Copper

Copper and its compounds are used in electrical wiring, water pipes, cooking utensils, and electroplating, and as algicides and food additives. Copper concentrations in drinking-water vary widely as a result of variations in pH, hardness, and copper availability in the distribution system. Levels of copper in running water tend to be low, whereas those of standing or partially flushed water samples are more variable and can be substantially higher, particularly in areas where the water is soft and corrosive. Adult intake of copper from food is usually 1-2 mg/day and may be considerably increased by consumption of standing or partially flushed water from a system that contains copper pipes or fittings.

Copper is an essential nutrient, required for the proper functioning of many important enzyme systems. In mammals, absorption of copper occurs in the upper gastrointestinal tract and is controlled by a complex homeostatic process. Absorption is influenced by the presence of competing metals, dietary proteins, fructose, and ascorbic acid. The major excretory pathway for absorbed copper is bile. In humans, the highest concentrations of copper are found in the liver, brain, heart, kidney, and adrenal glands. The liver of newborn infants contains about 10 times as much copper as the adult liver and accounts for 50-60% of the total body copper.

Copper utilization is affected by a number of genetic disorders. The genetic abnormalities associated with Menke syndrome, Wilson disease, and aceruloplasminaemia are fairly well understood, and there is some evidence to suggest a genetic basis for Indian childhood cirrhosis and idiopathic copper toxicosis.

Acute gastrointestinal effects may result from exposure to copper in drinking-water, although the levels at which such effects occur are not defined with any precision. Long-term intake of copper in the diet in the range 1.5-3 mg/day has no apparent adverse effects. Daily intake of copper below this range can lead to anaemia, neutropenia, and bone demineralization in malnourished children. Adults are more resistant than children to the symptoms of copper deficiency.

The IPCS Task Group responsible for preparation of the Environmental Health Criteria monograph for copper concluded that:

> "The upper limit of the AROI [acceptable range of oral intake] in adults is uncertain but it is most likely in the range of several but not many mg per day... (several meaning more than 2 or 3 mg/day). This evaluation is based solely on studies of gastrointestinal effects of copper-contaminated drinking-water. A more specific value for the upper AROI could not be confirmed for any segment of the general population... The available data on toxicity in animals were considered unhelpful in establishing the upper limit of the AROI, due to uncertainty about an appropriate model for humans."

A copper level of 2 mg/litre in drinking-water should not cause any adverse effects and provides an adequate margin of safety. The epidemiological and clinical studies conducted to date are too limited to allow a clear effect level to be established with any accuracy. Thus, it is recommended that this guideline value for copper of 2 mg/litre remain provisional as a result of uncertainties in the dose - response relationship between copper in drinking-water and acute gastrointestinal effects in humans. It is also noteworthy that copper is an essential element.

It is stressed that the outcome of epidemiological studies in process in Chile, Sweden, and the USA may permit more accurate quantification of effect levels for copper-induced toxicity in humans, including sensitive subpopulations.

Staining of laundry and sanitary ware occurs at copper concentrations above 1 mg/litre. At levels above 5 mg/litre, copper also imparts a colour and an undesirable bitter taste to water.

Copper

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In view of uncertainties regarding copper toxicity in humans, a provisional guideline value for copper of 2 mg/litre was established in the 1993 WHO *Guidelines for drinking-water quality*, based on a 10% allocation of the 1982 JECFA PMTDI to drinking-water. The PMTDI was established as 10 times the normal copper intake of 0.05 mg/kg of body weight per day, which was considered to be without adverse effect.[1] Copper was selected for re-evaluation by the Coordinating Committee for the updating of the *Guidelines* because of the provisional guideline value and because the development of an IPCS Environmental Health Criteria monograph for copper was under way.

[1] It was erroneously assumed in the 1993 *Guidelines* that the PMTDI established by JECFA was based on a study in dogs.

