studies, the spleen, liver, stomach, and bone marrow were examined for rats in the lower dose groups, and the forestomach was examined for mice. In the 13-week feed studies, the kidneys, liver, and forestomach were examined for rats in the lower dose groups, and the forestomach was examined for mice.
Supplemental Evaluations	Clinical Pathology Study (13-Week Feed Study): Blood samples were collected from supplemental rats on Days 5 and 21 and from base-study rats at study termination (Day 92). Hematology parameters evaluated were: hematocrit (HCT), hemoglobin (HGB), erythrocytes (RBCs), reticulocytes, nucleated erythrocytes, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets, and leukocyte (WBC) count and differential. Clinical chemistry parameters evaluated were: alanine aminotransferase (ALT), alkaline phosphatase (AP), 5'-nucleotidase (5Nase), sorbitol dehydrogenase (SDH), bile salts, total protein, albumin, creatinine, and urea nitrogen (UN). Urinalysis parameters evaluated were: creatinine, glucose, protein, aspartate aminotransferase (AST), *N*-acetyl-β-D-glucosaminidase (NAG), volume, and specific gravity. Tissue Metal Level Analyses: 2-Week feed studies: Blood was collected from the retroorbital sinus of special study rats administered 0, 1000, or 16,000 ppm cupric sulfate in the feed. Plasma and tissue samples (liver, kidney, and testis) were analyzed for copper, zinc, manganese, magnesium, and calcium. 13-Week feed studies: Samples of liver, kidney, testis, and plasma were collected from all male base-study rats at study termination. Blood was collected from the retroorbital sinus. Plasma and tissue samples were analyzed for copper, zinc, magnesium, and calcium.

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Studies of Cupric Sulfate (continued)

EXPERIMENTAL DESIGN (continued)

Supplemental Evaluations (continued)	Sperm Morphology and Vaginal Cytology Evaluations: Rats that were administered 0, 500, 2000, or 4000 ppm cupric sulfate in the 13-week feed study and mice that were administered 0, 1000, 4000, or 8000 ppm cupric sulfate in the 13-week feed study were evaluated. Males (10 animals per species per dose group) were evaluated for necropsy body and reproductive tissue weights and spermatozoal data. Females (10 animals per species per dose group) were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in various stages.

ANIMALS AND ANIMAL MAINTENANCE

Strain and Species	F344/N Rats, B6C3F$_1$ Mice
Animal Source	2-Week Drinking Water Studies: Taconic Laboratory Animals and Services (Germantown, NY) 2-Week Feed Studies: Simonsen Laboratories (Gilroy, CA) 13-Week Feed Studies: Simonsen Laboratories (Gilroy, CA)
Time Held Before Study	2-Week Drinking Water Studies: 12 days (rats), 16 days (mice) 2-Week Feed Studies: 12 days (rats), 14 days (mice) 13-Week Feed Studies: Base studies: 13 days (male rats and mice), 14 days (female rats and mice) Clinical pathology study: 13 days (males), 14 days (females)
Age When Study Began	2-Week Drinking Water Studies: 5 to 6 weeks 2-Week and 13-Week Feed Studies: 6 weeks
Age When Killed	2-Week Drinking Water Studies: 7 to 8 weeks 2-Week Feed Studies: 8 weeks 13-Week Feed Studies: Base studies: 19 weeks Clinical pathology study: 9 weeks
Method of Animal Distribution	Animals were weighed and were randomized using a Xybion® computer program.

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Studies of Cupric Sulfate (continued)

ANIMALS AND ANIMAL MAINTENANCE (continued)	
Diet	2-Week Drinking Water Studies: NIH-07 Open Formula Diet in pellet form (Zeigler Brothers, Inc., Gardners, PA) and dosed water *ad libitum*. 2-Week and 13-Week Feed Studies: Dosed feed and deionized water (filtered) available *ad libitum*.
Animal Room Environment	Rats were housed five animals per cage and mice were housed individually for all base studies. All animals were housed in polycarbonate cages with hardwood chip bedding. Temperature was maintained at 69°-79° F and relative humidity at 31%-73%, with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

Quality Assurance Methods

The animal studies of cupric sulfate were performed in compliance with U.S. Food and Drug Administration Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of Battelle performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

2-Week Drinking Water Study in F344/N Rats

One female rat in the 3000 ppm dose group and all male and female rats in the two highest dose groups (10,000 and 30,000 ppm) died or were killed moribund before the end of the study (Table 3). For male and female rats in the three highest dose groups (3000, 10,000, and 30,000 ppm), Day 8 mean body weights were significantly lower than those of the controls. Because all rats in the two highest dose groups died before the end of the study, no final mean body weights or weight gains were determined for these groups. However, final mean body weights and body weight changes for both sexes in the 3000 ppm groups were significantly lower than those of the controls. At the end of the 2-week study, males exposed to 3000 ppm cupric sulfate weighed 48% less than the controls and females weighed 46% less than the controls (Table 3).

Clinical signs of toxicity exhibited by rats in the three highest dose groups included ruffled fur, emaciation, abnormal posture, hypoactivity, and nasal discharge. These signs of toxicity are typical of those seen in moribund animals and are consistent with the effects of dehydration; thus, these clinical signs were attributed to a marked decrease in water consumption in the three highest dose groups. The average daily amount of water consumed by rats in the 3000 ppm groups was less than 30% of that consumed by the control groups, and the average daily amount of water consumed by rats in the 10,000 and 30,000 ppm groups was less than 6% of the control values (Table 3). This decrease in water consumption was attributed to poor palatability of the drinking water solutions.

The average daily compound consumption for each dose group, based on average water consumption and mean body weights, is given in Table 3. For animals in the 1000 to 30,000 ppm concentration range, cupric sulfate consumption increased variably and was below targeted levels due to reduced water intake.

TABLE 3 Survival, Weight Gain, Water Consumption, and Compound Consumption of F344/N Rats in the 2-Week Drinking Water Study of Cupric Sulfate

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change[2]			
MALE							
0	5/5	82	169	87		17.9	
300	5/5	82	171	89	101	17.3	41
1000	5/5	83	174	91	103	14.7	113
3000	5/5	82	88	6	52	4.8	175
10,000	0/5[4]	82	–	–	–	1.0	140
30,000	0/5[5]	83	–	–	–	0.9	379
FEMALE							
0	5/5	88	139	51		16.3	
300	5/5	89	141	52	101	15.3	39
1000	5/5	88	131	43	94	11.3	102
3000	4/5[6]	87	75	–14	54	3.2	121
10,000	0/5[7]	88	–	–	–	0.9	120
30,000	0/5[7]	89	–	–	–	0.7	279

[1] Number surviving at 15 days/number of animals per dose group. For groups with no survivors, no final mean body weights or body weight changes are given.
[2] Mean weight change of the survivors.
[3] (Dose group mean/control group mean) x 100.
[4] Day of death: 10, 11, 12, 12, 12.
[5] Day of death: 10, 12, 12, 12, 12.
[6] Day of death: 6.
[7] Day of death: 12, 12, 12, 12, 12.

Significant changes in absolute and relative organ weights were limited to rats in the highest surviving dose group (3000 ppm). For males and females at this exposure level, absolute organ weights were significantly lower than those of the controls. Relative organ weights showed variable changes and, in general, were elevated at this exposure level. All of these changes were attributed to the lower final mean body weights of animals receiving 3000 ppm cupric sulfate.

In the 2-week drinking water study, no chemical-related gross lesions were seen in male or female rats in any dose group. Microscopic lesions were limited to a minimal cytoplasmic alteration seen in the kidneys of male rats in the 300 and 1000 ppm groups. This alteration, demonstrated by Mallory-Heidenhain staining, consisted of an increase in the size and number of protein droplets in epithelial cells of the proximal convoluted tubule. No cytoplasmic changes were detected in H&E or Mallory-Heidenhain stained sections from female rats.

2-Week Drinking Water Study in B6C3F$_1$ Mice

One male mouse and three female mice in the 3000 ppm groups and all mice in the two highest dose groups (10,000 and 30,000 ppm) died before the end of the study (Table 4). Day 8 mean body weights for male and female mice in the three highest dose groups (3000, 10,000, and 30,000 ppm) were lower than those of the controls. Because all mice in the two highest dose groups died before the end of the study, no final mean body weights or weight changes were determined for these groups. However, final mean body weights and body weight changes for male and female mice receiving 3000 ppm cupric sulfate were significantly lower than those of the control groups. At the end of the 2-week study, males in this dose group weighed 22% less than controls, and females weighed 34% less than controls (Table 4).

Like rats in the 2-week drinking water study, male and female mice in the three highest dose groups exhibited clinical signs of toxicity consistent with the effects of dehydration, including ruffled fur, emaciation, abnormal posture, hypoactivity, dyspnea, tremors, and prostration. These findings, as well as the subsequent early deaths in these dose groups, were attributed to dehydration caused by low water consumption. Water consumption decreased with dose, and the average daily amount of water consumed by animals in the 3000, 10,000, and 30,000 ppm groups was less than 33% of that consumed by the control groups (Table 4). This decrease in water consumption was attributed to poor palatability of the drinking water solutions. Except for one male in the 1000 ppm group that appeared emaciated, no clinical signs of toxicity were observed in the 0, 300, or 1000 ppm groups.

The average daily compound consumption of each dose group is given in Table 4. Although the actual amount of compound consumed did increase with increasing concentrations of cupric sulfate in the drinking water, compound consumption amounts for all dose groups were below targeted levels because of the reduced water intake.

TABLE 4 Survival, Weight Gain, Water Consumption, and Compound Consumption of B6C3F$_1$ Mice in the 2-Week Drinking Water Study of Cupric Sulfate

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]	Water Consumption (g/day)	Compound Consumption (mg/kg/day)
		Initial	Final	Change[2]			
MALE							
0	5/5	25.1	27.2	2.1		4.8	
300	5/5	25.2	27.7	2.5	102	3.6	41
1000	5/5	25.0	26.5	1.5	97	2.4	95
3000	4/5[4]	25.1	21.1	-3.8	78	1.6	226
10,000	0/5[5]	25.2	–	–	–	1.1	524
30,000	0/5[6]	25.2	–	–	–	1.0	1442
FEMALE							
0	5/5	20.0	22.2	2.3		5.6	
300	5/5	20.0	21.5	1.5	97	4.1	58
1000	5/5	20.0	21.1	1.1	95	2.8	140
3000	2/5[7]	20.0	14.6	-5.0	66	1.3	245
10,000	0/5[8]	20.0	–	–	–	1.1	683
30,000	0/5[9]	19.9	–	–	–	0.7	1296

[1] Number surviving at 15 days/number of animals per dose group.
[2] Mean weight change of survivors.
[3] (Dose group mean/control group mean) x 100.
[4] Day of death: 12.
[5] Day of death: 10, 11, 12, 12, 12.
[6] Day of death: 9, 10, 10, 11, 12.
[7] Day of death: 10, 12, 13.
[8] Day of death: 9, 9, 9, 10, 10.
[9] Day of death: 8, 8, 10, 10, 10.

In the 2-week drinking water study, absolute organ weights were decreased for animals in the highest surviving dose group (3000 ppm), and many of these decreases were significant. Absolute thymus weights for males and females and absolute brain, kidney, liver, and lung weights for females in the 3000 ppm groups were significantly lower than those of the controls. Absolute brain and lung weights in females in the 1000 ppm group were also significantly lower than those of the controls. Relative organ weight changes were variable. All of these changes were attributed to the dehydration caused by the low water consumption of animals in these dose groups and the decreased body weights of mice in the 3000 ppm groups.

There were no gross or microscopic lesions attributed to the toxicity of cupric sulfate in the drinking water. Atrophy or cellular depletion was present in numerous tissues from most mice in the three highest dose groups (3000, 10,000, and 30,000 ppm) and was attributed

to the marked decrease in water consumption and body weight gain in these groups. Thus, while these cellular changes were considered to be treatment related, they were not considered to be specifically related to cupric sulfate toxicity.

2-Week Feed Study in F344/N Rats

No rats in any of the dose groups died or were killed before the end of the 2-week feed study (Table 5). Final mean body weights and body weight changes for rats receiving 8000 or 16,000 ppm cupric sulfate were significantly lower than those of the control groups; these differences were attributed in part to decreased feed consumption in animals in the 16,000 ppm groups during Week 1 of the study. The only clinical sign of toxicity that could be attributed to cupric sulfate consumption was thinness, which was observed in male and female rats in the 8000 and 16,000 ppm groups.

TABLE 5 Survival, Weight Gain, Feed Consumption, and Compound Consumption of F344/N Rats in the 2-Week Feed Study of Cupric Sulfate

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]	Feed Consumption (g/day)[4]	Compound Consumption (mg/kg/day)[5]
		Initial	Final	Change[2]			
MALE							
0	5/5	106	184	77		14.6	
1000	5/5	107	186	79	101	15.2	92
2000	5/5	107	183	76	100	14.7	180
4000	5/5	107	178	71	97	14.4	363
8000	5/5	106	151	45	82	13.3	777
16,000	5/5	106	122	16	66	9.2	1275
FEMALE							
0	5/5	98	138	41		11.4	
1000	5/5	99	139	41	101	11.6	89
2000	5/5	98	138	40	100	11.2	174
4000	5/5	98	136	38	98	11.7	367
8000	5/5	98	128	29	92	11.7	769
16,000	5/5	99	106	7	77	7.1	1121

[1] Number surviving at 15 days/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) x 100.
[4] (Week 1 mean + Week 2 mean)/2
[5] (Week 1 mean + Week 2 mean)/2. Weekly mean = (grams of feed consumed/kg body weight per day) x concentration, mg/g.

At the end of the 2-week study, final mean feed consumption for rats in the 1000 to 8000 ppm concentration range was similar to that of the controls; feed consumption for animals receiving 8000 ppm cupric sulfate was slightly decreased during Week 1, but this decrease was offset by a subsequent increase in feed consumption during Week 2. Due to a marked decrease in feed consumption by male and female rats in the 16,000 ppm groups during Week 1 of the study (and despite an increase in consumption by these animals during Week 2, particularly in high-dose males), final mean feed consumption values for animals in these groups were lower than those of the controls (Table 5). Decreases in feed consumption during the first week of the study were considered to be due at least partially to poor palatability of the feed mixture.

Average daily compound consumption increased proportionally with increasing concentrations of cupric sulfate for all but the 16,000 ppm groups. At this concentration, cupric sulfate consumption was slightly lower than the targeted amount due to the decrease in feed consumption during Week 1 of the study.

Generally, absolute organ weights were lower than those of the control groups for animals in the two highest dose groups, and the majority of these differences were significant for animals in the highest dose group. Absolute liver weights were significantly lower than those of the control groups for females in the 4000 ppm group as well as for males and females in the two highest dose groups. These changes in absolute organ weights were attributed to decreased feed consumption with decreased body weight gain, although systemic toxicity could not be excluded as a cause.

During the 2-week feed study, tissue metal level analyses were performed. For these analyses, three groups of three male rats were administered cupric sulfate in feed at concentrations of 0, 1000, or 16,000 ppm for 2 weeks. Analyses performed at the end of the study indicated that copper accumulated in the liver and kidneys of animals receiving 16,000 ppm cupric sulfate; copper concentrations in the liver and kidneys of rats in the 16,000 ppm group were, respectively, 130 and 25 times greater than control values. When compared to the control group, zinc concentration was also increased in the liver of animals in the 16,000 ppm group. Concentrations of manganese, calcium, and magnesium in the liver, kidney, testis, and plasma of treated groups were similar to those for the control group.

In all rats receiving cupric sulfate in the feed at concentrations of 2000 ppm or greater, there was hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach. In the more severely affected animals, the ridge appeared papilliferous, with an expanded stromal core and frond-like projections of the squamous mucosa. Less severely affected rats had hyperplasia with hyperkeratosis of the squamous mucosa and only minimal edema and inflammation in the stroma.

Inflammation of the liver (chronic-active) was present as a minimal to mild mononuclear inflammatory cell infiltrate in four males receiving 8000 ppm and in five males and three females receiving 16,000 ppm. This infiltrate was periportal to midzonal in distribution, and single-cell necrosis of hepatocytes was often seen within or adjacent to foci of inflammation.

Depletion of hematopoietic cells in bone marrow occurred in male and female rats in the 8,000 and 16,000 ppm groups. This depletion, which was minimal at 8,000 ppm and mild to moderate at 16,000 ppm, consisted of a decreased cellularity of bone marrow erythroid/myeloid elements and an increase in the prominence of fat cells normally present in the shaft of the bone. In many of the high-dose animals, bone mass (cortex and trabecular density) was reduced compared to controls. Similar reductions in bone mass have been reported in a number of short-term studies where there has been a marked reduction of body weight gain (NTP, 1990, 1992a). A minimal to mild decrease in erythroid hematopoiesis was also seen in the spleens of male and female rats in the 16,000 ppm groups.

Cytoplasmic alteration, characterized by an increased number and size of eosinophilic-staining protein droplets in the cytoplasm and lumen of the renal cortical tubules, was observed in the kidneys of male and female rats in the 4000, 8000, and 16,000 ppm groups. With the Mallory-Heidenhain method, there was a clear increase in the size (larger and abnormally shaped) and number of droplets, which stained strongly positive for protein but were negative by the PAS method. In females, the cytoplasmic alteration was not evident in the H&E-stained sections, while the Mallory-Heidenhain stain demonstrated a clear increase in the number of protein droplets in the cytoplasm of the renal tubule epithelium.

Based on stomach and liver lesions, hematopoietic cell depletion, and significant decreases in feed consumption and mean body weights at the 16,000 ppm concentration, the dietary concentrations selected for the 13-week study in male and female rats were 0, 500, 1000, 2000, 4000, and 8000 ppm.

13-Week Feed Study in F344/N Rats

Except for one female receiving 1000 ppm that was accidentally killed, all rats survived to the end of the study. Final mean body weights and body weight changes were lower than those of the controls for male rats in the 500, 4000, and 8000 ppm groups and for female rats in the 8000 ppm group (Table 6 and Figure 1). These differences were most pronounced in males in the high-dose (8000 ppm) group. No clinical signs of toxicity that could be directly attributed to cupric sulfate consumption were observed in male or female rats.

TABLE 6 Survival, Weight Gain, Feed Consumption, and Compound Consumption of F344/N Rats in the 13-Week Feed Study of Cupric Sulfate

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]	Feed Consumption (g/day)[4]	Compound Consumption (mg/kg/day)[5]
		Initial	Final	Change[2]			
MALE							
0	10/10	119	362	244		16.3	
500	10/10	120	335	215	92	16.6	32
1000	10/10	119	360	241	99	17.0	64
2000	10/10	119	354	234	98	16.5	129
4000	10/10	120	338	217	93	16.5	259
8000	10/10	119	275	156	76	14.4	551
FEMALE							
0	10/10	106	193	87		11.1	
500	10/10	106	196	90	101	11.0	34
1000	9/10[6]	105	199	94	103	11.3	68
2000	10/10	107	196	89	101	11.3	135
4000	10/10	107	188	81	97	10.8	267
8000	10/10	106	179	73	93	10.1	528

[1] Number surviving at 13 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) x 100.
[4] Average of group mean feed consumption values measured at Weeks 2-13.
[5] Average of group mean compound consumption values measured at Weeks 2-13.
[6] Week of death: 2 (accidental death).

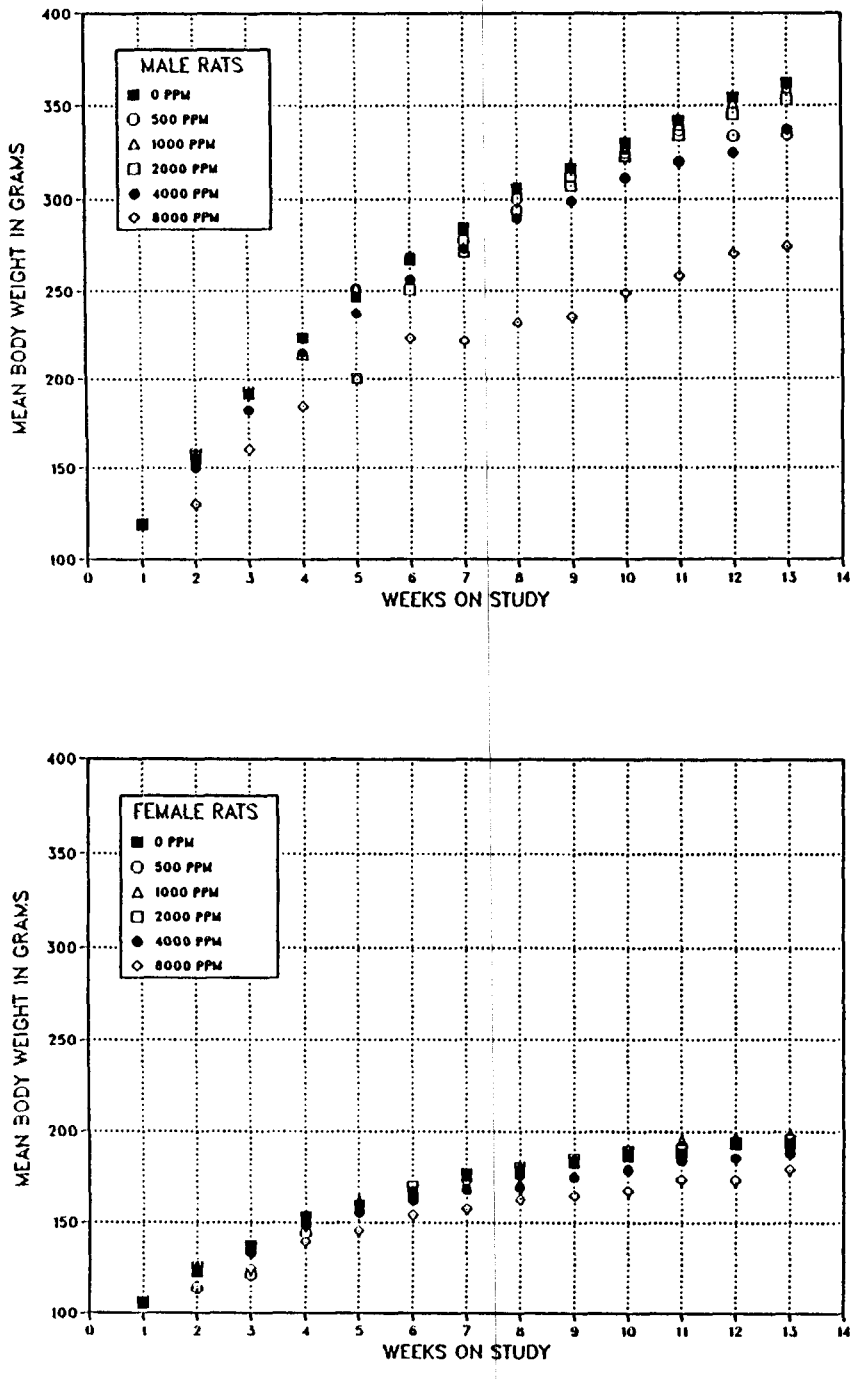

FIGURE 1 Body Weights of F344/N Rats Administered Cupric Sulfate in Feed for 13 Weeks

For male and female rats in the 500, 1000, 2000, and 4000 ppm groups, average daily feed consumption was similar to that of the controls. However, feed consumption by male and female rats in the 8000 ppm (high-dose) groups was slightly below that of the controls. Despite the slight decreases in feed consumption in high-dose rats, the average daily compound consumption increased proportionally with increasing concentrations of cupric sulfate in the feed (Table 6).

Significant changes in absolute organ weights were limited to males and females in the high-dose groups and included decreases in absolute brain, heart, kidney, liver, lung, and thymus weights in males and absolute kidney weights in females. Generally, relative organ weights for treated groups were similar to those of the controls or increased with decreasing mean body weights in the two highest dose groups (4000 and 8000 ppm). Complete organ weight data for rats are presented in Appendix B.

Hematology and clinical chemistry evaluations were conducted on Days 5 and 21 for supplemental rats and at the end of the 13-week study (Day 92) for base-study rats. Urinalysis was conducted on Day 19 for supplemental rats and on Day 90 for base-study rats.

Significant changes in hematology parameters were noted in male and female rats at all time points (Table 7; Appendix C, Table C1). At Day 5, significant increases in hematocrit (HCT) and hemoglobin (HGB) concentrations were noted in high-dose male and female rats. By Day 21, however, these parameters were significantly decreased for male rats in the two highest dose groups (4000 and 8000 ppm) and female rats in the three highest dose groups (2000, 4000, and 8000 ppm). At Day 92, HCT and HGB concentrations were significantly decreased in males in the two highest dose groups and in females in the highest dose group. At Day 5, significant increases in erythrocyte (RBC) counts were noted in males in the two highest dose groups and in high-dose females; on Day 92, the only significant increase in RBC count was noted in high-dose males. In males and females in the two highest dose groups, significant decreases in reticulocyte counts were noted on Day 5. However, by Day 21, reticulocyte counts in males and females in these same dose groups were significantly greater than those of the controls; at Day 92, this parameter was significantly increased only in high-dose males. The only significant change noted in nucleated erythrocytes was a marginal decrease in high-dose males at Day 5.

TABLE 7 Selected Hematology Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	Concentration (ppm)					
	0	500	1000	2000	4000	8000
MALE						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 5	39.2 ± 0.5[2]	39.3 ± 0.4[2]	39.0 ± 0.3	39.0 ± 0.2	39.5 ± 0.3	42.8 ± 0.7**
Day 21	46.0 ± 0.5	46.5 ± 0.4[2]	46.0 ± 0.4	44.2 ± 0.8[2]	36.4 ± 1.6**	34.8 ± 1.3**
Day 92	47.9 ± 0.4	46.5 ± 0.6	47.5 ± 0.2	48.1 ± 0.4	44.8 ± 1.2**	40.2 ± 1.7**
Hemoglobin (g/dL)						
Day 5	13.1 ± 0.2[2]	13.1 ± 0.1[2]	13.0 ± 0.1	12.9 ± 0.1	13.1 ± 0.1	14.1 ± 0.2**
Day 21	14.2 ± 0.1	14.3 ± 0.1[2]	14.3 ± 0.1	13.8 ± 0.2[2]	11.8 ± 0.5**	11.1 ± 0.3**
Day 92	14.3 ± 0.1	13.8 ± 0.2	14.0 ± 0.1	14.3 ± 0.1	13.4 ± 0.3**	12.2 ± 0.4**
Erythrocytes (10^6/μL)						
Day 5	6.60 ± 0.11[2]	6.62 ± 0.06[2]	6.62 ± 0.08	6.58 ± 0.04	6.81 ± 0.06*	7.41 ± 0.12**
Day 21	7.89 ± 0.08	8.00 ± 0.07[2]	8.00 ± 0.09	8.12 ± 0.12[2]	7.82 ± 0.12	7.67 ± 0.20
Day 92	8.88 ± 0.07	8.65 ± 0.11	8.84 ± 0.05	9.06 ± 0.06	9.23 ± 0.15	9.55 ± 0.10**
Reticulocytes (10^6/μL)						
Day 5	0.45 ± 0.03[2]	0.44 ± 0.02[2]	0.42 ± 0.02	0.41 ± 0.02	0.33 ± 0.02**	0.22 ± 0.02**
Day 21	0.20 ± 0.02	0.20 ± 0.02[2]	0.20 ± 0.02	0.24 ± 0.02[2]	0.38 ± 0.02**	0.41 ± 0.03**
Day 92	0.15 ± 0.01	0.20 ± 0.02	0.17 ± 0.01	0.15 ± 0.02	0.20 ± 0.02	0.27 ± 0.01**
Mean cell volume (fL)						
Day 5	59.4 ± 0.6[2]	59.4 ± 0.4[2]	58.8 ± 0.6	59.2 ± 0.3	58.2 ± 0.3*	57.8 ± 0.2**
Day 21	58.3 ± 0.5	58.1 ± 0.4[2]	57.7 ± 0.3	54.6 ± 0.8**[2]	46.4 ± 1.3**	45.2 ± 0.7**
Day 92	54.0 ± 0.2	53.7 ± 0.3	53.8 ± 0.3	53.1 ± 0.2*	48.8 ± 1.6**	42.1 ± 2.0**
Mean cell hemoglobin (pg)						
Day 5	19.9 ± 0.1[2]	19.8 ± 0.1[2]	19.6 ± 0.1	19.6 ± 0.1	19.2 ± 0.1**	19.1 ± 0.1**
Day 21	18.0 ± 0.1	17.9 ± 0.1[2]	17.9 ± 0.1	17.0 ± 0.2**[2]	15.0 ± 0.4**	14.5 ± 0.1**
Day 92	16.0 ± 0.1	15.9 ± 0.1	15.9 ± 0.1	15.7 ± 0.1**	14.5 ± 0.4**	12.8 ± 0.5**
Platelets (10^3/μL)						
Day 5	836.2 ± 10.6[2]	863.9 ± 10.5[2]	884.8 ± 17.2*	934.4 ± 11.1**	924.0 ± 53.2**	1009.0 ± 22.6**
Day 21	735.0 ± 14.9	775.0 ± 18.3[2]	776.6 ± 28.0	856.3 ± 23.3**[2]	1032.6 ± 44.8**	1039.5 ± 27.9**
Day 92	631.6 ± 17.9	712.4 ± 39.8	649.6 ± 14.8	616.4 ± 11.1	765.6 ± 24.1**	958.4 ± 62.7**
Leukocytes (10^3/μL)						
Day 5	5.93 ± 0.50[2]	5.47 ± 0.49[2]	6.17 ± 0.62	6.39 ± 0.65	7.32 ± 0.57	7.73 ± 0.51*
Day 21	6.15 ± 0.46	6.41 ± 0.44[2]	6.89 ± 0.60	7.52 ± 0.54*[2]	7.70 ± 0.46*	7.67 ± 0.72
Day 92	8.39 ± 0.42	8.04 ± 0.40	8.41 ± 0.45	7.87 ± 0.74	9.16 ± 0.39	10.27 ± 0.41*
FEMALE						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 5	43.3 ± 0.5	42.8 ± 0.5[2]	42.4 ± 0.6	42.2 ± 0.4	43.9 ± 0.4[2]	44.9 ± 0.3*
Day 21	49.3 ± 0.4[2]	49.5 ± 0.4[3]	48.4 ± 0.3	47.4 ± 0.4**[2]	42.9 ± 1.1**	41.2 ± 1.4**
Day 92	48.6 ± 0.6	46.7 ± 0.4*	47.9 ± 0.5[2]	47.9 ± 0.4	47.7 ± 0.7	43.9 ± 1.0**
Hemoglobin (g/dL)						
Day 5	13.9 ± 0.2	13.9 ± 0.1[2]	13.7 ± 0.2	13.6 ± 0.1	14.2 ± 0.1[2]	14.6 ± 0.1**
Day 21	15.2 ± 0.1[2]	15.3 ± 0.1[3]	14.9 ± 0.1	14.5 ± 0.1**[2]	13.4 ± 0.3**	13.1 ± 0.4**
Day 92	14.5 ± 0.2	14.0 ± 0.2	14.4 ± 0.1[2]	14.2 ± 0.1	14.2 ± 0.2	13.2 ± 0.3**
Erythrocytes (10^6/μL)						
Day 5	7.25 ± 0.12	7.11 ± 0.07[2]	7.11 ± 0.12	7.07 ± 0.07	7.41 ± 0.09[2]	7.63 ± 0.04*
Day 21	8.27 ± 0.09[2]	8.28 ± 0.07[3]	8.14 ± 0.07	8.17 ± 0.09[2]	8.27 ± 0.11	8.26 ± 0.16
Day 92	8.48 ± 0.11	8.15 ± 0.07	8.41 ± 0.09[2]	8.48 ± 0.05	8.44 ± 0.12	8.51 ± 0.13

TABLE 7 Selected Hematology Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate (continued)

	Concentration (ppm)					
	0	500	1000	2000	4000	8000
FEMALE (continued)						
Reticulocytes (10^6/μL)						
Day 5	0.34 ± 0.02	0.29 ± 0.02[2]	0.33 ± 0.03	0.29 ± 0.02	0.26 ± 0.03*[2]	0.19 ± 0.02**
Day 21	0.12 ± 0.01[2]	0.10 ± 0.02[3]	0.12 ± 0.01	0.10 ± 0.02[2]	0.19 ± 0.02*	0.21 ± 0.03**
Day 92	0.13 ± 0.01	0.14 ± 0.01	0.12 ± 0.01[2]	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.01
Mean cell volume (fL)						
Day 5	59.9 ± 0.4	60.1 ± 0.4[2]	59.8 ± 0.4	59.8 ± 0.3	59.2 ± 0.4[2]	58.8 ± 0.3*
Day 21	59.7 ± 0.4[2]	60.0 ± 0.3[3]	59.7 ± 0.3	58.1 ± 0.5*[2]	52.0 ± 1.2**	49.7 ± 1.0**
Day 92	57.2 ± 0.3	57.2 ± 0.2	57.0 ± 0.3[2]	56.3 ± 0.2*	56.5 ± 0.2*	51.5 ± 1.4**
Mean cell hemoglobin (pg)						
Day 5	19.2 ± 0.2	19.5 ± 0.1[2]	19.3 ± 0.1	19.3 ± 0.1	19.1 ± 0.1[2]	19.1 ± 0.1
Day 21	18.4 ± 0.1[2]	18.5 ± 0.1[3]	18.3 ± 0.1	17.8 ± 0.1*[2]	16.2 ± 0.3**	15.8 ± 0.3**
Day 92	17.0 ± 0.1	17.1 ± 0.1	17.2 ± 0.1[2]	16.8 ± 0.1*	16.9 ± 0.1	15.5 ± 0.4**
Platelets (10^3/μL)						
Day 5	823.6 ± 9.7	816.1 ± 18.9[2]	858.6 ± 14.8	879.6 ± 16.7*	875.6 ± 24.9*[2]	928.8 ± 21.1**
Day 21	696.7 ± 7.6[2]	656.4 ± 15.0[3]	682.3 ± 13.1	749.7 ± 10.8*[2]	852.0 ± 22.5**	915.7 ± 13.4**
Day 92	700.5 ± 23.5	671.2 ± 20.6	632.3 ± 18.9[2]	655.6 ± 18.2	715.7 ± 16.9	733.9 ± 25.0
Leukocytes (10^3/μL)						
Day 5	5.49 ± 0.68	5.34 ± 0.66[2]	5.38 ± 0.43	5.31 ± 0.45	6.68 ± 0.60[2]	6.34 ± 0.61
Day 21	6.81 ± 0.45[2]	7.10 ± 0.58[3]	6.63 ± 0.55	7.66 ± 0.38[2]	7.19 ± 0.39	7.55 ± 0.55
Day 92	7.78 ± 0.60	7.34 ± 0.17	6.19 ± 0.40[2]	7.14 ± 0.25	8.27 ± 0.25	9.88 ± 0.50*

[1] Data are given as mean ± standard error.
[2] n=9.
[3] n=8.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

On Day 5, mean cell volume (MCV) values were significantly decreased in males in the two highest dose groups and in females in the highest dose group; at this time point, mean cell hemoglobin (MCH) values were also significantly decreased for males in the two highest dose groups. At Days 21 and 92, decreases in MCV and MCH were noted in males and females in the three highest dose groups, and all of these decreases were significant with the exception of the Day 92 MCH value for females receiving 4000 ppm. The only significant changes in mean cell hemoglobin concentrations were increases noted on Day 21 in high-dose females and in males in the two highest dose groups.

At Days 5 and 21, significant increases in platelet counts were noted in males and females in the three highest dose groups; the Day 5 platelet count for males in the 1000 ppm group was also significantly increased compared to that of the control. At Day 92, increases in platelet counts were noted for males and females in the two highest dose groups, but these increases were significant only for males.

Leukocyte counts were increased at all time points in male and female rats in the two highest dose groups, with significant increases occurring at Day 5 in high-dose males, at Day 21 in males in the 4000 ppm dose group, and at Day 92 in high-dose males and females; leukocyte count was also significantly increased at Day 21 in males receiving 2000 ppm cupric sulfate. Significant increases in lymphocytes were noted at Day 5 in high-dose males, at Day 21 in males receiving 2000 or 4000 ppm cupric sulfate, and at Day 92 in high-dose females. The only other significant change noted in hematology parameters was an increase in segmented neutrophils at Day 92 in high-dose male rats.

Significant changes in serum chemistry parameters occurred in male and female rats at all time points, and, with a few exceptions, these changes were limited to the two highest dose groups (Table 8; Appendix C, Table C2). Alanine aminotransferase activities were significantly increased at all time points in male and female rats in the two highest dose groups; this parameter was also significantly increased at Day 92 in males receiving 1000 or 2000 ppm cupric sulfate. At Days 5 and 21, decreases in alkaline phosphatase (AP) activities were noted in males and females in the two highest dose groups; except for the Day 21 AP activity in males in the 4000 ppm group, all of these decreases were significant relative to the control values. Significant changes in sorbitol dehydrogenase (SDH) were limited to the Day 21 and 92 time points. At both of these time points, SDH activities were significantly elevated in males in the two highest dose groups and in high-dose females; significant increases in SDH activities were also noted at Day 92 in males in the 2000 ppm group and females in the 4000 ppm group. When compared to control values, 5′-nucleotidase was significantly decreased in high-dose females at Days 5 and 21 and in high-dose males at Day 5; at Day 92, however, this parameter was significantly increased in males receiving 4000 or 8000 ppm cupric sulfate.

At Day 5, slight increases in bile salts were noted in males in the three highest dose groups; however, female bile salt values were decreased for all treated groups at this time point, with significant decreases in the 1000 and 8000 ppm groups. By Day 21, no significant changes in this parameter were noted in females, but significant increases were noted in males in the two highest dose groups. At Day 92, significant increases in bile salts were noted in high-dose males and in females receiving 2000 or 4000 ppm cupric sulfate.