1. GENERAL DESCRIPTION

1.1 Identity

Copper (CAS no. 7440-50-8) is a transition metal that is stable in its metallic state and forms monovalent (cuprous) and divalent (cupric) cations. Common copper compounds include the following:

Compound	*CAS no.*
Copper(II) acetate monohydrate [$Cu(C_2H_3O_2)_2 \cdot H_2O$]	6046-93-1
Copper(II) chloride [$CuCl_2$]	7447-39-4
Copper(II) nitrate trihydrate [$Cu(NO_3)_2 \cdot 3H_2O$]	10031-43-3
Copper(II) oxide [CuO]	1317-38-0
Copper(II) sulfate pentahydrate [$CuSO_4 \cdot 5H_2O$]	7758-99-8

1.2 Physicochemical properties (Weast, 1983; ATSDR, 1990; Lewis, 1993)

Compound	*Density (g/cm^3)*	*Water solubility (g/litre)*
Copper(II) acetate monohydrate	1.88	72
Copper(II) chloride	3.39	706
Copper(II) nitrate trihydrate	2.32	1378
Copper(II) oxide	6.32	Insoluble
Copper(II) sulfate pentahydrate	2.28	316

1.3 Organoleptic properties

Dissolved copper imparts a light blue or blue-green colour and an unpleasant, metallic, bitter taste to drinking-water. The taste threshold is between 1 and 5 mg/litre and is influenced by the presence of other solutes (ATSDR, 1990; Olivares & Uauy, 1996a). Blue to green staining of porcelain sinks and plumbing fixtures occurs from copper dissolved in tap-water.

1.4 Major uses

Metallic copper is malleable, ductile, and a good thermal and electrical conductor. It has many commercial uses because of its versatility. Copper is used to make electrical wiring, pipes, valves, fittings, coins, cooking utensils, and building materials. It is present in munitions, alloys, and coatings. Copper compounds are used as or in fungicides, algicides, insecticides, wood preservatives, electroplating, azo dye manufacture, engraving, lithography, petroleum refining, and pyrotechnics. Fertilizers, animal feeds, and pharmaceuticals can contain copper compounds (ATSDR, 1990; Lewis, 1993). Copper compounds are also used as food additives (e.g. nutrient and/or colouring agent) (FAO/IPCS, 1994). Copper sulfate pentahydrate is sometimes added to surface water for the control of algae (NSF, 1996). Copper sulfate was once prescribed as an emetic, but this use has been discontinued owing to adverse health effects (Ellenhorn & Barceloux, 1988).

1.5 Environmental fate

The fate of elemental copper in water is complex and influenced by pH, dissolved oxygen, and the presence of oxidizing agents and complexing compounds or ions (US EPA, 1995). Surface oxidation of copper produces copper(I) oxide or hydroxide. In most instances, copper(I) ion is subsequently oxidized to copper(II) ion. However, copper(I) ammonium and copper(I) chloride complexes, when they form, are stable in aqueous solution.

In pure water, the copper(II) ion is the more common oxidation state (US EPA, 1995). Copper(II) ions will form complexes with hydroxide and carbonate ions. The formation of insoluble malachite [$Cu_2(OH)_2CO_3$] is a major factor in controlling the level of free copper(II) ion in aqueous solutions. Copper(II) ion is the major species in water at pHs up to 6; at pH 6-9.3, aqueous $CuCO_3$ is prevalent; and at pH 9.3-10.7, the aqueous $[Cu(CO_3)_2]^{2-}$ ion predominates (Stumm & Morgan, 1981).

In one copper-polluted, organic-depleted lake, soluble copper speciated in the order $CuOH^+ > Cu^{2+} > CuCO_3$ based on equilibrium calculations (Lopez & Lee, 1977). Dissolved copper ions are removed from solution by sorption to clays, minerals, and organic solids or by precipitation (Callahan et al., 1979). Copper has been reported to strongly adsorb to clay materials in a pH-dependent fashion; such adsorption is increased by the presence of particulate organic materials (Payne & Pickering, 1975; Huang et al., 1977; Brown et al., 1983). Copper discharged to wastewater is concentrated in sludge during treatment. Sorption can be reversed in a reducing or acidic environment; thus, sediments and sludge can act as a reservoir for copper ions (Lopez & Lee, 1977). Copper ions form chelates with humic acids and polyvalent organic anions (Cotton & Wilkinson, 1980). The presence of chelating agents increases the solubility of copper in an aqueous medium.