TABLE 8 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	Concentration (ppm)					
	0	500	1000	2000	4000	8000
MALE						
n	10	10	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 5	42 ± 1	42 ± 1	41 ± 2	42 ± 1	53 ± 3**	62 ± 5**[2]
Day 21	44 ± 2	42 ± 2	50 ± 3	48 ± 2	90 ± 7**	379 ± 45**
Day 92	51 ± 2	69 ± 8	78 ± 11	108 ± 15**	494 ± 60**	563 ± 49**
Alkaline phosphatase (IU/L)						
Day 5	1596 ± 22	1592 ± 27	1575 ± 26	1531 ± 31	1406 ± 36**	1007 ± 27**[2]
Day 21	1131 ± 17	1149 ± 44	1146 ± 22	1107 ± 30	1056 ± 35	883 ± 37**
Day 92	503 ± 7	549 ± 17	541 ± 14	498 ± 23	513 ± 15	525 ± 25
5′-nucleotidase (IU/L)						
Day 5	36.5 ± 0.9	38.9 ± 0.9	36.7 ± 0.5	36.2 ± 0.5	34.7 ± 0.9	27.4 ± 1.0**[2]
Day 21	31.8 ± 0.8	33.5 ± 1.0	31.3 ± 0.9	30.2 ± 0.7	32.0 ± 0.8	34.2 ± 1.2
Day 92	33.6 ± 0.7	33.8 ± 0.7	33.3 ± 0.9	32.5 ± 0.5	39.3 ± 1.0**	36.5 ± 0.8**
Sorbitol dehydrogenase (IU/L)						
Day 5	18 ± 1	20 ± 2	19 ± 1	16 ± 1	18 ± 1	19 ± 1[2]
Day 21	22 ± 1	26 ± 4	22 ± 1	22 ± 1	44 ± 6**	162 ± 25**
Day 92	22 ± 1	27 ± 3	32 ± 6	42 ± 6**	197 ± 20**	282 ± 29**
Bile salts (μmol/L)						
Day 5	15.83 ± 2.20	18.32 ± 3.43	12.16 ± 1.25	19.18 ± 3.66	19.36 ± 2.80	16.81 ± 3.23[2]
Day 21	15.62 ± 2.79	11.18 ± 1.97	13.13 ± 2.89	13.20 ± 1.21	30.62 ± 2.81**	36.91 ± 4.73**
Day 92	14.07 ± 2.09	11.23 ± 1.43	11.72 ± 2.18	14.78 ± 2.52	21.67 ± 2.85	25.02 ± 2.84*
Total protein (g/dL)						
Day 5	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.3 ± 0.0**	5.0 ± 0.1**[2]
Day 21	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	5.6 ± 0.1**	5.4 ± 0.1**
Day 92	6.6 ± 0.0	6.6 ± 0.0	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.1 ± 0.1**
Albumin (g/dL)						
Day 5	4.2 ± 0.1	4.2 ± 0.0	4.1 ± 0.0	4.0 ± 0.0*	3.9 ± 0.0**	3.8 ± 0.1**[2]
Day 21	4.3 ± 0.0	4.4 ± 0.0	4.4 ± 0.1	4.2 ± 0.0	4.0 ± 0.0**	3.9 ± 0.0**
Day 92	4.6 ± 0.0	4.6 ± 0.0	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.3 ± 0.0**
Urea nitrogen (mg/dL)						
Day 5	21.1 ± 0.4	21.9 ± 0.6	21.0 ± 0.6	22.4 ± 0.5	23.5 ± 0.3**	23.6 ± 0.7**[2]
Day 21	20.0 ± 0.3	20.1 ± 0.5	21.0 ± 0.5	21.7 ± 0.2**	20.8 ± 0.5*	21.4 ± 0.4*
Day 92	21.6 ± 0.3	21.8 ± 0.4	20.6 ± 0.4	20.7 ± 0.7	22.1 ± 0.5	23.5 ± 0.6*
FEMALE						
n	10	10	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 5	39 ± 2	39 ± 2	39 ± 1	38 ± 2	46 ± 2*	48 ± 3*
Day 21	37 ± 2	35 ± 1	34 ± 1	35 ± 1	50 ± 4*	119 ± 17**
Day 92	44 ± 2	38 ± 2	38 ± 1[2]	37 ± 2	84 ± 10*	214 ± 20**
Alkaline phosphatase (IU/L)						
Day 5	1226 ± 40	1316 ± 45	1317 ± 30	1238 ± 39	1040 ± 35*	844 ± 27**
Day 21	893 ± 27	903 ± 18	861 ± 16	858 ± 29	775 ± 30**	624 ± 27**
Day 92	408 ± 35	439 ± 17	516 ± 11[2]	443 ± 18	412 ± 13	385 ± 8
5′-nucleotidase (IU/L)						
Day 5	39.0 ± 1.5	40.6 ± 0.9	41.1 ± 1.5	39.8 ± 1.4	36.7 ± 1.2	31.1 ± 1.3**
Day 21	35.4 ± 1.2	40.1 ± 1.1[2]	38.5 ± 1.2	38.4 ± 1.3	36.3 ± 1.3	28.4 ± 1.2*
Day 92	34.5 ± 2.0	38.7 ± 0.9	40.0 ± 0.9	36.7 ± 0.8	36.8 ± 0.8	31.8 ± 1.0

TABLE 8 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate (continued)

	Concentration (ppm)					
	0	500	1000	2000	4000	8000
FEMALE (continued)						
Sorbitol dehydrogenase (IU/L)						
Day 5	24 ± 2	24 ± 2	20 ± 1	20 ± 1	21 ± 2	21 ± 2
Day 21	22 ± 1	24 ± 1	20 ± 1	20 ± 1	23 ± 1	39 ± 4**
Day 92	16 ± 1	15 ± 1	19 ± 2[2]	16 ± 1	34 ± 5**	96 ± 12**
Bile salts (µmol/L)						
Day 5	19.28 ± 1.96	16.85 ± 1.44	13.12 ± 0.85*	15.33 ± 1.12	15.24 ± 1.26	13.36 ± 1.11*
Day 21	17.45 ± 2.08	12.74 ± 2.42	13.15 ± 2.61	20.78 ± 3.37	17.61 ± 2.66	20.79 ± 2.36
Day 92	13.41 ± 2.06	12.43 ± 2.36	13.03 ± 1.12[2]	23.57 ± 1.67**	18.87 ± 2.67*	15.99 ± 2.01
Total protein (g/dL)						
Day 5	5.7 ± 0.1	5.6 ± 0.0	5.6 ± 0.1	5.5 ± 0.1*	5.3 ± 0.1**	5.2 ± 0.1**
Day 21	5.9 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.6 ± 0.0*	5.5 ± 0.1**	5.2 ± 0.1**
Day 92	6.6 ± 0.3	6.7 ± 0.1	6.8 ± 0.1[2]	6.9 ± 0.1	6.3 ± 0.1**	5.7 ± 0.1**
Albumin (g/dL)						
Day 5	4.3 ± 0.1	4.2 ± 0.0	4.2 ± 0.1	4.1 ± 0.0*	4.0 ± 0.0**	4.0 ± 0.1**
Day 21	4.4 ± 0.1	4.5 ± 0.1	4.4 ± 0.0	4.2 ± 0.0*	4.2 ± 0.1**	3.9 ± 0.0**
Day 92	4.8 ± 0.2	4.9 ± 0.1	4.9 ± 0.1[2]	5.1 ± 0.1	4.6 ± 0.1**	4.0 ± 0.1**
Urea nitrogen (mg/dL)						
Day 5	21.9 ± 0.6	22.3 ± 0.6	23.1 ± 0.7	22.9 ± 0.9	25.8 ± 0.5**	24.8 ± 0.8**
Day 21	22.1 ± 0.4	20.7 ± 0.5	21.3 ± 0.6	22.1 ± 0.6	24.2 ± 0.8	24.9 ± 0.7*
Day 92	17.1 ± 0.4	18.0 ± 0.4	19.7 ± 0.3**[2]	18.0 ± 0.5*	20.6 ± 0.5**	22.9 ± 0.5**

[1] Data are given as mean ± standard error.
[2] n=9.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

At all time points, total protein was significantly decreased in high-dose males and in females in the two highest dose groups; at Days 5 and 21, total protein was also significantly decreased in males receiving 4000 ppm cupric sulfate and in females receiving 2000 ppm cupric sulfate. At Days 5 and 21, decreases in albumin concentrations were noted in males and females in the three highest dose groups, and all of these decreases were significant, excluding the Day 21 albumin concentration for males receiving 2000 ppm cupric sulfate. At Day 92, this parameter was significantly decreased in high-dose males and in females in the two highest dose groups.

Urea nitrogen (UN) was significantly increased for males and females in the two highest dose groups at Day 5, and by Day 21, this parameter was significantly increased in males in the three highest dose groups and females in the highest dose group. At Day 92, UN was significantly elevated in high-dose males and females as well as in females receiving

1000, 2000, or 4000 ppm cupric sulfate. The only significant change in creatinine was an increase noted in high-dose females on Day 92.

Significant changes in urinalysis parameters were noted in supplemental-study rats at Day 19 and in base-study rats at Day 90 (Appendix C, Table C3). Significant increases in urinary aspartate aminotransferase (AST) activities, expressed in IU/L or IU/mg creatinine, occurred at Days 19 and 90 in male and female rats in the highest dose groups. Generally, increases in this parameter also occurred at both time points in male and female rats in the 4000 ppm groups, and most of these increases were significant. A few significant increases in AST activities occurred in animals in the lower dose groups (500 to 2000 ppm). Significant increases in *N*-acetyl-β-D-glucosaminidase activities, expressed in IU/L or IU/mg creatinine, were noted in high-dose male and female rats on Day 90; at this time point, increases also occurred in males and females in the 4000 ppm groups, and the increases for the parameter expressed in IU/mg creatinine were significant. Glucose output (mg/mg creatinine) was significantly increased at Day 19 in males in the 2000 ppm group, and at Day 90, this parameter was significantly elevated in males in the two highest dose groups. A significant decrease in protein output (mg/mg creatinine) was noted in high-dose males at Day 19; however, at the Day 90 evaluation in base-study rats, this parameter was significantly increased relative to the control in males in the two highest dose groups. No significant changes in glucose or protein output were noted in females at either time point.

At the end of the 13-week feed study, samples of liver, kidney, testis, and plasma were collected from all base-study male rats and analyzed for copper, zinc, magnesium, and calcium. The results of these analyses indicated that copper accumulated in the liver and kidney in a dose-related manner and was accompanied by an accumulation of zinc in these tissues. Copper concentrations were significantly increased in the kidney and liver of rats in all treated groups (Table 9). Copper levels were also significantly elevated in the plasma and testis of rats in the three highest dose groups (2000, 4000, and 8000 ppm). Significant increases in zinc concentration in the kidney and liver were noted in animals in the three highest dose groups, and concentrations of calcium in plasma were significantly decreased in the 4000 and 8000 ppm groups. Significant increases in magnesium were noted in the kidney and plasma of rats receiving 2000 ppm cupric sulfate as well as in the plasma of rats receiving 8000 ppm cupric sulfate.

TABLE 9 Tissue Metal Concentrations in Male F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	Concentration (ppm)					
	0	500	1000	2000	4000	8000
n	10	10	10	10	10	10
Copper						
Kidney	0.62 ± 0.29	4.81 ± 0.51**	3.45 ± 0.42**	7.65 ± 0.53**	52.89 ± 8.35**	181.03 ± 17.55**
Liver	0.24 ± 0.06	1.83 ± 0.42**	6.11 ± 1.14**	17.90 ± 3.00**	127.31 ± 23.24**	372.12 ± 47.70**
Plasma	0.09 ± 0.09	0.09 ± 0.06	0.02 ± 0.02	0.18 ± 0.06*	0.29 ± 0.06**	0.85 ± 0.09**
Testis	0.12 ± 0.05	0.11 ± 0.05	0.26 ± 0.16	1.25 ± 0.12**	1.24 ± 0.08**	1.21 ± 0.04**
Calcium						
Kidney	15.48 ± 3.56	14.24 ± 2.07	12.23 ± 2.65	10.59 ± 1.08	12.25 ± 1.19	9.78 ± 1.85
Liver	3.08 ± 0.72	5.42 ± 1.69	3.33 ± 1.36[2]	2.64 ± 0.90	3.93 ± 1.64	5.70 ± 1.52
Plasma	3.15 ± 1.20	2.31 ± 0.77	2.49 ± 1.34	0.74 ± 0.53	0.62 ± 0.49*	0.17 ± 0.17**
Testis	1.61 ± 0.39[2]	0.29 ± 0.29[2]	1.00 ± 0.67[2]	1.69 ± 0.89	3.25 ± 1.38	0.12 ± 0.12
Magnesium						
Kidney	3.95 ± 1.71	6.80 ± 2.35	7.56 ± 3.76	15.16 ± 2.69*	9.28 ± 1.58	3.27 ± 2.12
Liver	17.94 ± 4.40	16.95 ± 6.08	14.83 ± 4.54	10.04 ± 3.15	20.48 ± 4.62	13.23 ± 2.52
Plasma	0.00 ± 0.00	0.09 ± 0.09	0.11 ± 0.11	0.16 ± 0.08*	0.05 ± 0.05	0.86 ± 0.47**
Testis	23.29 ± 3.57	20.55 ± 2.52	19.73 ± 3.39	21.54 ± 4.71	30.91 ± 6.01	21.53 ± 3.16
Zinc						
Kidney	3.22 ± 0.42	4.34 ± 0.54	3.97 ± 0.69	5.35 ± 0.31**	5.48 ± 0.28**	6.38 ± 0.55**
Liver	0.31 ± 0.21[2]	0.63 ± 0.32	0.07 ± 0.07	3.68 ± 0.58**	2.71 ± 0.67**	4.43 ± 0.74**
Plasma	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03	0.27 ± 0.25	0.00 ± 0.00
Testis	6.50 ± 0.77	5.44 ± 0.48	4.67 ± 0.73	6.02 ± 1.06	6.11 ± 1.57	5.63 ± 0.64

[1] Data are given as mean ± standard error. Tissue metal concentrations are given in parts per million.
[2] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's test or Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

In the 13-week feed study, treatment-related gross lesions were present in the forestomach of male and female rats receiving cupric sulfate at concentrations of 2000 ppm or greater. The limiting ridge that forms the junction of the forestomach squamous mucosa with the glandular gastric mucosa appeared enlarged in all rats in the 4000 and 8000 ppm groups and in seven females and nine males in the 2000 ppm groups.

Histopathologic findings that corresponded to the gross lesions consisted of minimal to moderate hyperplasia of the squamous mucosa at the site of the limiting ridge. This lesion was characterized by a thickening and increased folding of the squamous mucosa; hyperkeratosis was also a component of the squamous cell hyperplasia (Plate 1). The

increased incidence and severity of this lesion were dose related (Table 10). When this lesion was more severe (moderate grade), there was often an increase in the number of inflammatory cells and/or edema in the lamina propria of the limiting ridge. There was no evidence of erosion/ulceration, and no lesions were present in other areas of the squamous mucosa.

Other histopathologic findings related to treatment were present in the liver and kidney of male and female rats (Table 10). There was a dose-related increase in the incidence and severity of chronic-active inflammation in the liver of male and female rats. This lesion was present in most rats in the 4000 and 8000 ppm groups and in one male in the 2000 ppm group and was characterized by multiple foci of a mixture of mononuclear inflammatory cells, primarily macrophages (Plates 2 and 3). These foci of inflammation occurred primarily in the periportal portion of the hepatic lobules. Necrosis of one to several hepatocytes was often observed adjacent to or within the foci of inflammation.

Chemical-related cytoplasmic alteration (an increase in size and number of cytoplasmic protein droplets) was present in the kidneys of male and female rats at doses of 2000 ppm and greater (Table 10). This lesion was morphologically similar in both sexes but was less severe in females. A few droplets were also present in the tubule lumina of female rats. In treated male rats, the protein droplets were much larger and more numerous than those in control males or in treated females, and many large droplets were present in the tubule lumina. These droplets stained strongly positive for protein but were negative by iron, PAS, and acid-fast (lipofuscin) staining methods. Results of α-2-microglobulin staining of kidney sections from male and female control and high-dose rats were inconclusive. While the kidneys of male rats stained positive for α-2-microglobulin, there were no clear qualitative differences in staining between treated and control rats. Also present in the kidneys of rats in the high-dose groups was minimal nuclear enlargement (karyomegaly) in renal tubule cells. Degeneration of the renal tubule epithelium was present in three females in the 8000 ppm group.

TABLE 10 Incidence and Severity of Selected Histopathologic Lesions in F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	Concentration (ppm)					
	0	500	1000	2000	4000	8000
MALE						
n	10	10	10	10	10	10
Liver						
Inflammation, chronic active	0	–[2]	0	1 (1.0)	10 (1.0)	10 (1.9)
Forestomach						
Hyperplasia	0	0[3]	0	10 (1.6)	10 (2.8)	10 (2.8)
Kidney						
Cytoplasmic alteration	0	0[4]	0	3 (1.0)	10 (2.0)	10 (2.5)
Karyomegaly, renal tubule epithelium	0	0[4]	0	0	0	10 (1.0)
FEMALE						
n	10	10	10	10	10	10
Liver						
Inflammation, chronic active	0	0[3]	0[5]	0	6 (1.2)	10 (1.9)
Forestomach						
Hyperplasia	0	–	0	7 (1.3)	10 (2.5)	10 (2.5)
Kidney						
Cytoplasmic alteration	0	0	1 (1.0)	9 (1.0)	10 (1.0)	10 (1.0)
Pigmentation	0	0	0	0	0	2 (1.0)
Karyomegaly, renal tubule epithelium	0	0	0	0	0	10 (1.1)
Renal tubule degeneration	0	0	0	0	0	3 (1.3)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions; 1=minimal, 2=mild, 3=moderate, and 4=marked.
[2] *Not applicable; tissue not examined for animals in this dose group.*
[3] n=1.
[4] n=9.
[5] n=2.

Livers and kidneys of male and female rats in all dose groups were stained for the presence of copper. The results are shown in Table 11. Positive staining in liver sections was limited to the 4000 and 8000 ppm groups. At 8000 ppm, staining in the liver had a clear periportal to midzonal distribution and consisted of a few to numerous (10 to 20) red granules of 1 to 2 mm size in the cytoplasm of hepatocytes (scored as +3). In addition, there was minimal staining of the cytoplasm in some of the cells in the inflammatory foci (Plate 4). At 4000 ppm, staining of hepatocytes was limited to the periportal area, and there was a marked reduction in the number of cells stained and the number of granules per cell (scored as +2).

TABLE 11 Results of Copper Staining of Liver and Kidney Sections from F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	Concentration (ppm)					
	0	500	1000	2000	4000	8000
MALE						
n	2	2	2	2	2	2
Liver	–	–	–	–	+2	+3
Kidney	–	–	–	–	+2	+3
FEMALE						
n	2	2	2	2	2	2
Liver	–	–	–	–	+2	+3
Kidney	–	–	–	–	+2	+3

[1] Scoring for degree of copper staining is described in text.

Kidney sections also stained positive for copper only in the two highest dose groups (+3 for 8000 ppm and +2 for 4000 ppm). Staining consisted of red granules in the cytoplasm of the renal tubule epithelium and a diffuse or stippled red staining of the protein droplets in the cytoplasm and the tubule lumen. However, many of these protein droplets, especially in the 4000 ppm groups, did not stain positive for copper. Positive staining of kidney tubule cells was limited to the cortex; there was no staining in the medullary rays, outer medulla, or inner medulla. In addition to kidney and liver, sections of heart and spleen were present on all copper-stained slides; however, no positive staining was present in either of these tissues in any dose group.

Sections of spleen from four rats per dose group were evaluated for the presence of iron (hemosiderin) with Perl's iron stain. In the 8000 ppm groups of males and females, there were only a few iron-positive granules in the cytoplasm of macrophages in the red pulp. The reduction in iron-positive material in spleens of rats from the 2000 and 4000 ppm groups was much less prominent than in the 8000 ppm group, but a minimal decrease was evident when compared to the normal amount of iron-positive granules in the spleen of control animals.

Transmission electron microscopy (TEM) of the livers of male and female rats showed that within the cytoplasm of hepatocytes in the periportal area, there were degenerative changes consisting of increased numbers of secondary lysosomes, many of which were enlarged and contained clear, non-staining crystalline structures and electron-dense material (Plates 5 and 6). Examination of the kidneys of rats using TEM revealed similar ultrastructural alterations. Kidneys from rats of both sexes had mild (females) to marked (males) increases in the number and size of electron-dense protein droplets in the cytoplasm of the proximal convoluted tubule epithelium. In addition to changes in size and number, many of the droplets in the kidneys of male rats had irregular crystalline shapes; however, the large crystalline structures seen in the kidneys of males were not present in females (Plates 7-10). Other ultrastructural changes in kidney epithelium were minimal; there was minimal degeneration evidenced by margination of nuclear chromatin in a few renal tubule cells.

A summary of nonneoplastic lesions in rats in the 13-week feed study is presented in Appendix A, Tables A1 and A2.

Sperm morphology and vaginal cytology evaluations were performed on base-study rats in the 0, 500, 2000, and 4000 ppm groups. There were no significant findings in males or females (Appendix D).

2-Week Feed Study in B6C3F$_1$ Mice

No mice in any of the dose groups died or were killed before the end of the 2-week feed study (Table 12). Final mean body weights for male and female mice receiving 8000 or 16,000 ppm cupric sulfate and for female mice receiving 2000 ppm cupric sulfate were lower than those of the control groups but remained within 6% of control values. Mean body weight gains were also lower than those of the controls for male mice in the 8,000 and 16,000 ppm groups (Table 12). The slightly lower final mean body weights for the 8000 and 16,000 ppm groups could be related to decreased feed consumption in animals during Week 1 of the study.

The only clinical sign of toxicity that could be attributed to cupric sulfate consumption was thinness, which was observed in one male and two females receiving 8000 ppm cupric sulfate and in one female receiving 16,000 ppm cupric sulfate.

TABLE 12 Survival, Weight Gain, Feed Consumption, and Compound Consumption of B6C3F$_1$ Mice in the 2-Week Feed Study of Cupric Sulfate

Concentration (ppm)	Survival[1]	Mean Body Weight (grams) Initial	Final	Change[2]	Final Weight Relative to Controls (%)[3]	Feed Consumption (g/day)[4]	Compound Consumption (mg/kg/day)[5]
MALE							
0	5/5	22.5	25.1	2.6		4.4	
1000	5/5	22.0	25.1	3.1	100	4.1	168
2000	5/5	22.2	25.4	3.2	102	4.5	362
4000	5/5	22.0	24.6	2.5	98	4.7	773
8000	5/5	22.2	23.6	1.4	94	3.3	1154
16,000	5/5	22.6	23.6	1.0	94	4.0	2817
FEMALE							
0	5/5	18.2	21.2	3.0		4.1	
1000	5/5	18.2	21.4	3.2	101	4.3	210
2000	5/5	18.5	20.2	1.8	96	4.0	408
4000	5/5	18.3	20.8	2.5	98	4.3	849
8000	5/5	17.6	20.2	2.6	96	3.8	1563
16,000	5/5	17.6	20.0	2.4	95	3.7	3068

[1] Number surviving at 15 days/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) x 100.
[4] (Week 1 mean + Week 2 mean)/2.
[5] (Week 1 mean + Week 2 mean)/2. Weekly mean = (grams of feed consumed/kg body weight per day) x concentration, mg/g.

At the end of Week 1, feed consumption for male mice in all dose groups and female mice in the two highest dose groups (8000 and 16,000 ppm) was lower than that of the control groups. However, during Week 2 of the study, all mice except males in the two highest dose groups and females in the 8000 ppm group consumed amounts of feed similar to the control amounts. At the end of the study, final mean feed consumption amounts for male mice in the 1000, 8000, and 16,000 ppm groups and female mice in the 8000 and 16,000 ppm groups were lower than those of the controls (Table 12). All other final group mean feed consumption amounts were similar to those of the controls. In the 2-week feed study, average daily compound consumption increased proportionally with increasing concentrations of cupric sulfate.

Male and female mice in the high-dose (16,000 ppm) groups had relative brain and liver weights that were significantly greater than those of the control groups. Additionally, relative brain weight was significantly increased in males in the 8000 ppm group, and relative liver weights were significantly increased in males in the 4000 and 8000 ppm groups. All changes in absolute and relative organ weights were considered incidental and not chemical related.

There were no chemical-related gross lesions in male or female mice. Histopathologic findings were limited to the forestomach of both sexes of mice and consisted of minimal hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge of the forestomach at its junction with the glandular gastric mucosa. This lesion was present in all treated mice in the two highest dose groups (8000 and 16,000 ppm) and in three of five males and two of five females in the 4000 ppm groups.

Based on the absence of mortality and the lack of life-threatening changes that could be attributed to cupric sulfate consumption, the concentrations selected for the 13-week feed study in mice were the same as those used in the 2-week study.

13-Week Feed Study in B6C3F$_1$ Mice

No mice in any of the dose groups died or were killed before the end of the 13-week feed study (Table 13). Mice exhibited a dose-related growth depression that occurred earlier and resulted in more severe body weight depression at the higher dose levels. Final mean body weights and body weight gains were slightly lower than those of the control group for males in the 4000 ppm group and were significantly lower for males and females in the 8000 and 16,000 ppm groups (Table 13 and Figure 2). No clinical signs of toxicity considered to be chemical related were observed in male or female mice during the course of the study.

TABLE 13 Survival, Weight Gain, Feed Consumption, and Compound Consumption of B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate

Concentration (ppm)	Survival[1]	Mean Body Weight (grams) Initial	Final	Change[2]	Final Weight Relative to Controls (%)[3]	Feed Consumption (g/day)[4]	Compound Consumption (mg/kg/day)[5]
MALE							
0	10/10	21.6	32.9	11.3		4.2	
1000	10/10	20.9	32.6	11.7	99	4.8	173
2000	10/10	21.0	31.1	10.1	95	5.1	382
4000	10/10	20.9	29.5	8.7	90	4.8	736
8000	10/10	21.1	29.0	7.9	88	5.1	1563
16,000	10/10	21.6	26.8	5.2	82	5.0	3201
FEMALE							
0	10/10	17.7	28.5	10.9		5.0	
1000	10/10	18.6	28.5	9.9	100	5.0	205
2000	10/10	17.2	27.8	10.6	97	5.9	494
4000	10/10	17.1	26.9	9.8	94	6.2	1048
8000	10/10	18.3	25.0	6.7	88	5.9	2106
16,000	10/10	17.8	21.7	3.9	76	5.4	4157

[1] Number surviving at 13 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) x 100.
[4] Average of group mean feed consumption values measured at Weeks 2-13.
[5] Average of group mean compound consumption values measured at Weeks 2-13.

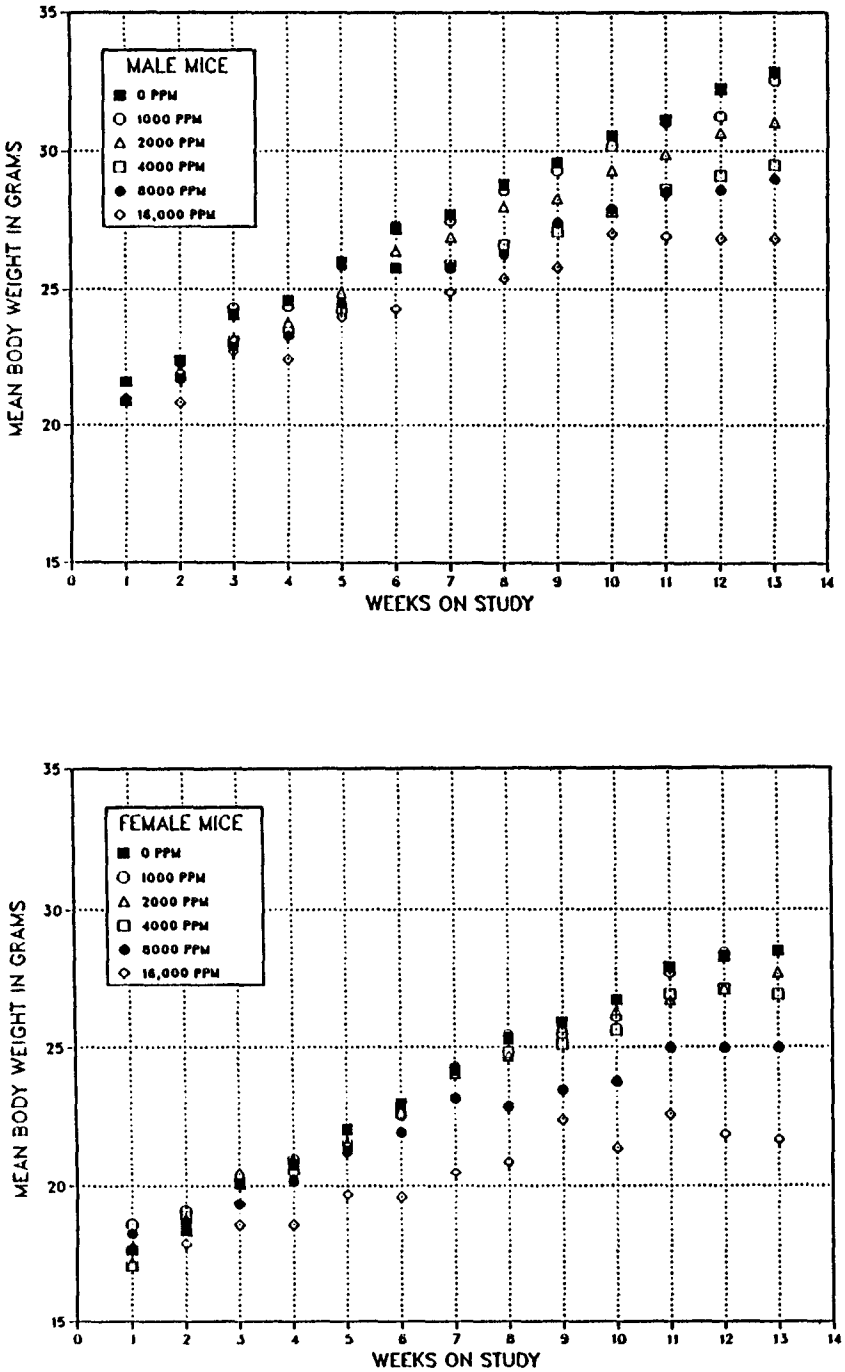

FIGURE 2 **Body Weights of B6C3F$_1$ Mice Administered Cupric Sulfate in Feed for 13 Weeks**

For male and female mice in all dosed groups, average daily feed consumption was similar to or exceeded that of the controls, and average daily compound consumption increased proportionally with increasing concentrations of cupric sulfate in the feed (Table 13).

Significant decreases in absolute organ weights were noted for the heart and kidney of high-dose (16,000 ppm) male mice and the thymus and kidney of high-dose female mice. In addition, dose-related decreases in absolute liver weights were noted for males and females, with significant decreases occurring in both sexes in the 8000 and 16,000 ppm groups and in males in the 4000 ppm group. Generally, relative organ weights for males and females in all dosed groups were greater than those of the controls, and many of these increases were significant for the higher dose groups. These changes in absolute and relative organ weights could be attributed to the lower final mean body weights of mice in the higher dose groups. Complete organ weight data for mice are presented in Appendix B.

In the 13-week feed study in mice, chemical-related gross lesions were limited to the forestomach of seven male and four female mice in the 16,000 ppm groups. This lesion was characterized as a focal white discoloration of the squamous mucosa in the area of the limiting ridge where it forms a junction with the glandular gastric mucosa. Histopathologic findings included minimal to mild squamous cell hyperplasia with hyperkeratosis of the forestomach mucosa at the site of the limiting ridge (Table 14). This lesion was present in male and female mice receiving 4000 ppm cupric sulfate or greater. There was no evidence of inflammation or erosion/ulceration in the forestomach, and there was no increase in hyperplasia or hyperkeratosis in other portions of the forestomach mucosa.

The livers and kidneys of male mice in all dose groups and female mice in the control and 16,000 ppm groups were stained for the presence of copper. Positive staining was limited to the livers of high-dose male and female mice. Staining was extremely minimal (+1) and consisted of only a few positive staining hepatocytes in the entire liver section. Hepatocytes staining positive for copper contained a maximum of approximately 10 red granules per cell. Due to the limited number of cells stained, no periportal distribution of copper was apparent. There was no staining of livers in the lower doses or in controls, and no staining was present in the kidneys of any mice.

TABLE 14 Incidence and Severity of Selected Histopathologic Lesions in B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate[1]

	Concentration (ppm)					
	0	1000	2000	4000	8000	16,000
MALE						
n	10	10	10	10	10	10
Forestomach Hyperplasia	0	0	0	2 (1.0)	6 (1.0)	10 (1.6)
FEMALE						
n	10	10	10	10	10	10
Forestomach Hyperplasia	0	–[2]	0	5 (1.0)	8 (1.0)	10 (1.7)

[1] Incidences are given as the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions; 1=minimal, 2=mild, 3=moderate, and 4=marked.
[2] Not applicable; tissue not examined for animals in this dose group.

Because of the reduction in iron in the spleen of rats, additional sections of spleen from four mice in each dosed and control group were stained for iron. There was no difference between dosed and control mice in the amount of iron-positive granules in the spleen.

A summary of nonneoplastic lesions in mice in the 13-week feed study is presented in Appendix A, Tables A3 and A4.

Sperm morphology and vaginal cytology evaluations were performed on mice in the 0, 1000, 4000, and 8000 ppm groups. No significant findings were noted in males or females (Appendix D).

PLATE 1

Forestomach mucosa from a female rat administered 8000 ppm cupric sulfate in the feed for 13 weeks. At the limiting ridge (the junction between the glandular stomach (G) and forestomach) there is moderate hyperkeratosis (K) and hyperplasia of the squamous epithelium characterized by an increased thickness and folding of the epithelial surface. Minimal edema (*) is present in the superficial lamina propria of the squamous mucosa. H&E, 70×.

PLATE 2

Liver from a male rat administered 8000 ppm cupric sulfate in the feed for 13 weeks. Although the portion of the hepatic lobule surrounding the central vein (C) is normal, there is a focal accumulation of inflammatory cells (large arrows) in the portal area around the bile duct (small arrow). The severity grade of this lesion was +2 (mild). H&E, 120×.

PLATE 3

Liver from a male rat administered 8000 ppm cupric sulfate in the feed for 13 weeks. Detail of the portal area of the hepatic lobule shows cross-sections through bile ducts (arrows) and an accumulation of macrophages with abundant pale eosinophilic cytoplasm. H&E, 310×.

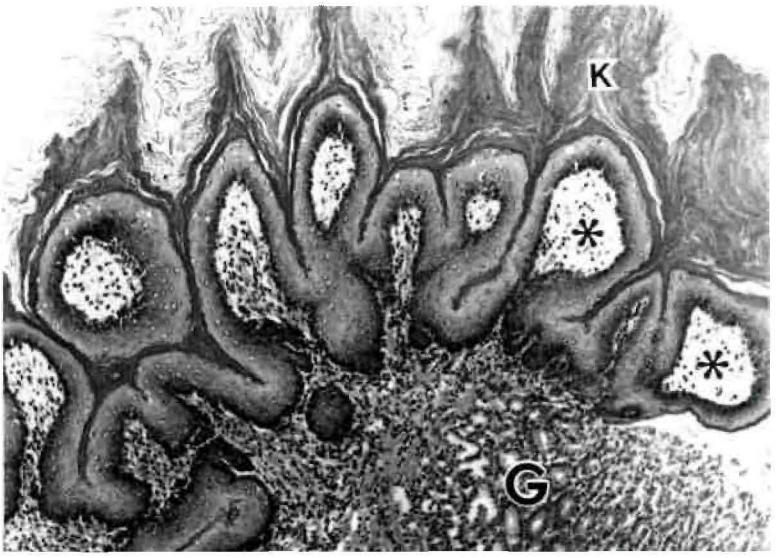

PLATE 1

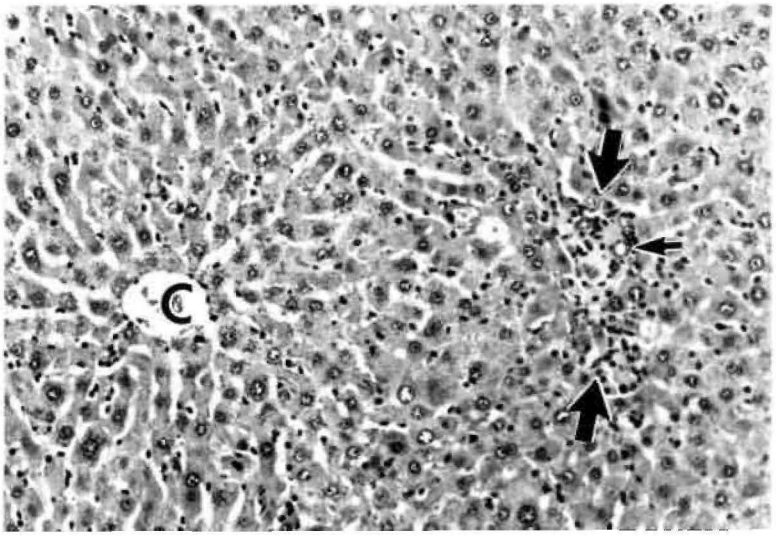

PLATE 2

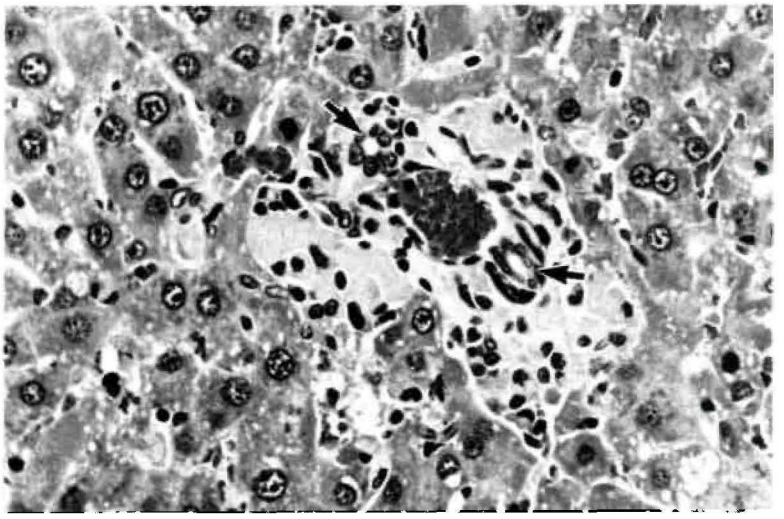

PLATE 3

PLATE 4

Section of rat liver stained for copper showing the accumulation of darkly stained cytoplasmic granules (arrows) in the hepatocytes adjacent to the portal area (P) of the hepatic lobule. Rhodanine stain, 310×.

PLATE 5

Ultrastructural appearance of the liver from a female rat administered 8000 ppm cupric sulfate in the feed for 13 weeks. Hepatocytes in the periportal area of the hepatic lobule contain numerous irregular shaped electron-dense inclusions (arrows). TEM, 3600×.

PLATE 6

Higher magnification of hepatocyte showing detail of cytoplasmic inclusions (*) adjacent to the nucleus (N). Note the presence of densely stained droplets and the nonstained crystalline clefts within the membrane bound (arrows) inclusions. TEM, 9000×.