2. ANALYTICAL METHODS

The most important analytical methods for the detection of copper in water are atomic absorption spectrometry (AAS) with flame detection, graphite furnace atomic absorption spectroscopy, inductively coupled plasma atomic emission spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS), and stabilized temperature platform graphite furnace atomic absorption (ISO, 1986, 1996; ASTM, 1992, 1994; US EPA, 1994). The ICP-MS technique has the lowest detection limit (0.02 µg/litre) and the AAS technique the highest (10 µg/litre). Detection limits for the other three techniques range from 0.7 to 3 µg/litre. Measurement of dissolved copper requires sample filtration; results from unfiltered samples include dissolved and particulate copper.

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Copper is present in the atmosphere from wind dispersion of particulate geological materials and particulate matter from smokestack emissions. In a nationwide study by the US EPA for the years 1977-1983, the range of copper concentrations in 23 814 air samples was 0.003-7.32 μg/m^3 (US EPA, 1987).[2] Median values for different cities and years ranged from 0.004 to 1.79 μg/m^3, and mean values ranged from 0.0043 to 1.96 μg/m^3. Concentrations of copper determined in over 3800 samples of ambient air at 29 sites in Canada over the period 1984-1993 averaged 0.014 μg/m^3. The maximum value was 0.418 μg/m^3 (WHO, in press). Atmospheric copper is removed by gravitational settling, dry disposition, rain, and snow.

[2] Additional source: Computer printout of frequency distribution listing of copper in air, 1977-1983. Research Triangle Park, NC, US Environmental Protection Agency, Environmental Monitoring Systems Laboratory.

3.2 Water

Because copper is a naturally occurring element, it is found ubiquitously in surface water, groundwater, seawater, and drinking-water (ATSDR, 1990; US EPA, 1991). In a 1969 survey of 678 groundwater supplies in the USA, the maximum reported copper concentration was 0.47 mg/litre, whereas the mean concentration in samples exceeding the detection limit (0.010 mg/litre) was 0.075 mg/litre (US EPA, 1991). Comparative values for 109 surface water supplies were 0.304 and 0.066 mg/litre, respectively. Copper levels in surface water samples were reported to range from 0.0005 to 1 mg/litre, with a median of 0.01 mg/litre (ATSDR, 1990). In the United Kingdom, the mean copper concentration in the River Stour was 0.006 mg/litre (range 0.003-0.019 mg/litre). Background levels were 0.001 mg/litre, derived from an upper catchment control site. Fourfold increases in copper concentrations were apparent downstream of a sewage treatment plant. In an unpolluted zone of the River Periyar in India, copper concentrations ranged from 0.0008 to 0.010 mg/litre (WHO, in press).

Copper concentrations in drinking-water vary widely as a result of variations in pH, hardness, and copper availability in the distribution system. A number of studies indicate that copper levels in drinking-water samples can range from ≤0.005 to 18 mg/litre, with the primary source most often being the corrosion of interior copper plumbing (ATSDR, 1990; US EPA, 1991). Levels of copper in running or fully flushed water tend to be low, whereas those of standing or partially flushed water samples are more variable and can be substantially higher (frequently ≥1 mg/litre) (ATSDR, 1990). In the Netherlands, copper concentrations between 0.2 and 3.8 mg/litre were reported in water standing for 16 hours. Average copper levels in water from municipalities were between 0.04 and 0.69 mg/litre. In two cities in Sweden, the mean standing water copper level was 0.7 mg/litre, with a 90th percentile of 2.1 mg/litre; in water for consumption, the mean copper concentration was 0.6 mg/litre, with a 90th percentile of 1.6 mg/litre. In distributed water from 70 municipalities across Canada, mean concentrations of copper ranged from 0.02 to 0.075 mg/litre; maximum values ranged up to 0.56 mg/litre. In about 20% of the distributed water supplies, the level of copper was significantly higher than in the corresponding treated water samples. Furthermore, the increase was higher in those areas where the water was soft and corrosive (WHO, in press). In another survey in Canada, the median copper concentration in distributed water was 0.27 mg/litre (range >0.01-0.9 mg/litre) for 27 supplies with acid or neutral pH (Health Canada, 1992). Some of the copper in drinking-water may derive from treatment of surface water sources with copper sulfate algicides (US EPA, 1991).