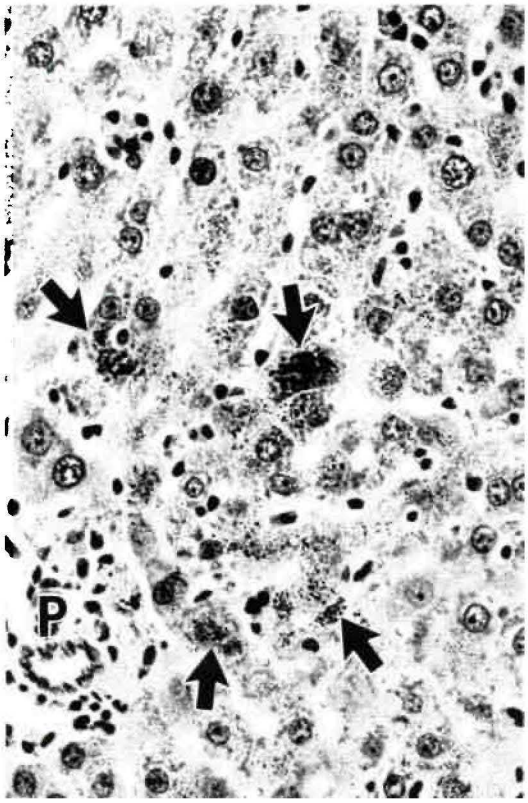

PLATE 4

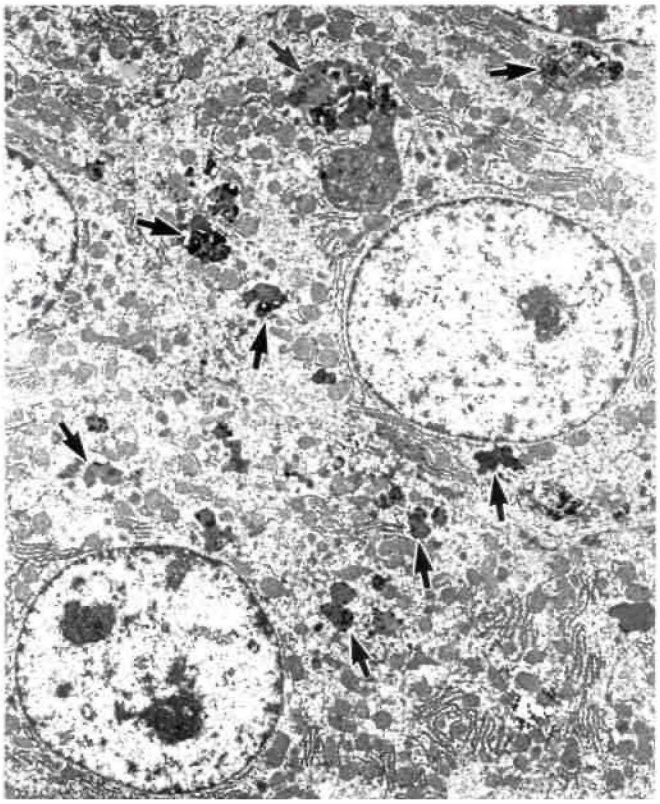

PLATE 5

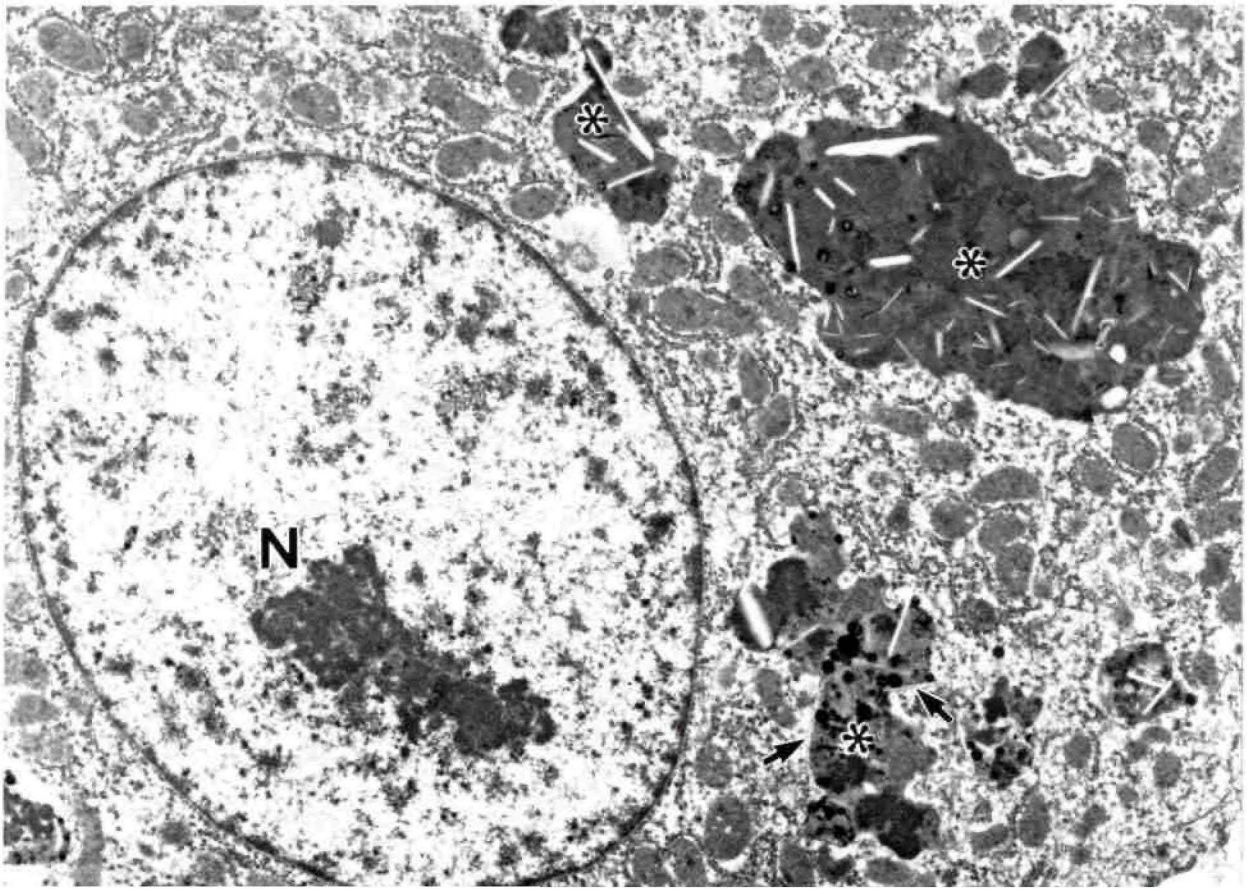

PLATE 6

PLATE 7

Kidney from a control male rat showing the typical size and shape of protein droplets (arrows) in the cytoplasm of the proximal tubule epithelium. Compare to the dosed male rat in Plate 8. TEM, 2700×.

PLATE 8

Kidney from a male rat administered 8000 ppm cupric sulfate in the feed for 13 weeks. Note the increased size and irregular shape of protein droplets (arrows) compared to those seen in the control male rat in Plate 7. TEM, 2700×.

PLATE 9

Kidney from a control female rat showing the typical size and shape of protein droplets (arrows) in the cytoplasm of the proximal tubule epithelium. Compare to the dosed female rat in Plate 10. TEM, 2700×.

PLATE 10

Kidney from a female rat administered 8000 ppm cupric sulfate in the feed for 13 weeks. Note the increased number and size and the irregular shape of the protein droplets (arrows) in the cytoplasm of the proximal tubule epithelium compared to the control female rat in Plate 9. TEM, 2700×.

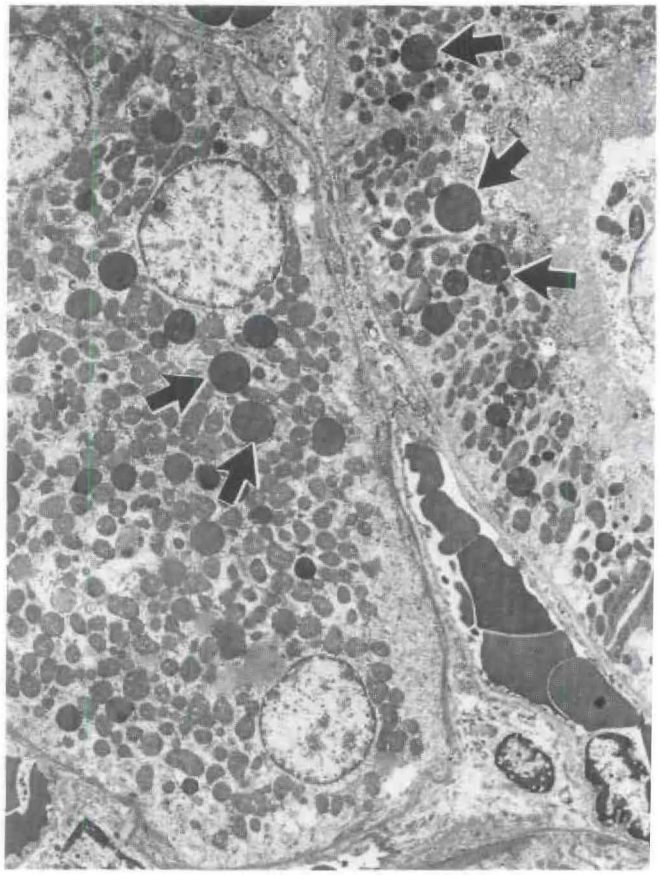

PLATE 7

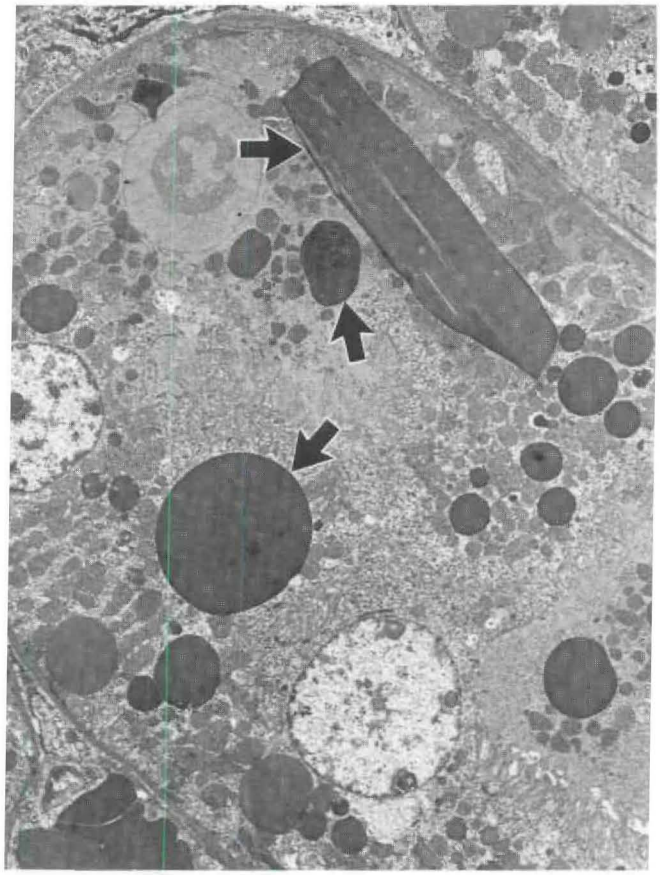

PLATE 8

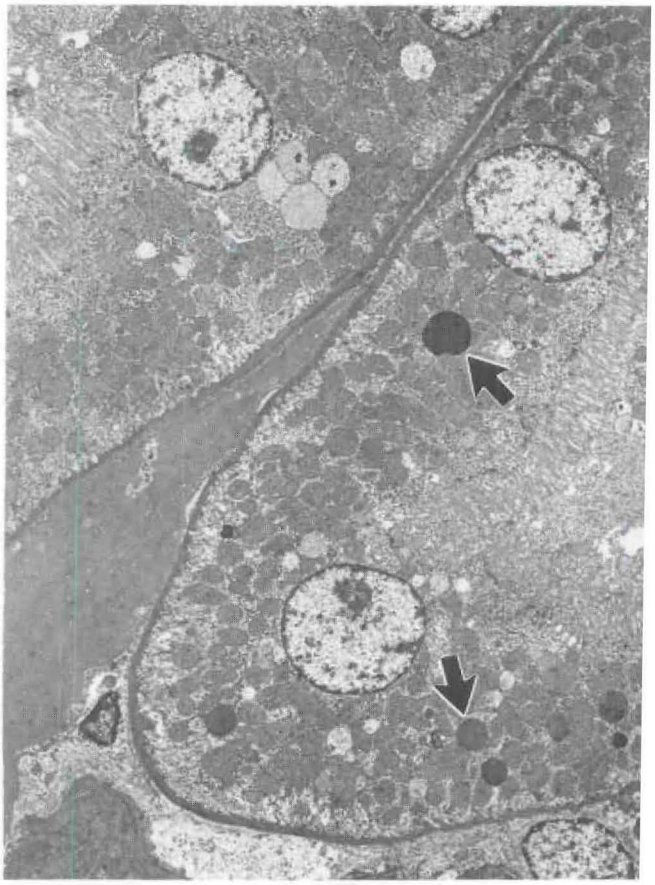

PLATE 9

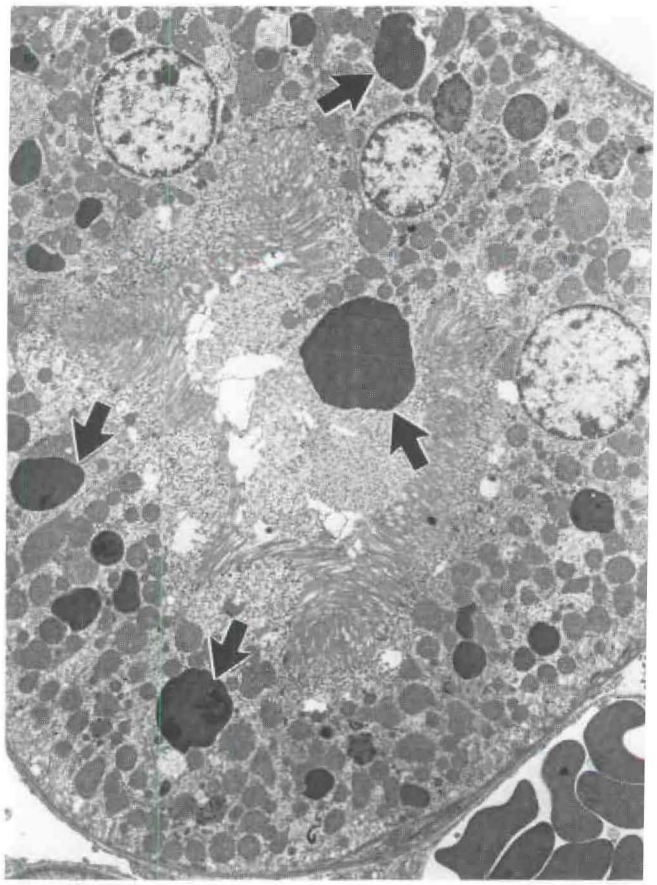

PLATE 10

DISCUSSION

In the 2-week drinking water studies with cupric sulfate, deaths occurred in the 3000, 10,000, and 30,000 ppm groups. No treatment-related deaths occurred in the 2-week feed studies. However, cupric sulfate concentrations of 4000 to 8000 ppm and above in the feed caused significant reductions in body weight gain in rats and mice. Decreases in water and feed consumption at early time points were seen in the same dose groups and were probably the result of the decreased palatability of the cupric sulfate preparations. The reductions in body weight gain, the early deaths seen in the drinking water studies, and the clinical signs of toxicity noted in the 2-week drinking water and feed studies were attributed to voluntary water or feed deprivation and not to specific cupric sulfate toxicity. Likewise, changes in absolute organ weights and various organ-weight-to-body-weight ratios were attributed to the markedly reduced feed or water consumption and the accompanying reductions in body weight gain.

A comparison of average compound consumption between the 2-week drinking water and 2-week dosed feed studies indicated that much higher doses of cupric sulfate could be achieved using the dosed feed vehicle. Animals refused drinking water containing cupric sulfate concentrations of 3000 ppm and greater (net achieved doses of 121 to 175 mg/kg per day for rats and 226 to 245 mg/kg per day for mice), which resulted in severe dehydration, significant decrements in body weight gain, and death and limited the detection of toxic effects that might be directly attributable to cupric sulfate. Consequently, the dosed feed route was used in the 13-week studies.

The only consistent lesion occurring as a result of cupric sulfate ingestion in both rats and mice was a hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge between the forestomach and the glandular stomach. This lesion was present in animals of both species in the 2-week and the 13-week feed studies but had no detectable adverse effects on the health of the animals. However, the hyperplasia persisted for at least 13 weeks with continuous exposure to cupric sulfate. Cupric sulfate is a gastric irritant (the irritation is the cause of vomiting seen in poisoned animals and humans), and it is possible that with chronic administration, a persistent irritation, along with a sustained increase in cell proliferation, could contribute to an increased incidence of forestomach tumors.

The kidney was identified as a target organ for cupric sulfate toxicity in rats in the dosed water and dosed feed studies. Increases in serum urea nitrogen, urine aspartate aminotransferase (AST) and *N*-acetyl-β-D-glucosaminidase (NAG) activities, and urine protein and glucose in the 13-week feed study are all consistent with renal injury. The elevated urine AST and NAG activities in male and female rats at Days 19 and 90 is indicative of renal epithelial damage, with the damaged epithelial cells releasing these enzymes. Likewise, the increased urine glucose level that occurred in the 4000 and 8000 ppm male rats is indicative of proximal renal tubule damage and a resultant decrease in renal glucose resorption.

Microscopic changes specifically related to cupric sulfate toxicity in the kidney were characterized by a dose-related increase in the size and number of eosinophilic protein droplets in the epithelial cytoplasm and the lumina of the renal proximal convoluted tubules. These droplets were present in male rats in the 300 and 1000 ppm dosed drinking water groups, in rats of each sex in the 4000 ppm and higher dosed feed groups in the 2-week study, and in rats of each sex in the 2000 ppm and higher dosed feed groups in the 13-week study. This lesion was morphologically similar in both sexes but was less severe in females than in males. Specific stains were performed on sections of affected kidneys, but the identity of the contents of the droplets could not be ascertained. In the 13-week study in rats, these droplets stained positive for protein, and some droplets in males stained positive for α-2-microglobulin. The droplets did not stain with the iron, PAS, or acid-fast (lipofuscin) staining methods. As reported by Haywood (1980), copper was present in some, but not all, of the eosinophilic droplets in this study. A similar protein droplet accumulation has been described in the kidneys of sheep and Wistar and Sprague-Dawley rats treated subchronically with cupric sulfate (Wolff, 1960; Gopinath *et al.*, 1974; Haywood, 1980, 1985); however, the droplets in the sheep kidney are described as being PAS-positive, whereas those in the present study were PAS-negative. It has been suggested that these eosinophilic droplets may contain secretory or excretory products or copper-containing proteins (such as ceruloplasmin) that have been absorbed from the bloodstream (Haywood, 1980). Transmission electron microscopy of kidneys from rats in the 13-week study confirmed the light microscopy results, revealing mild (females) to moderate (males) increases in the number and size of electron-dense protein droplets in the cytoplasm of the proximal convoluted tubule epithelium. Many of the droplets in male, but not female, rat kidneys were large and had irregular crystalline

shapes, similar to those seen in the toxic nephropathy related to the accumulation of α-2-microglobulin (NTP, 1992b).

Additional morphologic evidence of kidney toxicity in rats included nuclear enlargement (karyomegaly) and degeneration (high-dose females) of renal tubule epithelium. A morphologic change similar to karyomegaly (described as cellular pleomorphism) has been reported in rats after copper-induced degeneration and necrosis of renal tubule epithelium (Haywood *et al.*, 1985). These nuclear changes were considered to be a part of the regenerative process in the epithelium.

The liver was also identified as a target organ for cupric sulfate toxicity in male and female rats in both the 2-week and 13-week feed studies. Increases in serum alanine aminotransferase and sorbitol dehydrogenase activities in the dosed rats at all time points in the 13-week study are consistent with hepatocellular injury. Similar enzyme changes have been observed in rats and other species poisoned with cupric sulfate, including humans (Todd and Thompson, 1963; Singh and Singh, 1968; Kumar and Sharma, 1987). At later time points, the degree of change was more pronounced, suggesting that the hepatocellular damage was continuous and/or progressive. These findings were supported by the histologic evidence of chronic-active inflammation with necrosis that was observed in the tissues of the two highest dose groups of male and female rats. Increases in serum bile acid concentrations and the activity of 5′-nucleotidase in the high-dose rats on Day 92 are indicative of cholestasis.

The primary microscopic lesion in the livers of rats fed cupric sulfate was a chronic-active inflammation, which was present with a periportal to midzonal distribution and was often accompanied by necrosis of individual hepatocytes within or adjacent to areas of inflammation. There was a dose-related increase in both the incidence and the severity of the inflammation at 2 and 13 weeks. Haywood (1980, 1985) described similar inflammatory changes with isolated necrotic hepatocytes in male Wistar rats fed diets containing up to 6000 ppm cupric sulfate. Rhodanine staining of livers from cupric sulfate-treated rats in the present 13-week study revealed that copper was distributed in the liver in a periportal to midzonal pattern. Additionally, transmission electron microscopy of the livers of rats of each sex revealed degenerative changes consisting of increases in the number of secondary lysosomes in hepatocytes in the periportal area. Many of these lysosomes contained clear crystalline structures and electron-dense

material. This is consistent with the observations of Haywood *et al.* (1985), who reported that copper appears to become localized in lysosomes in rat liver.

It has been suggested that cupric sulfate-induced hepatic damage in the rat is precipitated by the rupture of copper-laden lysosomes and subsequent redistribution of the copper to the nuclei of the cells (Haywood *et al.*, 1985). A similar lysosomal accumulation of copper with eventual rupture and release of the copper stores is believed to lead to the acute hemolytic crisis identified with copper toxicity in sheep (Buck *et al.*, 1976). Rats, however, differ from sheep in that they are more resistant to the toxic effects of copper and are able to adapt to long-term administration of high levels of copper. Hemolysis was not evident in rats in the present study nor in the Haywood studies (Haywood, 1980, 1985; Haywood *et al.*, 1985). Most human deaths from cupric sulfate poisoning occur directly or indirectly as the result of acute hemolysis and subsequent renal damage. Hemolysis in humans occurs in cases of acute poisoning and is thought to result from inhibition by copper of glycolytic enzymes and/or precipitation of hemoglobin (Fairbanks, 1967; Singh and Singh, 1968). Chronic copper poisoning in humans would almost certainly lead to accumulation in the liver. However, it is not known whether the human liver can adapt to chronic high levels of copper, like the rat liver, or whether such accumulation would eventually lead to a sudden release of copper and precipitation of an acute crisis, as in sheep.

In the Haywood studies (Haywood, 1980, 1985; Haywood *et al.*, 1985), liver and kidney damage from cupric sulfate treatment in rats was most pronounced at early time points (2 to 5 weeks), with progressive recovery taking place from the fifth week of dosing up to at least the fifteenth week. Recovery was virtually complete by the 15-week time point at doses of 3000 ppm cupric sulfate and below, whereas at higher doses (up to 6000 ppm) the onset of recovery was delayed and the extent of recovery was less complete. In the present studies, rats did not appear to adapt to the liver- or kidney-damaging effects of cupric sulfate. Liver toxicity at 13 weeks was evident at lower doses than at 2 weeks, and the severity of the lesions at higher doses was about the same at both time points. Female rats in this study appeared to be somewhat more resistant to cupric sulfate toxicity than males.

In general, the changes that occurred in the hematology endpoints in rats during the 13-week study indicate an ineffective erythropoiesis resulting in a microcytic anemia. The increases in erythrocytes (RBCs), hematocrit (HCT), and hemoglobin (HGB) on Day 5 in the 8000 ppm rats are consistent with a relative polycythemia, which can occur as a result of dehydration. However, depletion of hematopoietic cells was evident in rats of each sex in the bone marrow (8000 and 16,000 ppm) and spleen (16,000 ppm) in the 2-week feed study, and the observed polycythemia on Day 5 of the 13-week study could also be a result of increased release of blood cells from bone marrow and other sites. The observed decreases in reticulocyte counts, serum albumin, total protein, alkaline phosphatase, and 5′-nucleotidase are often seen in cases of altered nutritional status and are probably the result of decreased feed consumption by rats in the higher dose groups. The elevation of platelet counts in male and female rats on Days 5 and 21 is consistent with a reactive thrombocytosis, which could have been due to a variety of factors, including trauma, iron deficiency, chronic hemorrhage, and/or inflammation.

By Day 21, the initial polycythemia that was seen on Day 5 was replaced by a responsive microcytic anemia, with normal RBC counts, reductions in mean cell volume and mean cell hemoglobin, and increases in mean cell hemoglobin concentration. Reductions in HCT and HGB indicate an anemia, with the bone marrow attempting to compensate for the anemia by releasing increased numbers of smaller-than-normal (microcytic) RBCs. Classically, microcytic anemia has been observed with iron deficiency, wherein iron levels are insufficient to support complete heme synthesis. The result is a prolonged erythropoietic cycle, during which RBCs undergo an additional cell division in an attempt to achieve critical HGB concentrations. This additional cell division produces RBCs which are smaller than normal. By Day 92, the microcytic anemia was still present but was not as pronounced as on Day 21. Elevated levels of copper in the diet have been shown to interfere with iron absorption and utilization in other species (Gipp *et al.*, 1973; Theil and Calvert, 1978) and produce microcytic anemia in pigs (Gipp *et al.*, 1973). Furthermore, results from iron staining of the rat spleens indicate that there was a profound dose-related depletion of iron stores in the spleen. Therefore, it is likely that the microcytic anemia seen in the present study is due to an iron deficiency that resulted from interference of copper with iron absorption and/or metabolism. The decreased severity of the anemia (and the responsive thrombocytosis) on Day 92 relative to Day 21 indicates that these treatment-related responses had moderated and were either stabilized or resolving. This observation indicates that the rats were able to adapt to the damaging

effects of copper on the hematopoietic system and is consistent with the results of the Haywood studies.

In mice in the feed studies, the only morphological change which was specifically attributed to cupric sulfate was a minimal to mild hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach. This lesion occurred in both the 2-week and 13-week feed studies and was not considered to be life threatening. With the exception of a trend toward decreases in absolute liver weights at higher doses in the 13-week study, no evidence of damage to liver, kidney, or the hematopoietic system was evident.

In summary, under the exposure conditions employed in this study, ingestion of cupric sulfate in drinking water or feed produced similar forestomach lesions in rats and mice that could be attributed to the irritant effects of the compound. The no-observed-adverse-effect level (NOAEL) for this effect was 1000 ppm in feed for rats and 2000 ppm in feed for mice. Cupric sulfate was more toxic to rats than to mice, producing damage to the liver, kidney, and hematopoietic system when administered in the feed. Liver and kidney lesions were similar to those previously described by other investigators. For rats in the 13-week study, the NOAEL for liver damage was 1000 ppm for males and 2000 ppm for females, and the NOAEL for kidney damage was 1000 ppm for males and 500 ppm for females. A NOAEL for the kidney effects seen in male rats in the 2-week drinking water study could not be determined because these changes were seen only at the two lowest doses tested and not at the higher dose levels. Toxic effects on the hematopoietic system were primarily related to a responsive microcytic anemia that was probably secondary to disturbances of iron metabolism. Rats appeared to be able to adapt to the hematopoietic effects but not to the hepatotoxic and renotoxic effects of cupric sulfate. Ingestion of cupric sulfate did not cause any adverse reproductive effects in either sex of either species.

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APPENDIX A

Summary of Nonneoplastic Lesions in Rats and Mice

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)		(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	1 (10%)		1 (10%)	1 (10%)		
Inflammation, chronic active				1 (10%)	10 (100%)	10 (100%)
Pancreas	(10)					(10)
Atrophy	2 (20%)					1 (10%)
Stomach, forestomach	(10)	(1)	(10)	(10)	(10)	(10)
Hyperplasia				10 (100%)	10 (100%)	10 (100%)
Stomach, glandular	(10)		(10)	(10)	(10)	(10)
Mineralization			1 (10%)			
Cardiovascular System						
Heart	(10)					(10)
Inflammation, chronic active	10 (100%)					5 (50%)
Endocrine System						
Pituitary gland	(10)					(10)
Cyst						1 (10%)
General Body System						
None						
Genital System						
Epididymis	(10)					(10)
Inflammation, chronic active	1 (10%)					
Preputial gland	(10)					(10)
Inflammation, chronic active	7 (70%)					8 (80%)
Prostate	(10)					(10)
Inflammation, chronic active	1 (10%)					1 (10%)
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Feed Study of Cupric Sulfate (continued)

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Inflammation, chronic active	1 (10%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)	(9)	(10)	(10)	(10)	(10)
Cytoplasmic alteration				3 (30%)	10 (100%)	10 (100%)
Nephropathy	10 (100%)	9 (100%)	10 (100%)	8 (80%)	9 (90%)	6 (60%)
Proximal convoluted renal tubule, karyomegaly						10 (100%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidently killed			1			
Survivors						
Terminal sacrifice	10	10	9	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine small, jejunum	(10)		(1)			(10)
Inflammation, acute	1 (10%)					
Liver	(10)	(1)	(2)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	2 (20%)	1 (100%)	2 (100%)	2 (20%)		
Inflammation, chronic active					6 (60%)	10 (100%)
Inflammation, focal	2 (20%)					
Mesentery		(1)				
Fat, necrosis		1 (100%)				
Pancreas	(10)		(1)			(10)
Atrophy	1 (10%)					1 (10%)
Stomach, forestomach	(10)		(10)	(10)	(10)	(10)
Cyst epithelial inclusion						1 (10%)
Hyperplasia				7 (70%)	10 (100%)	10 (100%)
Cardiovascular System						
Heart	(10)					(10)
Inflammation, chronic active						1 (10%)
Endocrine System						
None						
General Body System						
None						
Genital System						
Clitoral gland	(10)		(1)			(10)
Inflammation, chronic active	9 (90%)					10 (100%)
Ovary	(10)	(1)	(1)			(10)
Cyst		1 (100%)				
Hematopoietic System						
None						
Integumentary System						
None						

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Feed Study of Cupric Sulfate (continued)

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
Musculoskeletal System						
None						
Nervous System						
Brain	(10)		(1)			(10)
Gliosis	1 (10%)					
Respiratory System						
Lung	(10)					(10)
Inflammation, chronic active	1 (10%)					1 (10%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Cyst	1 (10%)					
Cytoplasmic alteration			1 (10%)	9 (90%)	10 (100%)	10 (100%)
Mineralization		1 (10%)				
Nephropathy			1 (10%)	1 (10%)		2 (20%)
Pigmentation						2 (20%)
Proximal convoluted renal tubule, karyomegaly						10 (100%)
Renal tubule, degeneration						3 (30%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate[1]

	0 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm	16,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(1)				(10)
Infarct		1 (100%)				
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia				2 (20%)	6 (60%)	10 (100%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate (continued)

	0 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm	16,000 ppm
Special Senses System						
None						
Urinary System						
None						

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate[1]

	0 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm	16,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10		10	10	10	10
Alimentary System						
Stomach, forestomach	(10)		(10)	(10)	(10)	(10)
Hyperplasia				5 (50%)	8 (80%)	10 (100%)
Cardiovascular System						
Heart	(10)					(10)
Myocardium, mineralization	1 (10%)					
Endocrine System						
None						
General Body System						
None						
Genital System						
Clitoral gland	(10)					(8)
Cyst	1 (10%)					
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate (continued)

	0 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm	16,000 ppm
Special Senses System						
None						
Urinary System						
Urinary bladder	(10)					(10)
Transitional epithelium, mineralization	3 (30%)					

[1] Number of animals examined microscopically at site and number of animals with lesion.

APPENDIX B

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE B1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	361 ± 5	345 ± 9	362 ± 6	352 ± 11	339 ± 5*	275 ± 6**
Brain						
Absolute	1.957 ± 0.015	1.940 ± 0.015	1.948 ± 0.012	1.914 ± 0.023	1.969 ± 0.024	1.898 ± 0.019*
Relative	5.43 ± 0.06	5.65 ± 0.14	5.39 ± 0.08	5.47 ± 0.12	5.81 ± 0.06**	6.92 ± 0.10**
Heart						
Absolute	1.082 ± 0.024	1.047 ± 0.028	1.049 ± 0.018	1.074 ± 0.037	1.062 ± 0.024	0.918 ± 0.029**
Relative	3.00 ± 0.06	3.04 ± 0.03	2.90 ± 0.03	3.05 ± 0.06	3.13 ± 0.06	3.34 ± 0.08**
Right kidney						
Absolute	1.319 ± 0.022	1.321 ± 0.032	1.322 ± 0.034	1.269 ± 0.047	1.320 ± 0.030	1.105 ± 0.027**
Relative	3.66 ± 0.05	3.84 ± 0.09	3.65 ± 0.05	3.60 ± 0.04	3.89 ± 0.05**	4.02 ± 0.03**
Liver						
Absolute	13.914 ± 0.287	14.172 ± 0.537	14.683 ± 0.381	13.422 ± 0.617	12.946 ± 0.363	10.271 ± 0.233**
Relative	38.54 ± 0.46	41.05 ± 0.96	40.52 ± 0.63	38.02 ± 0.83	38.16 ± 0.65	37.37 ± 0.52
Lungs						
Absolute	1.780 ± 0.051	1.815 ± 0.062	1.899 ± 0.061	1.873 ± 0.073	1.914 ± 0.073	1.532 ± 0.050*
Relative	4.93 ± 0.14	5.27 ± 0.16	5.25 ± 0.16	5.33 ± 0.18	5.63 ± 0.14**	5.57 ± 0.16**
Right testis						
Absolute	1.466 ± 0.017	1.429 ± 0.033	1.479 ± 0.040	1.460 ± 0.041	1.481 ± 0.028	1.414 ± 0.029
Relative	4.06 ± 0.03	4.15 ± 0.08	4.08 ± 0.08	4.15 ± 0.04	4.37 ± 0.03**	5.15 ± 0.07**
Thymus						
Absolute	0.402 ± 0.029	0.353 ± 0.010	0.393 ± 0.016	0.348 ± 0.027	0.383 ± 0.017	0.296 ± 0.011**
Relative	1.11 ± 0.07	1.03 ± 0.03	1.09 ± 0.05	0.98 ± 0.06	1.13 ± 0.05	1.08 ± 0.03
FEMALE						
n	10	10	9	10	10	10
Necropsy body wt	196 ± 2	194 ± 3	201 ± 4	196 ± 3	190 ± 3	180 ± 3**
Brain						
Absolute	1.786 ± 0.010	1.761 ± 0.024	1.787 ± 0.019	1.775 ± 0.011	1.793 ± 0.015	1.780 ± 0.012
Relative	9.12 ± 0.10	9.11 ± 0.13	8.93 ± 0.14	9.10 ± 0.14	9.45 ± 0.17	9.94 ± 0.18**
Heart						
Absolute	0.644 ± 0.011	0.665 ± 0.009	0.667 ± 0.012	0.680 ± 0.016	0.623 ± 0.013	0.618 ± 0.015
Relative	3.29 ± 0.05	3.44 ± 0.05	3.33 ± 0.06	3.48 ± 0.07	3.28 ± 0.04	3.44 ± 0.05
Right kidney						
Absolute	0.746 ± 0.011	0.731 ± 0.011	0.738 ± 0.014	0.753 ± 0.018	0.706 ± 0.017	0.674 ± 0.013**
Relative	3.81 ± 0.04	3.78 ± 0.05	3.68 ± 0.04	3.85 ± 0.05	3.72 ± 0.07	3.75 ± 0.05
Liver						
Absolute	7.080 ± 0.232	6.842 ± 0.207	6.780 ± 0.083	6.768 ± 0.133	6.612 ± 0.145	6.849 ± 0.099
Relative	36.16 ± 1.21	35.34 ± 0.90	33.86 ± 0.46	34.65 ± 0.66	34.80 ± 0.59	38.21 ± 0.56
Lungs						
Absolute	1.265 ± 0.019	1.297 ± 0.049	1.320 ± 0.030	1.354 ± 0.028	1.347 ± 0.035	1.267 ± 0.049
Relative	6.46 ± 0.08	6.70 ± 0.23	6.60 ± 0.17	6.93 ± 0.11	7.10 ± 0.18*	7.05 ± 0.19*
Thymus						
Absolute	0.271 ± 0.013	0.259 ± 0.014	0.275 ± 0.011	0.256 ± 0.013	0.262 ± 0.011	0.255 ± 0.005
Relative	1.38 ± 0.06	1.33 ± 0.06	1.37 ± 0.05	1.31 ± 0.07	1.38 ± 0.06	1.42 ± 0.02

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different (P≤0.05) from the control group by Dunnett's or Williams' test.

** Significantly different (P≤0.01) from the control group by Williams' test.

TABLE B2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate[1]

	0 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm	16,000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	33.5 ± 0.7	32.8 ± 0.7	31.4 ± 0.8*	29.7 ± 0.6**	29.2 ± 0.7**	26.4 ± 0.4**
Brain						
Absolute	0.444 ± 0.005	0.442 ± 0.006	0.445 ± 0.005	0.445 ± 0.004	0.446 ± 0.006	0.445 ± 0.005
Relative	13.30 ± 0.24	13.51 ± 0.30	14.26 ± 0.42*	15.06 ± 0.27**	15.30 ± 0.27**	16.90 ± 0.30**
Heart						
Absolute	0.168 ± 0.008	0.160 ± 0.002	0.162 ± 0.004	0.155 ± 0.004	0.158 ± 0.003	0.154 ± 0.005*
Relative	5.01 ± 0.17	4.90 ± 0.07	5.19 ± 0.13	5.25 ± 0.16	5.44 ± 0.15	5.84 ± 0.24**
Right kidney						
Absolute	0.303 ± 0.009	0.320 ± 0.006	0.308 ± 0.007	0.302 ± 0.011	0.298 ± 0.005	0.264 ± 0.007**
Relative	9.10 ± 0.32	9.77 ± 0.16*	9.84 ± 0.16*	10.16 ± 0.22**	10.24 ± 0.19**	10.02 ± 0.18**
Liver						
Absolute	1.644 ± 0.063	1.588 ± 0.038	1.530 ± 0.029	1.466 ± 0.044**	1.399 ± 0.040**	1.204 ± 0.037**
Relative	49.02 ± 1.21	48.48 ± 1.11	48.92 ± 1.14	49.38 ± 0.68	47.87 ± 0.72	45.61 ± 1.05*
Lungs						
Absolute	0.226 ± 0.008	0.207 ± 0.004	0.226 ± 0.006	0.237 ± 0.014	0.217 ± 0.013	0.211 ± 0.007
Relative	6.76 ± 0.18	6.35 ± 0.20	7.23 ± 0.25	8.03 ± 0.53*	7.39 ± 0.34*	8.02 ± 0.30**
Right testis						
Absolute	0.123 ± 0.003	0.120 ± 0.002	0.120 ± 0.003	0.122 ± 0.004	0.125 ± 0.003	0.118 ± 0.002
Relative	3.67 ± 0.10	3.66 ± 0.06	3.85 ± 0.12	4.12 ± 0.15**	4.28 ± 0.12**	4.48 ± 0.09**
Thymus						
Absolute	0.049 ± 0.003	0.048 ± 0.003	0.044 ± 0.002	0.046 ± 0.003	0.043 ± 0.002	0.043 ± 0.002
Relative	1.47 ± 0.06	1.47 ± 0.07	1.41 ± 0.08	1.55 ± 0.09	1.47 ± 0.05	1.62 ± 0.08
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	30.1 ± 0.9	29.5 ± 1.0	28.5 ± 0.8	27.8 ± 0.6*	26.2 ± 0.6**	22.6 ± 0.5**
Brain						
Absolute	0.463 ± 0.005	0.466 ± 0.004	0.466 ± 0.005	0.472 ± 0.005	0.465 ± 0.005	0.459 ± 0.006
Relative	15.51 ± 0.53	15.94 ± 0.51	16.46 ± 0.47	17.08 ± 0.41*	17.87 ± 0.48**	20.45 ± 0.62**
Heart						
Absolute	0.138 ± 0.002	0.138 ± 0.002	0.145 ± 0.003	0.138 ± 0.003	0.135 ± 0.004	0.138 ± 0.005
Relative	4.61 ± 0.14	4.73 ± 0.16	5.12 ± 0.16	4.97 ± 0.15	5.16 ± 0.13*	6.14 ± 0.25**
Right kidney						
Absolute	0.221 ± 0.005	0.224 ± 0.004	0.220 ± 0.005	0.215 ± 0.004	0.209 ± 0.006	0.191 ± 0.002**
Relative	7.38 ± 0.22	7.66 ± 0.26	7.76 ± 0.23	7.79 ± 0.21	7.99 ± 0.20	8.48 ± 0.18**
Liver						
Absolute	1.407 ± 0.035	1.435 ± 0.050	1.337 ± 0.032	1.303 ± 0.043	1.276 ± 0.029*	1.067 ± 0.036**
Relative	46.92 ± 1.20	48.75 ± 1.03	46.99 ± 0.93	46.91 ± 1.12	48.86 ± 0.92	47.17 ± 0.86
Lungs						
Absolute	0.208 ± 0.006	0.214 ± 0.005	0.216 ± 0.004	0.211 ± 0.008	0.213 ± 0.005	0.214 ± 0.007
Relative	6.94 ± 0.27	7.29 ± 0.20	7.62 ± 0.18	7.64 ± 0.33	8.18 ± 0.25**	9.52 ± 0.43**
Thymus						
Absolute	0.066 ± 0.003	0.066 ± 0.004	0.062 ± 0.003	0.057 ± 0.002	0.062 ± 0.002	0.049 ± 0.003**
Relative	2.21 ± 0.09	2.23 ± 0.07	2.16 ± 0.09	2.07 ± 0.06	2.36 ± 0.08	2.19 ± 0.12

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different (P≤0.05) from the control group by Williams' test.