3.3 Food

Food is a principal source of copper exposure for humans. Liver and other organ meats, seafood, nuts, and seeds are good sources of dietary copper (NAS, 1989). Vitamin/mineral preparations

for children and adults generally contain 2 mg of copper as copper oxide. Infant formula contains 0.6-2 µg of copper per kcal (Olivares & Uauy, 1996b).

Based on data collected during the US Food and Drug Administration's Total Diet Study (1982-1986), the average dietary intake of copper for adult males was 1.2 mg/day, whereas that for adult females was 0.9 mg/day. The average intake for infants (6 months to 1 year) was 0.45 mg/day, and that for 2-year-olds was 0.57 mg/day (Pennington et al., 1989).

In Scandinavian countries, intakes are in the range of 1.0-2.0 mg/day for adults, 2 mg/day for lactovegetarians, and 3.5 mg/day for vegans (Pettersson & Sandstrum, 1995; WHO, in press). The United Kingdom reported intakes of 1.2 and 1.6 mg/day for adult females and males, respectively, and 0.5 mg/day for children 1½ to 4½ years old. Australia reported intakes of 2.2 and 1.9 mg/day for adult females and males, respectively, and 0.8 mg/day for the 2-year-old. In Germany, the dietary intake of adults was 0.95 mg/day (WHO, in press).

Copper is an essential nutrient. In the USA, the estimated safe and adequate dietary intake for copper is 1.5-3 mg/day for adults, 0.4-0.6 mg/day for infants, and 0.7-2 mg/day for children; although average intakes for adults appear to be suboptimum, there is little evidence of copper deficiency in the population (NAS, 1989). Estimates of average copper requirements are 12.5 µg/kg of body weight per day for adults and about 50 µg/kg of body weight per day for infants (WHO, 1996).

3.4 Estimated total exposure and relative contribution of drinking-water

Food and water are the primary sources of copper exposure in developed countries. In general, dietary copper intakes for adults range from 1 to 2 mg/day (WHO, in press); use of a vitamin/mineral supplement will increase exposure by 2 mg/day. Drinking-water contributes 0.1-1 mg/day in most situations. Thus, daily copper intakes for adults usually range from 1 to 3 mg/day. Consumption of standing or partially flushed water from a system that contains copper pipes or fittings can considerably increase total daily copper exposure, especially for infants fed formula reconstituted with tap-water.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Absorption of orally administered copper in mammals occurs in the upper gastrointestinal tract and is controlled by a complex homeostatic process involving active and passive transport, plus binding sites on metallothionein and other proteins in the mucosal cells (Linder & Hazegh-Azam, 1996). Mucosal and serosal transport mechanisms differ. Movement of copper across the mucosa occurs by diffusion or facilitated transport. The presence of competing metals, dietary proteins, anions, fructose, and ascorbic acid influences copper uptake from the gastrointestinal tract (Lonnerdal, 1996). Using an *in vivo* intestinal perfusion system in rats, it was demonstrated that excess concentrations of cations having an electronic configuration similar to that of copper [i.e. iron(II), zinc, tin, and cobalt] in the presence of a high-affinity ligand such as *L*-histidine can significantly inhibit the intestinal absorption and/or retention of copper (Wapnir et al., 1993).

Within the mucosal cells, 80% of the copper is found in the cytosol bound to proteins, especially metallothionein. Transport from the mucosal cells is mediated by a *p*-type ATPase active transport system. Serosal transport of copper is inhibited in Menke's syndrome, one of several genetic diseases related to copper. Copper in the portal blood is bound to albumin or transcuprin; a small amount may be chelated by peptides and amino acids (Linder & Hazegh-Azam, 1996).

Copper uptake from the blood by the liver and distribution within the liver are not well understood. They are presumed to involve a transport process that differs from that in the intestines (Linder & Hazegh-Azam, 1996). Within the liver, copper becomes incorporated in ceruloplasmin, as well as the enzymes superoxide dismutase and cytochrome oxidase. Excess copper is bound to hepatic metallothionein. Ceruloplasmin is the primary copper transport protein in systemic circulation and

contains about 75% of the plasma copper (Luza & Speisky, 1996). Ceruloplasmin carries copper to the cells for uptake. It also has enzymatic activity as a ferrooxidase and functions in the synthesis of haemoglobin.

In humans, the highest concentrations of copper are found in the liver, brain, heart, kidney, and adrenal gland; moderate levels are found in the intestine, lung, and spleen; and low concentrations occur in endocrine glands, bone, muscle, larynx, trachea, aorta, and testes (Schroeder et al., 1966; Evans, 1973). Approximately 50% of the body's copper is found in muscle and bone tissue, whereas about 10% is stored in the liver (Evans, 1973; Luza & Speisky, 1996). The liver of newborn infants contains about 10 times the copper of the adult liver and accounts for 50-60% of the total body copper (Luza & Speisky, 1996).

Copper is an essential nutrient and is required for the proper functioning of many important enzyme systems. Copper-containing enzymes include ceruloplasmin, superoxide dismutase, cytochrome oxidase, tyrosinase, monoamine oxidase, lysyl oxidase, and phenylalanine hydroxylase (Linder & Hazegh-Azam, 1996). The activity of the enzyme superoxide dismutase can be used in the assessment of copper status (Olivares & Uauy, 1996b). Ceruloplasmin and serum copper concentrations are also employed as indicators of copper status.

Copper is excreted from the body in bile, faeces, sweat, hair, menses, and urine (Gollan & Deller, 1973; Luza & Speisky, 1996). In humans, the major excretory pathway for absorbed copper is bile, where copper is bound to both low-molecular-weight and macromolecular species. Biliary copper travels to the intestine; after minimal reabsorption, it is eliminated in the faeces. In normal humans, <3% of the daily copper intake is excreted in the urine (Luza & Speisky, 1996). Urinary copper may originate from amino acid-bound metal or from dissociated copper-albumin complexes; erythrocyte or ceruloplasmin-bound copper generally will not permeate the glomerulus, thus preventing its excretion (Evans, 1973).

There are several genetic disorders that affect copper utilization. The genetic abnormalities associated with Menke syndrome, Wilson's disease, and aceruloplasminaemia are fairly well understood. There is some evidence to suggest a genetic basis for Indian childhood cirrhosis and idiopathic copper toxicosis (Muller et al., 1996; Olivares & Uauy, 1996a). In the case of Menke syndrome and Wilson's disease, the affected genes have been identified. Data suggest that the predisposing genetic component for idiopathic copper toxicosis may be an autosomal recessive gene (Muller et al., 1996). Individuals with a glucose-6-phosphate dehydrogenase deficiency disorder have an increased susceptibility to copper-induced oxidation reactions and methaemoglobin formation (Moore & Calabrese, 1980), but there is no direct effect of the disorder on copper uptake, distribution, or metabolism.

In Menke syndrome, there is minimal copper absorption from the intestines, leading to death during early childhood (Harris & Gitlin, 1996; Olivares & Uauy, 1996a). Children suffering from Menke syndrome (an X chromosome-linked disorder) exhibit mental deterioration, failure to thrive, hypothermia, and connective tissue abnormalities (Harris & Gitlin, 1996). The defective protein is a *p*-type ATPase responsible for intestinal transport of copper; a child with Menke syndrome suffers from a profound copper deficiency, despite adequate dietary copper. There is no effective treatment for Menke syndrome, although administration of copper as the dihistidine complex delays the development of symptoms (Linder & Hazegh-Azam, 1996).