** Significantly different (P≤0.01) from the control group by Williams' test.

APPENDIX C

Hematology, Clinical Chemistry, and Urinalysis Results

TABLE C1 Hematology Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
MALE						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 5	39.2 ± 0.5[2]	39.3 ± 0.4[2]	39.0 ± 0.3	39.0 ± 0.2	39.5 ± 0.3	42.8 ± 0.7**
Day 21	46.0 ± 0.5	46.5 ± 0.4[2]	46.0 ± 0.4	44.2 ± 0.8[2]	36.4 ± 1.6**	34.8 ± 1.3**
Day 92	47.9 ± 0.4	46.5 ± 0.6	47.5 ± 0.2	48.1 ± 0.4	44.8 ± 1.2**	40.2 ± 1.7**
Hemoglobin (g/dL)						
Day 5	13.1 ± 0.2[2]	13.1 ± 0.1[2]	13.0 ± 0.1	12.9 ± 0.1	13.1 ± 0.1	14.1 ± 0.2**
Day 21	14.2 ± 0.1	14.3 ± 0.1[2]	14.3 ± 0.1	13.8 ± 0.2[2]	11.8 ± 0.5**	11.1 ± 0.3**
Day 92	14.3 ± 0.1	13.8 ± 0.2	14.0 ± 0.1	14.3 ± 0.1	13.4 ± 0.3**	12.2 ± 0.4**
Erythrocytes (10^6/μL)						
Day 5	6.60 ± 0.11[2]	6.62 ± 0.06[2]	6.62 ± 0.08	6.58 ± 0.04	6.81 ± 0.06*	7.41 ± 0.12**
Day 21	7.89 ± 0.08	8.00 ± 0.07[2]	8.00 ± 0.09	8.12 ± 0.12[2]	7.82 ± 0.12	7.67 ± 0.20
Day 92	8.88 ± 0.07	8.65 ± 0.11	8.84 ± 0.05	9.06 ± 0.06	9.23 ± 0.15	9.55 ± 0.10**
Reticulocytes (10^6/μL)						
Day 5	0.45 ± 0.03[2]	0.44 ± 0.02[2]	0.42 ± 0.02	0.41 ± 0.02	0.33 ± 0.02**	0.22 ± 0.02**
Day 21	0.20 ± 0.02	0.20 ± 0.02[2]	0.20 ± 0.02	0.24 ± 0.02[2]	0.38 ± 0.02**	0.41 ± 0.03**
Day 92	0.15 ± 0.01	0.20 ± 0.02	0.17 ± 0.01	0.15 ± 0.02	0.20 ± 0.02	0.27 ± 0.01**
Nucleated erythrocytes (10^3/μL)						
Day 5	0.05 ± 0.01[2]	0.04 ± 0.01[2]	0.05 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.02 ± 0.02*
Day 21	0.00 ± 0.00	0.03 ± 0.01[2]	0.01 ± 0.01	0.00 ± 0.00[2]	0.02 ± 0.01	0.01 ± 0.01
Day 92	0.00 ± 0.00	0.03 ± 0.01	0.04 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.02
Mean cell volume (fL)						
Day 5	59.4 ± 0.6[2]	59.4 ± 0.4[2]	58.8 ± 0.6	59.2 ± 0.3	58.2 ± 0.3*	57.8 ± 0.2**
Day 21	58.3 ± 0.5	58.1 ± 0.4[2]	57.7 ± 0.3	54.6 ± 0.8**[2]	46.4 ± 1.3**	45.2 ± 0.7**
Day 92	54.0 ± 0.2	53.7 ± 0.3	53.8 ± 0.3	53.1 ± 0.2*	48.8 ± 1.6**	42.1 ± 2.0**
Mean cell hemoglobin (pg)						
Day 5	19.9 ± 0.1[2]	19.8 ± 0.1[2]	19.6 ± 0.1	19.6 ± 0.1	19.2 ± 0.1**	19.1 ± 0.1**
Day 21	18.0 ± 0.1	17.9 ± 0.1[2]	17.9 ± 0.1	17.0 ± 0.2**[2]	15.0 ± 0.4**	14.5 ± 0.1**
Day 92	16.0 ± 0.1	15.9 ± 0.1	15.9 ± 0.1	15.7 ± 0.1**	14.5 ± 0.4**	12.8 ± 0.5**
Mean cell hemoglobin concentration (g/dL)						
Day 5	33.4 ± 0.2[2]	33.3 ± 0.2[2]	33.3 ± 0.2	33.0 ± 0.3	33.1 ± 0.3	33.1 ± 0.2
Day 21	30.9 ± 0.2	30.9 ± 0.2[2]	31.1 ± 0.2	31.3 ± 0.2[2]	32.3 ± 0.2**	32.0 ± 0.3**
Day 92	29.7 ± 0.2	29.6 ± 0.3	29.5 ± 0.1	29.7 ± 0.2	29.9 ± 0.2	30.4 ± 0.4
Platelets (10^3/μL)						
Day 5	836.2 ± 10.6[2]	863.9 ± 10.5[2]	884.8 ± 17.2*	934.4 ± 11.1**	924.0 ± 53.2**	1009.0 ± 22.6**
Day 21	735.0 ± 14.9	775.0 ± 18.3[2]	776.6 ± 28.0	856.3 ± 23.3**[2]	1032.6 ± 44.8**	1039.5 ± 27.9**
Day 92	631.6 ± 17.9	712.4 ± 39.8	649.6 ± 14.8	616.4 ± 11.1	765.6 ± 24.1**	958.4 ± 62.7**
Leukocytes (10^3/μL)						
Day 5	5.93 ± 0.50[2]	5.47 ± 0.49[2]	6.17 ± 0.62	6.39 ± 0.65	7.32 ± 0.57	7.73 ± 0.51*
Day 21	6.15 ± 0.46	6.41 ± 0.44[2]	6.89 ± 0.60	7.52 ± 0.54*[2]	7.70 ± 0.46*	7.67 ± 0.72
Day 92	8.39 ± 0.42	8.04 ± 0.40	8.41 ± 0.45	7.87 ± 0.74	9.16 ± 0.39	10.27 ± 0.41*
Segmented neutrophils (10^3/μL)						
Day 5	0.80 ± 0.10[2]	0.60 ± 0.08[2]	0.88 ± 0.12	0.79 ± 0.09	0.82 ± 0.08	1.09 ± 0.10
Day 21	0.61 ± 0.05	0.62 ± 0.04[2]	0.56 ± 0.06	0.64 ± 0.08[2]	0.76 ± 0.05	0.80 ± 0.11
Day 92	1.38 ± 0.06	1.58 ± 0.16	1.25 ± 0.09	1.73 ± 0.34	1.58 ± 0.13	2.14 ± 0.11**
Lymphocytes (10^3/μL)						
Day 5	5.00 ± 0.43[2]	4.74 ± 0.42[2]	5.18 ± 0.54	5.45 ± 0.57	6.40 ± 0.55	6.43 ± 0.45*
Day 21	5.37 ± 0.43	5.65 ± 0.44[2]	6.20 ± 0.59	6.72 ± 0.51*[2]	6.80 ± 0.46*	6.70 ± 0.66
Day 92	6.65 ± 0.31	6.26 ± 0.31	6.98 ± 0.38	6.00 ± 0.52	7.38 ± 0.35	7.99 ± 0.42

TABLE C1 Hematology Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate (continued)

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
MALE (continued)						
Monocytes (10^3/μL)						
Day 5	0.11 ± 0.03[2]	0.10 ± 0.03[2]	0.09 ± 0.02	0.13 ± 0.03	0.10 ± 0.03	0.16 ± 0.02
Day 21	0.13 ± 0.03	0.08 ± 0.02[2]	0.10 ± 0.02	0.09 ± 0.03[2]	0.13 ± 0.03	0.13 ± 0.03
Day 92	0.06 ± 0.02	0.11 ± 0.02	0.08 ± 0.01	0.06 ± 0.02	0.07 ± 0.02	0.11 ± 0.03
Eosinophils (10^3/μL)						
Day 5	0.03 ± 0.01[2]	0.02 ± 0.01[2]	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.05 ± 0.02
Day 21	0.04 ± 0.01	0.05 ± 0.02[2]	0.04 ± 0.01	0.09 ± 0.03[2]	0.01 ± 0.01	0.05 ± 0.01
Day 92	0.11 ± 0.04	0.10 ± 0.04	0.09 ± 0.03	0.08 ± 0.02	0.12 ± 0.03	0.03 ± 0.02
FEMALE						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 5	43.3 ± 0.5	42.8 ± 0.5[2]	42.4 ± 0.6	42.2 ± 0.4	43.9 ± 0.4[2]	44.9 ± 0.3*
Day 21	49.3 ± 0.4[2]	49.5 ± 0.4[3]	48.4 ± 0.3	47.4 ± 0.4**[2]	42.9 ± 1.1**	41.2 ± 1.4**
Day 92	48.6 ± 0.6	46.7 ± 0.4*	47.9 ± 0.5[2]	47.9 ± 0.4	47.7 ± 0.7	43.9 ± 1.0**
Hemoglobin (g/dL)						
Day 5	13.9 ± 0.2	13.9 ± 0.1[2]	13.7 ± 0.2	13.6 ± 0.1	14.2 ± 0.1[2]	14.6 ± 0.1**
Day 21	15.2 ± 0.1[2]	15.3 ± 0.1[3]	14.9 ± 0.1	14.5 ± 0.1**[2]	13.4 ± 0.3**	13.1 ± 0.4**
Day 92	14.5 ± 0.2	14.0 ± 0.2	14.4 ± 0.1[2]	14.2 ± 0.1	14.2 ± 0.2	13.2 ± 0.3**
Erythrocytes (10^6/μL)						
Day 5	7.25 ± 0.12	7.11 ± 0.07[2]	7.11 ± 0.12	7.07 ± 0.07	7.41 ± 0.09[2]	7.63 ± 0.04*
Day 21	8.27 ± 0.09[2]	8.28 ± 0.07[3]	8.14 ± 0.07	8.17 ± 0.09[2]	8.27 ± 0.11	8.26 ± 0.16
Day 92	8.48 ± 0.11	8.15 ± 0.07	8.41 ± 0.09[2]	8.48 ± 0.05	8.44 ± 0.12	8.51 ± 0.13
Reticulocytes (10^6/μL)						
Day 5	0.34 ± 0.02	0.29 ± 0.02[2]	0.33 ± 0.03	0.29 ± 0.02	0.26 ± 0.03*[2]	0.19 ± 0.02**
Day 21	0.12 ± 0.01[2]	0.10 ± 0.02[3]	0.12 ± 0.01	0.10 ± 0.02[2]	0.19 ± 0.02*	0.21 ± 0.03**
Day 92	0.13 ± 0.01	0.14 ± 0.01	0.12 ± 0.01[2]	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.01
Nucleated erythrocytes (10^3/μL)						
Day 5	0.02 ± 0.01	0.03 ± 0.01[2]	0.02 ± 0.01	0.05 ± 0.02	0.01 ± 0.01[2]	0.00 ± 0.00
Day 21	0.00 ± 0.00	0.00 ± 0.00[3]	0.02 ± 0.01	0.03 ± 0.01[2]	0.02 ± 0.01	0.00 ± 0.00
Day 92	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.02[2]	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
Mean cell volume (fL)						
Day 5	59.9 ± 0.4	60.1 ± 0.4[2]	59.8 ± 0.4	59.8 ± 0.3	59.2 ± 0.4[2]	58.8 ± 0.3*
Day 21	59.7 ± 0.4[2]	60.0 ± 0.3[3]	59.7 ± 0.3	58.1 ± 0.5*[2]	52.0 ± 1.2**	49.7 ± 1.0**
Day 92	57.2 ± 0.3	57.2 ± 0.2	57.0 ± 0.3[2]	56.3 ± 0.2*	56.5 ± 0.2*	51.5 ± 1.4**
Mean cell hemoglobin (pg)						
Day 5	19.2 ± 0.2	19.5 ± 0.1[2]	19.3 ± 0.1	19.3 ± 0.1	19.1 ± 0.1[2]	19.1 ± 0.1
Day 21	18.4 ± 0.1[2]	18.5 ± 0.1[3]	18.3 ± 0.1	17.8 ± 0.1*[2]	16.2 ± 0.3**	15.8 ± 0.3**
Day 92	17.0 ± 0.1	17.1 ± 0.1	17.2 ± 0.1[2]	16.8 ± 0.1*	16.9 ± 0.1	15.5 ± 0.4**
Mean cell hemoglobin concentration (g/dL)						
Day 5	32.1 ± 0.1	32.4 ± 0.2[2]	32.3 ± 0.2	32.3 ± 0.2	32.2 ± 0.2[2]	32.6 ± 0.1
Day 21	30.8 ± 0.2[2]	31.0 ± 0.2[3]	30.8 ± 0.2	30.6 ± 0.1[2]	31.2 ± 0.2	31.7 ± 0.2**
Day 92	29.7 ± 0.2	29.9 ± 0.2	30.1 ± 0.2[2]	29.7 ± 0.2	29.9 ± 0.2	30.0 ± 0.2
Platelets (10^3/μL)						
Day 5	823.6 ± 9.7	816.1 ± 18.9[2]	858.6 ± 14.8	879.6 ± 16.7*	875.6 ± 24.9*[2]	928.8 ± 21.1**
Day 21	696.7 ± 7.6[2]	656.4 ± 15.0[3]	682.3 ± 13.1	749.7 ± 10.8*[2]	852.0 ± 22.5**	915.7 ± 13.4**
Day 92	700.5 ± 23.5	671.2 ± 20.6	632.3 ± 18.9[2]	655.6 ± 18.2	715.7 ± 16.9	733.9 ± 25.0

TABLE C1 Hematology Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate (continued)

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
FEMALE (continued)						
Leukocytes (10^3/μL)						
Day 5	5.49 ± 0.68	5.34 ± 0.66[2]	5.38 ± 0.43	5.31 ± 0.45	6.68 ± 0.60[2]	6.34 ± 0.61
Day 21	6.81 ± 0.45[2]	7.10 ± 0.58[3]	6.63 ± 0.55	7.66 ± 0.38[2]	7.19 ± 0.39	7.55 ± 0.55
Day 92	7.78 ± 0.60	7.34 ± 0.17	6.19 ± 0.40[2]	7.14 ± 0.25	8.27 ± 0.25	9.88 ± 0.50*
Segmented neutrophils (10^3/μL)						
Day 5	0.69 ± 0.13	0.76 ± 0.09[2]	0.76 ± 0.09	0.63 ± 0.07	0.87 ± 0.14[2]	0.88 ± 0.10
Day 21	0.78 ± 0.14[2]	0.78 ± 0.13[3]	0.71 ± 0.11	0.61 ± 0.05[2]	0.73 ± 0.09	0.73 ± 0.09
Day 92	1.48 ± 0.40	1.45 ± 0.15	1.07 ± 0.12[2]	1.35 ± 0.12	1.28 ± 0.07	1.50 ± 0.12
Lymphocytes (10^3/μL)						
Day 5	4.73 ± 0.59	4.50 ± 0.59[2]	4.53 ± 0.36	4.61 ± 0.40	5.72 ± 0.51[2]	5.37 ± 0.53
Day 21	5.94 ± 0.32[2]	6.17 ± 0.49[3]	5.78 ± 0.44	6.92 ± 0.37[2]	6.32 ± 0.37	6.68 ± 0.54
Day 92	6.17 ± 0.32	5.75 ± 0.24	5.02 ± 0.41[2]	5.75 ± 0.26	6.85 ± 0.22	8.23 ± 0.42**
Monocytes (10^3/μL)						
Day 5	0.04 ± 0.01	0.06 ± 0.03[2]	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.02[2]	0.05 ± 0.02
Day 21	0.05 ± 0.02[2]	0.09 ± 0.03[3]	0.07 ± 0.02	0.07 ± 0.02[2]	0.11 ± 0.02	0.10 ± 0.03
Day 92	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.01[2]	0.01 ± 0.01	0.04 ± 0.01	0.05 ± 0.03
Eosinophils (10^3/μL)						
Day 5	0.03 ± 0.02	0.02 ± 0.01[2]	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.02[2]	0.05 ± 0.02
Day 21	0.04 ± 0.02[2]	0.06 ± 0.03[3]	0.07 ± 0.02	0.06 ± 0.02[2]	0.04 ± 0.02	0.05 ± 0.02
Day 92	0.11 ± 0.03	0.10 ± 0.02	0.08 ± 0.01[2]	0.04 ± 0.02	0.12 ± 0.03	0.10 ± 0.02