Wilson's disease is an autosomal recessive disorder that affects the hepatic intracellular transport of copper and its subsequent inclusion into ceruloplasmin and bile. A *p*-type ATPase is again affected, but the enzyme is different from that affected in Menke syndrome. Because copper is not incorporated into ceruloplasmin, its normal systemic distribution is impaired, and copper accumulates in the liver, brain, and eyes (Pennington et al., 1989; Harris & Gitlin, 1996). Wilson disease generally appears in late childhood and is accompanied by hepatic cirrhosis, neurologic degeneration, and copper deposits in the cornea of the eye (Kayser-Fleischer rings). Patients with Wilson disease are treated with chelating agents, such as penicillamine, to promote copper

excretion (Yarze et al., 1992). Patients that follow their therapeutic regime can expect to live a normal life (Scheinberg & Sternlieb, 1996). Restriction of dietary copper alone cannot influence the progression of the disease.

Aceruloplasminaemia is an autosomal recessive disorder caused by changes in the ceruloplasmin gene that affect the ability of ceruloplasmin to bind copper (Harris & Gitlin, 1996). The symptoms of aceruloplasminaemia do not become apparent until adulthood. They include dementia, diabetes, retinal degeneration, and increased tissue iron stores.

The etiologies of Indian childhood cirrhosis and idiopathic copper toxicosis are complex and may involve a combination of genetic, developmental, and environmental factors (Muller et al., 1996; Pandit & Bhave, 1996). Both disorders are characterized by liver enlargement, elevated copper deposits in liver cells, pericellular fibrosis, and necrosis. Both disorders are generally fatal. Exposure to elevated levels of copper in milk or infant formula prepared in copper-containing vessels or with water containing elevated copper concentrations is believed to contribute to the hepatic copper overload. Poor biliary excretion of copper may also play a role in the etiology of the disease.

5. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

Acute responses to copper vary with species and copper compound. Sheep and dogs are more sensitive to copper than rodents, pigs, and poultry (Linder & Hazegh-Azam, 1996). Soluble copper salts are more toxic than insoluble compounds.

In a classic study, Wang & Borison (1951) evaluated the acute emetic response of 107 mongrel dogs to a single dose of copper sulfate pentahydrate in aqueous solution. In this group, 20 (19%) responded to 20 mg of copper sulfate [5 mg of copper(II)] with a mean response latency of 19 ± 11 minutes. Ninety-one dogs (85%) responded to 40 mg of copper sulfate [10 mg of copper(II)] with a response latency of 16 ± 9 minutes, and all animals responded to an 80-mg dose of copper sulfate [20 mg of copper(II)] (latency 19 ± 7 minutes). Some of the animals were then subjected to a vagotomy, sympathectomy, or vagotomy and sympathectomy. After severing of the neural pathways, the dogs were again exposed to copper sulfate. The acute dose required to elicit the emetic response increased in the vagotomized and in the vagotomized/sympathectomized dogs, as did the response latency time. The greatest effects on response threshold and latency time were seen in the vagotomized/sympathectomized dogs. The authors postulated that the emetic action of copper sulfate in dogs is biphasic. The initial rapid response event is caused by the action of copper sulfate on the peripheral nervous system and is the most sensitive response. The secondary response is elicited by the central nervous system and is responsive to absorbed copper. The requirement of the secondary response for absorption increases its latency time. More recent studies in dogs and ferrets (Bhandari & Andrews, 1991; Makale & King, 1992; Fukui et al., 1994) confirm the importance of gastrointestinal neural pathways and receptors in copper sulfate-induced emesis.

In one study, copper sulfate solution was infused into the stomach and duodenum of groups of four or five ferrets with ligated pyloric sphincters (Makale & King, 1992). In one group (four ferrets), the stomach infusion preceded the duodenal infusion; in the other group (five ferrets), the duodenal infusion preceded the stomach infusion. Infusion to the stomach resulted in vomiting in seven of nine ferrets with a mean latency of 4.4 minutes. Infusion to the duodenum resulted in vomiting in one of nine animals. The authors concluded that the primary site of the emetic response to copper sulfate is in the stomach of the ferret.

5.2 Short-term exposure

Several short-term studies of copper toxicity have been conducted in rats and mice. Effects were

largely the same in both species, but rats were slightly more sensitive than mice (Hébert et al., 1993).

Groups of five male and five female F 344/N rats were administered copper sulfate in their drinking-water for 2 weeks at estimated doses up to 97 mg of copper per kg of body weight per day (Hébert et al., 1993). A LOAEL of 10 mg of copper per kg of body weight per day observed in male rats was based on an increase in the size and number of protein droplets in the epithelial cells of the proximal convoluted tubules of the males. No renal effects were seen in the females receiving the same dose. There was no NOAEL for males in this study; the NOAEL for females was 26 mg of copper per kg of body weight per day.

When copper (as copper sulfate) was administered by gavage to 10 male albino rats at a dose of 25 mg/kg of body weight for 20 days, the kidneys displayed necrosis and tubular engorgement. Centrilobular necrosis, perilobular sclerosis, and periportal copper disposition were seen in the liver; haemoglobin and haematocrit values were decreased (Rana & Kumar, 1980).

Copper was less toxic to rats when administered in the diet than when administered in drinking-water or by gavage. This was true for 2-week and 13-week exposures. A dietary concentration of 1000 mg/kg of feed (estimated doses of 23 mg/kg of body weight per day for 2 weeks and 16 mg/kg of body weight per day for 13 weeks) had no adverse effects in male or female F 344/N rats (Hébert et al., 1993). Dietary concentrations of 2000 mg/kg of feed (44 mg/kg of body weight per day for 2 weeks and 33 mg/kg of body weight per day for 13 weeks) were associated with hyperplasia and hyperkeratosis of the squamous epithelium of the limiting ridge of the rat forestomach.

With the 13-week exposure and the 2000 mg/kg of feed (33 mg/kg of body weight per day) dietary concentration, protein droplets were present in the kidneys, and liver inflammation was noted (Hébert et al., 1993). Changes in liver and kidney histopathology were dose-related; males were affected more than females. Staining of the kidney cells for $\alpha_{2\mu}$ globulin was negative. Dose-related decreases in haematological parameters at 4000 mg/kg of feed (66 mg/kg of body weight per day) and 8000 mg/kg of feed (134 mg/kg of body weight per day) were indicative of a microcytic anaemia, whereas increases in serum enzymes were indicative of liver damage. There was some evidence of significant biochemical effects with the 2000 mg/kg of feed (33 mg/kg of body weight per day) exposure concentration and definitive effects at the 4000 mg/kg of feed (66 mg/kg of body weight per day) and 8000 mg/kg of feed (134 mg/kg of body weight per day) concentrations. Iron stores in the spleen were depleted, especially for the highest exposure concentration.

5.3 Long-term exposure

Male weanling Wistar rats (four per group) were given either a normal diet containing 10-20 mg of copper per kg of feed (controls) or diets supplemented with 3000, 4000, or 5000 mg of copper per kg of feed for 15 weeks (Haywood & Loughran, 1985). The animals receiving 3000 mg of copper per kg of feed were then allowed to continue the experimental regime for the remainder of the year. Assuming that rats consume 5% of their body weight per day in food, these dietary copper concentrations would correspond to approximate doses of 0.5-1.0, 150, 200, and 250 mg of copper per kg of body weight per day. All copper-supplemented groups exhibited reductions in body-weight gains relative to the control group that persisted until the end of the 15-week exposure period. For the 3000, 4000, and 5000 mg/kg of feed groups, copper concentrations in the liver peaked at 3-4 weeks, declined significantly by 6 weeks, but were still elevated at 15 weeks. Although the timing and duration varied somewhat, all supplemented groups evidenced hepatocellular necrosis during weeks 1-6, followed by a regeneration process that began after 3-5 weeks. The adaptation process noted during the latter part of the first 15 weeks of exposure continued during the 3000 mg/kg of feed group extension period. The average body weight recovered to 80% of that of the control group, and the copper concentration in the liver dropped from 1303 µg/g at 15 weeks to 440 µg/g at 52 weeks. However, even at 52 weeks, hepatic copper