[1] Mean ± standard error.
[2] n=9.
[3] n=8.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE C2 Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
MALE						
n	10	10	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 5	42 ± 1	42 ± 1	41 ± 2	42 ± 1	53 ± 3**	62 ± 5**[2]
Day 21	44 ± 2	42 ± 2	50 ± 3	48 ± 2	90 ± 7**	379 ± 45**
Day 92	51 ± 2	69 ± 8	78 ± 11*	108 ± 15**	494 ± 60**	563 ± 49**
Alkaline phosphatase (IU/L)						
Day 5	1596 ± 22	1592 ± 27	1575 ± 26	1531 ± 31	1406 ± 36**	1007 ± 27**[2]
Day 21	1131 ± 17	1149 ± 44	1146 ± 22	1107 ± 30	1056 ± 35	883 ± 37**
Day 92	503 ± 7	549 ± 17	541 ± 14	498 ± 23	513 ± 15	525 ± 25
5'-nucleotidase (IU/L)						
Day 5	36.5 ± 0.9	38.9 ± 0.9	36.7 ± 0.5	36.2 ± 0.5	34.7 ± 0.9	27.4 ± 1.0**[2]
Day 21	31.8 ± 0.8	33.5 ± 1.0	31.3 ± 0.9	30.2 ± 0.7	32.0 ± 0.8	34.2 ± 1.2
Day 92	33.6 ± 0.7	33.8 ± 0.7	33.3 ± 0.9	32.5 ± 0.5	39.3 ± 1.0**	36.5 ± 0.8**
Sorbitol dehydrogenase (IU/L)						
Day 5	18 ± 1	20 ± 2	19 ± 1	16 ± 1	18 ± 1	19 ± 1[2]
Day 21	22 ± 1	26 ± 4	22 ± 1	22 ± 1	44 ± 6**	162 ± 25**
Day 92	22 ± 1	27 ± 3	32 ± 6	42 ± 6**	197 ± 20**	282 ± 29**
Bile salts (μmol/L)						
Day 5	15.83 ± 2.20	18.32 ± 3.43	12.16 ± 1.25	19.18 ± 3.66	19.36 ± 2.80	16.81 ± 3.23[2]
Day 21	15.62 ± 2.79	11.18 ± 1.97	13.13 ± 2.89	13.20 ± 1.21	30.62 ± 2.81**	36.91 ± 4.73**
Day 92	14.07 ± 2.09	11.23 ± 1.43	11.72 ± 2.18	14.78 ± 2.52	21.67 ± 2.85	25.02 ± 2.84*
Total protein (g/dL)						
Day 5	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.3 ± 0.0**	5.0 ± 0.1**[2]
Day 21	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	5.6 ± 0.1**	5.4 ± 0.1**
Day 92	6.6 ± 0.0	6.6 ± 0.0	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.1 ± 0.1**
Albumin (g/dL)						
Day 5	4.2 ± 0.1	4.2 ± 0.0	4.1 ± 0.0	4.0 ± 0.0*	3.9 ± 0.0**	3.8 ± 0.1**[2]
Day 21	4.3 ± 0.0	4.4 ± 0.0	4.4 ± 0.1	4.2 ± 0.0	4.0 ± 0.0**	3.9 ± 0.0**
Day 92	4.6 ± 0.0	4.6 ± 0.0	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.3 ± 0.0**
Creatinine (mg/dL)						
Day 5	0.68 ± 0.01	0.71 ± 0.01	0.70 ± 0.00	0.68 ± 0.02	0.65 ± 0.02	0.67 ± 0.02[2]
Day 21	0.63 ± 0.02	0.66 ± 0.02	0.64 ± 0.02	0.64 ± 0.02	0.64 ± 0.02	0.61 ± 0.01
Day 92	0.79 ± 0.05	0.83 ± 0.03	0.71 ± 0.02	0.82 ± 0.04	0.72 ± 0.01	0.76 ± 0.02
Urea nitrogen (mg/dL)						
Day 5	21.1 ± 0.4	21.9 ± 0.6	21.0 ± 0.6	22.4 ± 0.5	23.5 ± 0.3**	23.6 ± 0.7**[2]
Day 21	20.0 ± 0.3	20.1 ± 0.5	21.0 ± 0.5	21.7 ± 0.2**	20.8 ± 0.5*	21.4 ± 0.4*
Day 92	21.6 ± 0.3	21.8 ± 0.4	20.6 ± 0.4	20.7 ± 0.7	22.1 ± 0.5	23.5 ± 0.6*
FEMALE						
n	10	10	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 5	39 ± 2	39 ± 2	39 ± 1	38 ± 2	46 ± 2*	48 ± 3*
Day 21	37 ± 2	35 ± 1	34 ± 1	35 ± 1	50 ± 4*	119 ± 17**
Day 92	44 ± 2	38 ± 2	38 ± 1[2]	37 ± 2	84 ± 10*	214 ± 20**
Alkaline phosphatase (IU/L)						
Day 5	1226 ± 40	1316 ± 45	1317 ± 30	1238 ± 39	1040 ± 35*	844 ± 27**
Day 21	893 ± 27	903 ± 18	861 ± 16	858 ± 29	775 ± 30**	624 ± 27**
Day 92	408 ± 35	439 ± 17	516 ± 11[2]	443 ± 18	412 ± 13	385 ± 8

TABLE C2 Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate (continued)

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
FEMALE (continued)						
5'-nucleotidase (IU/L)						
Day 5	39.0 ± 1.5	40.6 ± 0.9	41.1 ± 1.5	39.8 ± 1.4	36.7 ± 1.2	31.1 ± 1.3**
Day 21	35.4 ± 1.2	40.1 ± 1.1[2]	38.5 ± 1.2	38.4 ± 1.3	36.3 ± 1.3	28.4 ± 1.2*
Day 92	34.5 ± 2.0	38.7 ± 0.9	40.0 ± 0.9[2]	36.7 ± 0.8	36.8 ± 0.8	31.8 ± 1.0
Sorbitol dehydrogenase (IU/L)						
Day 5	24 ± 2	24 ± 2	20 ± 1	20 ± 1	21 ± 2	21 ± 2
Day 21	22 ± 1	24 ± 1	20 ± 1	20 ± 1	23 ± 1	39 ± 4**
Day 92	16 ± 1	15 ± 1	19 ± 2[2]	16 ± 1	34 ± 5**	96 ± 12**
Bile salts (μmol/L)						
Day 5	19.28 ± 1.96	16.85 ± 1.44	13.12 ± 0.85*	15.33 ± 1.12	15.24 ± 1.26	13.36 ± 1.11*
Day 21	17.45 ± 2.08	12.74 ± 2.42	13.15 ± 2.61	20.78 ± 3.37	17.61 ± 2.66	20.79 ± 2.36
Day 92	13.41 ± 2.06	12.43 ± 2.36	13.03 ± 1.12[2]	23.57 ± 1.67**	18.87 ± 2.67*	15.99 ± 2.01
Total protein (g/dL)						
Day 5	5.7 ± 0.1	5.6 ± 0.0	5.6 ± 0.1	5.5 ± 0.1*	5.3 ± 0.1**	5.2 ± 0.1**
Day 21	5.9 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.6 ± 0.0*	5.5 ± 0.1**	5.2 ± 0.1**
Day 92	6.6 ± 0.3	6.7 ± 0.1	6.8 ± 0.1[2]	6.9 ± 0.1	6.3 ± 0.1**	5.7 ± 0.1**
Albumin (g/dL)						
Day 5	4.3 ± 0.1	4.2 ± 0.0	4.2 ± 0.1	4.1 ± 0.0*	4.0 ± 0.0**	4.0 ± 0.1**
Day 21	4.40 ± 0.06	4.51 ± 0.05	4.44 ± 0.04	4.24 ± 0.03*	4.16 ± 0.05**	3,91 ± 0.04**
Day 92	4.8 ± 0.2	4.9 ± 0.1	4.9 ± 0.1[2]	5.1 ± 0.1	4.6 ± 0.1**	4.0 ± 0.1**
Creatinine (mg/dL)						
Day 5	0.71 ± 0.02	0.71 ± 0.01	0.70 ± 0.02	0.69 ± 0.02	0.73 ± 0.02	0.70 ± 0.02
Day 21	0.69 ± 0.01	0.64 ± 0.02	0.65 ± 0.02	0.66 ± 0.02	0.67 ± 0.03	0.64 ± 0.02
Day 92	0.68 ± 0.01	0.69 ± 0.02	0.68 ± 0.02[2]	0.68 ± 0.01	0.69 ± 0.01	0.77 ± 0.02**
Urea nitrogen (mg/dL)						
Day 5	21.9 ± 0.6	22.3 ± 0.6	23.1 ± 0.7	22.9 ± 0.9	25.8 ± 0.5**	24.8 ± 0.8**
Day 21	22.1 ± 0.4	20.7 ± 0.5	21.3 ± 0.6	22.1 ± 0.6	24.2 ± 0.8	24.9 ± 0.7*
Day 92	17.1 ± 0.4	18.0 ± 0.4	19.7 ± 0.3**[2]	18.0 ± 0.5*	20.6 ± 0.5**	22.9 ± 0.5**

[1] Mean ± standard error.
[2] n=9.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE C3 Urinalysis Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
MALE						
n	10	10	10	10	10	10
Creatinine (mg/dL)						
Day 19	52.75 ± 8.99	42.68 ± 6.37	53.38 ± 6.17	66.52 ± 6.27	38.79 ± 3.05[2]	48.25 ± 5.02
Day 90	109.05 ± 21.53	92.15 ± 11.11	96.59 ± 9.40	91.71 ± 9.53	91.59 ± 12.69	85.59 ± 6.89
Glucose (mg/dL)						
Day 19	13 ± 2	11 ± 2	14 ± 2	19 ± 1	10 ± 1[2]	13 ± 2
Day 90	19 ± 3	18 ± 2	18 ± 2	16 ± 2	18 ± 3	18 ± 2
Glucose output (mg/mg creatinine)						
Day 19	0.25 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.30 ± 0.02*	0.26 ± 0.00	0.26 ± 0.01
Day 90	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.20 ± 0.01*	0.21 ± 0.01**
Protein (mg/dL)						
Day 19	225 ± 44	171 ± 30	231 ± 30	291 ± 27	155 ± 13[2]	139 ± 16
Day 90	282 ± 52	271 ± 38	270 ± 27	259 ± 26	268 ± 46	269 ± 24
Protein output (mg/mg creatinine)						
Day 19	4.10 ± 0.14	3.84 ± 0.18	4.26 ± 0.08	4.37 ± 0.09	4.04 ± 0.10	2.88 ± 0.12**
Day 90	2.59 ± 0.07	2.87 ± 0.11	2.79 ± 0.07	2.83 ± 0.09	2.85 ± 0.09*	3.14 ± 0.09**
Aspartate aminotransferase (IU/L)						
Day 19	4 ± 1[2]	4 ± 0[2]	5 ± 0	6 ± 1*[2]	11 ± 2**	7 ± 1**
Day 90	8 ± 2	7 ± 1	9 ± 1	8 ± 1	10 ± 2	37 ± 8**
Aspartate aminotransferase (IU/mg creatinine)						
Day 19	0.09 ± 0.01	0.10 ± 0.01[2]	0.09 ± 0.01	0.09 ± 0.01[2]	0.21 ± 0.03**[2]	0.15 ± 0.03**
Day 90	0.077 ± 0.004	0.076 ± 0.006	0.096 ± 0.010	0.090 ± 0.004	0.101 ± 0.007*	0.427 ± 0.081**
N-acetyl-β-D-glucosaminidase (IU/L)						
Day 19	6.7 ± 1.0	5.0 ± 0.6	7.0 ± 0.7	7.9 ± 0.9	5.9 ± 0.8	6.7 ± 0.7
Day 90	8.9 ± 1.1	9.2 ± 1.1	8.7 ± 0.6	8.3 ± 0.5	9.8 ± 1.2	18.1 ± 1.7**
N-acetyl-β-D-glucosaminidase (in IU/mg creatinine)						
Day 19	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
Day 90	0.09 ± 0.00	0.10 ± 0.00*	0.09 ± 0.00	0.10 ± 0.01	0.11 ± 0.01**	0.22 ± 0.02**
Volume (mL/16 hr)						
Day 19	9.4 ± 1.3	10.1 ± 1.3	8.9 ± 1.1	6.0 ± 0.9	9.5 ± 1.1	6.7 ± 0.9
Day 90	7.9 ± 0.9	8.1 ± 1.5	6.2 ± 0.7	7.6 ± 0.9	7.7 ± 1.2	5.6 ± 0.7
Specific gravity						
Day 19	1.023 ± 0.004	1.019 ± 0.003	1.025 ± 0.003	1.032 ± 0.003	1.021 ± 0.003	1.023 ± 0.002
Day 90	1.035 ± 0.005	1.038 ± 0.005	1.032 ± 0.003	1.031 ± 0.003	1.034 ± 0.005	1.036 ± 0.003
FEMALE						
n	10	10	10	10	10	10
Creatinine (mg/dL)						
Day 19	27.22 ± 7.16	20.55 ± 1.86	18.02 ± 1.67	19.84 ± 3.17	19.95 ± 1.54	17.03 ± 1.67
Day 90	63.77 ± 11.03	47.11 ± 6.30	55.36 ± 7.77[2]	93.35 ± 10.96	53.87 ± 7.51	54.54 ± 7.47
Glucose (mg/dL)						
Day 19	7 ± 2	5 ± 0	4 ± 0	5 ± 1	5 ± 0	4 ± 0
Day 90	7 ± 1	6 ± 1[2]	6 ± 1[2]	12 ± 2	6 ± 1	6 ± 1
Glucose output (mg/mg creatinine)						
Day 19	0.23 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.23 ± 0.01
Day 90	0.11 ± 0.01	0.11 ± 0.01[2]	0.11 ± 0.01[2]	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.01
Protein (mg/dL)						
Day 19	34 ± 16	22 ± 4	17 ± 3	21 ± 6	21 ± 3	15 ± 3
Day 90	67 ± 15	57 ± 7[2]	55 ± 12[2]	106 ± 14	50 ± 11	55 ± 9

TABLE C3 Urinalysis Data for F344/N Rats in the 13-Week Feed Study of Cupric Sulfate (continued)

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
FEMALE (continued)						
Protein output (mg/mg creatinine)						
Day 19	0.86 ± 0.19	0.99 ± 0.10	0.87 ± 0.12	0.97 ± 0.12	1.07 ± 0.06[2]	0.82 ± 0.12
Day 90	0.94 ± 0.09	1.07 ± 0.04[2]	0.88 ± 0.12[2]	1.12 ± 0.03	0.83 ± 0.08	0.98 ± 0.04
Aspartate aminotransferase (IU/L)						
Day 19	2 ± 0[2]	2 ± 0	2 ± 0	2 ± 0	2 ± 0[2]	6 ± 1**[2]
Day 90	3 ± 0	3 ± 0	3 ± 1[2]	6 ± 1**	8 ± 1**	51 ± 8**
Aspartate aminotransferase (IU/mg creatinine)						
Day 19	0.08 ± 0.01[2]	0.11 ± 0.02*	0.08 ± 0.01[2]	0.10 ± 0.01	0.11 ± 0.01*[2]	0.36 ± 0.04**[2]
Day 90	0.044 ± 0.004	0.061 ± 0.008	0.064 ± 0.005**[2]	0.067 ± 0.006**	0.150 ± 0.012**	0.959 ± 0.110**
N-acetyl-β-D-glucosaminidase (IU/L)						
Day 19	4.2 ± 0.8	3.0 ± 0.4	2.2 ± 0.3	3.4 ± 0.7	3.3 ± 0.4	2.8 ± 0.4
Day 90	6.3 ± 1.0	5.5 ± 0.6	5.8 ± 0.8[2]	9.8 ± 0.9*	8.9 ± 1.3	19.8 ± 2.7**
N-acetyl-β-D-glucosaminidase (IU/mg creatinine)						
Day 19	0.17 ± 0.02	0.15 ± 0.02	0.13 ± 0.01	0.17 ± 0.02	0.17 ± 0.02	0.17 ± 0.02
Day 90	0.10 ± 0.01	0.13 ± 0.02	0.11 ± 0.01[2]	0.11 ± 0.01	0.16 ± 0.01**	0.38 ±0.03**
Volume (mL/16 hr)						
Day 19	11.5 ± 1.5	12.3 ± 1.0	13.3 ± 1.7	12.7 ± 1.1	11.5 ± 0.8	12.8 ± 1.4
Day 90	7.3 ± 1.4	7.6 ± 1.1	7.4 ± 1.3[2]	3.8 ± 0.6	6.8 ± 1.0	6.6 ± 1.3
Specific gravity						
Day 19	1.016 ± 0.004	1.010 ± 0.001	1.010 ± 0.001	1.010 ± 0.001	1.010 ± 0.001	1.009 ± 0.001
Day 90	1.021 ± 0.003	1.016 ± 0.002	1.018 ± 0.002[2]	1.029 ± 0.004	1.018 ± 0.002	1.018 ± 0.002

[1] Mean ± standard error.
[2] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

APPENDIX D

Reproductive Tissue Evaluations and Estrous Cycle Characterization

TABLE D1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

Study Parameters	0 ppm	500 ppm	2000 ppm	4000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	361 ± 5	345 ± 9	352 ± 11	339 ± 5*
Left epididymis	0.440 ± 0.009	0.428 ± 0.004	0.444 ± 0.013	0.432 ± 0.007
Left cauda epididymis	0.145 ± 0.006	0.139 ± 0.005	0.146 ± 0.004	0.138 ± 0.004
Left testis	1.51 ± 0.02	1.49 ± 0.03	1.52 ± 0.04	1.59 ± 0.08
Spermatid measurements				
Spermatid heads (10^7/g testis)	10.83 ± 0.42	11.39 ± 0.83	12.66 ± 0.49	10.76 ± 0.57
Spermatid heads (10^7/testis)	8.05 ± 0.27	8.20 ± 0.62	9.20 ± 0.39	8.10 ± 0.36
Spermatid count (mean/10^{-4}mL suspension)	80.48 ± 2.74	82.03 ± 6.16	92.03 ± 3.89	81.03 ± 3.60
Spermatozoal measurements				
Motility (%)	71.44 ± 1.95	72.98 ± 1.60	67.14 ± 2.16	70.09 ± 2.02
Concentration (10^6/g cauda epididymal tissue)	885.6 ± 66.5	810.7 ± 48.2	773.3 ± 37.3	782.2 ± 25.0

[1] Data presented as mean ± standard error. Differences from the control group for testis, epididymal, and cauda epididymal weights, spermatid measurements, and spermatozoal measurements are not significant by Dunn's test.

* Significantly different (P≤0.05) from the control group by Williams' test.

TABLE D2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

Study Parameters	0 ppm	500 ppm	2000 ppm	4000 ppm
n	10	10	10	10
Necropsy body weight (g)	196 ± 2	194 ± 3	196 ± 3	190 ± 3**
Estrous cycle length (days)	4.85 ± 0.11	4.75 ± 0.11	4.95 ± 0.09	5.20 ± 0.13
Estrous stages (% of cycle)				
Diestrus	33.3	37.5	36.7	42.5
Proestrus	10.8	11.7	10.0	10.8
Estrus	33.3	31.7	31.7	25.8
Metestrus	22.5	19.2	20.8	20.0
Uncertain diagnoses (%)	0.0	0.0	0.8	0.8

[1] Data presented as mean ± standard error. Estrous cycle lengths are not significant by Shirley's test. By multivariate analysis of variance (MANOVA), dosed groups do not differ significantly from controls in cycle length or in the relative length of time spent in the estrous stages.

** Significantly different (P≤0.01) from the control group by Williams' test.

TABLE D3 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate[1]

Study Parameters	0 ppm	1000 ppm	4000 ppm	8000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	33.5 ± 0.7	32.8 ± 0.7	29.7 ± 0.6**	29.2 ± 0.7**
Left epididymis	0.040 ± 0.001	0.040 ± 0.001	0.040 ± 0.001	0.040 ± 0.001
Left cauda epididymis	0.024 ± 0.011	0.013 ± 0.001	0.013 ± 0.001	0.013 ± 0.000
Left testis	0.115 ± 0.002	0.112 ± 0.001	0.116 ± 0.004	0.117 ± 0.003
Spermatid measurements				
Spermatid heads (10^7/g testis)	18.75 ± 1.25	21.21 ± 0.78	18.24 ± 1.65	18.38 ± 0.94
Spermatid heads (10^7/testis)	2.16 ± 0.14	2.39 ± 0.10	2.08 ± 0.15	2.16 ± 0.14
Spermatid count (mean/10^{-4}mL suspension)	67.43 ± 4.48	74.68 ± 3.26	65.10 ± 4.82	67.63 ± 4.37
Spermatozoal measurements				
Motility (%)	75.67 ± 1.37	70.81 ± 2.20	70.43 ± 1.33	77.45 ± 1.14
Concentration (10^6/g cauda epididymal tissue)	1374 ± 88	1225 ± 58	1341 ± 113	1242 ± 90

[1] Data presented as mean ± standard error. Differences from the control group for testis, epididymal, and cauda epididymal weights, spermatid measurements, and spermatozoal measurements are not significant by Dunn's test.
** Significantly different (P≤0.01) from the control group by Williams' test.

TABLE D4 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate[1]

Study Parameters	0 ppm	1000 ppm	4000 ppm	8000 ppm
n	10	10	10	10
Necropsy body weight (g)	30.1 ± 0.9	29.5 ± 1.0	27.8 ± 0.6*	26.2 ± 0.6**
Estrous cycle length (days)	4.05 ± 0.05	4.00 ± 0.00	4.00 ± 0.00	4.10 ± 0.07
Estrous stages (% of cycle)				
Diestrus	28.3	29.2	29.2	25.0
Proestrus	16.7	15.0	11.7	19.2
Estrus	30.8	33.3	38.3	32.5
Metestrus	22.5	20.8	20.8	23.3
Uncertain diagnoses (%)	1.7	1.7	0.0	0.0

[1] Data presented as mean ± standard error. Estrous cycle lengths are not significant by Dunn's test. By multivariate analysis of variance (MANOVA), dosed groups do not differ significantly from controls in cycle length or in the relative length of time spent in the estrous stages.
* Significantly different (P≤0.05) from the control group by Williams' test.
** Significantly different (P≤0.01) from the control group by Williams' test.

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