5. EFFECTS ON HUMANS

5.1 Acute exposure

Paragraph 1

The acute lethal dose for adults lies between 4 and 400 mg of copper(II) ion per kg of body weight, based on data from accidental ingestion and suicide cases (Chuttani et al., 1965; Jantsch et al., 1984; Agarwal et al., 1993). Individuals ingesting large doses of copper present with gastrointestinal bleeding, haematuria, intravascular haemolysis, methemoglobinaemia, hepatocellular toxicity, acute renal failure, and oliguria (Agarwal et al., 1993).

Paragraph 2

At lower doses, copper ions can cause symptoms typical of food poisoning (headache, nausea, vomiting, diarrhoea). Records from case-study reports of gastrointestinal illness induced by copper from contaminated water or beverages plus public health department reports for 68 incidents indicate an acute onset of symptoms. Symptoms generally appear after 15–60 minutes of exposure; nausea and vomiting are more common than diarrhoea (Wyllie, 1957; Spitalny et al., 1984; US EPA, 1987; Knobeloch et al. 1994; Low et al., 1996; Stenhammar, 1999). Among outbreaks with quantitative data, the lowest copper concentrations associated with effects were about 4 mg/L or higher; background information on the amount of beverage consumed, sample collection, and analytical methods is limited. Some studies (Knobeloch et al. 1994; Stenhammar, 1999) reported lower effect levels in children.

Paragraph 3

Araya et al. (2001) conducted a double-blinded clinical study with a total of 179 subjects from three different international sites: Santiago, Chile (60); Grand Forks, North Dakota, USA (61); and Coleraine, Northern Ireland (58). Subjects fasted overnight and came to their test facility one morning per week for five weeks where they were given 200 mL of copper sulfate solution with randomly assigned concentrations of 0, 2, 4, 6, or 8 mg/L copper to drink. Subjects completed a "symptoms-signs" questionnaire immediately prior to treatment, 15 minutes after, and 24 hours later by telephone; they were also directly observed in a facility lounge for 1 hour after treatment. Of the GI symptoms (nausea, abdominal pain, vomiting, diarrhea), nausea (alone or in combination) was by far most commonly reported (by 27.3%), generally during the first 15 minutes after exposure; abdominal pain was the next most common. The nausea was transient, passing quickly in most cases. Vomiting in combination with nausea occurred in 5 instances; one each for 4 and 6 mg/L concentrations and 3 for the 8 mg/L concentration. One case of vomiting alone was experienced at the 6 mg/L concentration but was not felt to be related to the copper exposure. Diarrhea was reported to occur 1-24 hours after treatment by 5 subjects (2.8%; one was a control subject), and was not associated with nausea or vomiting. The authors concluded that site, sex, age, and dose order did not significantly affect response.

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Paragraph 4

Using logistic regression analysis and a statistical significance level of $p \leq 0.05$, data for nausea and for total GI symptoms defined a no observable adverse effect level (NOAEL) of 4 mg/L (14 incidents) and a lowest observable adverse effect level (LOAEL) of 6 mg/L (24 incidents). However, the number of individuals reporting symptoms for the 4 mg/L concentration (14) were about twice the numbers for the control and 2 mg/L groups (8 and 7 incidents) although not statistically significant. Odds ratios at 4 mg/L were 3.53 ($p = 0.07$) and 1.83 ($p = 0.2$) for nausea and total GI symptoms, respectively. Upper confidence levels on dose-response curves generated by a two-staged polynomial regression model indicated that 3% of the population would respond at 2.5-3 mg/L, while 5% would respond at 3.5-4 mg/L.

Paragraph 5

A second study of very similar design was conducted in Chile by Olivares et al. (2001). Purified water containing 0, 2, 4, 6, 8, and 12 mg/L copper as copper sulfate was ingested by 61 apparently healthy volunteers. The NOAEL was determined to be the 2 mg/L concentration and the LOAEL was 4 mg/L based on nausea incidence. The lowest concentration at which vomiting occurred was 6 mg/L. When copper at the same concentrations was ingested in an orange flavored drink the NOAEL increased to 6 mg/L and the LOAEL to 8 mg/L.

Paragraph 6

In a follow-up to the Araya et al. (2001) study discussed above, 269 females at the three original locations in Chile, Ireland and the United States plus a fourth location in China were tested using an adaptation of original design. Subjects ingested 0.4, 0.8, or 1.2 mg copper as its sulfate in volumes of 100, 150, or 200 mL solution (Poirier et al., 2002). Additional samples of 0 and 1.6 mg copper were added for the 200 mL volume. Nausea was the most frequently reported symptom. For any of the three volumes, the incidence of nausea increased with an increase in copper concentration. For a given copper mass, the nausea incidence increased as the volume decreased. There was a significant increase in nausea at a concentration of 6 mg/L with a 200 mL sample volume, identifying 4 mg/L as a statistical NOAEL. Using factorial analysis, the acute nausea concentration threshold was determined to be 5.3 mg/L.

5.2 Longer-term exposure

Paragraph 1

In a prospective study that was blind for the subjects, 60 healthy adult women from Santiago, Chile were randomized into four groups (Pizarro et al., 1999). Using a "Latin square" design, each group was sequentially exposed for two weeks to drinking-water containing copper sulfate at concentrations of 0, 1, 3, and 5 mg/L copper; the order of concentrations given differed for each group, and each two-week exposure period was separated from the next by a week of rest. Indicators of copper homeostasis (serum copper and serum ceruloplasmin) and liver status (serum levels of hepatic enzymes) did not significantly change during the study. The incidence of gastrointestinal symptoms (one or

more of diarrhea, nausea, abdominal pain or vomiting) was significantly correlated ($p < 0.007$) to copper concentrations in drinking water and was 5, 8, 23, and 22% at 0, 1, 3, and 5 mg/L, respectively. The apparent response threshold was between 1 and 3 mg/L. There was some evidence of an adaptive response for diarrhea, but not the other symptoms.

Paragraph 2

In a second study, Pizarro et al. (2001) compared the abilities of soluble copper sulfate and insoluble copper oxide (the most common copper form in vitamin supplements) to induce gastrointestinal symptoms (one or more of nausea, abdominal pain, vomiting or diarrhea). The double-blinded study used 45 healthy adult women (ages 18-55) from Santiago, Chile, who were randomized into 3 groups that differed in the order to which they were exposed to the different copper solutions (Latin square design). The study consisted of five exposure weeks alternating with four "break" weeks. Drinking water containing 5 mg/L copper was provided with one of the following ratios of copper from its sulfate to copper from its oxide: 0:5, 1:4, 2:3, 3:2, and 5:0. Subjects recorded the amounts of test water consumed and any symptoms experienced. No significant changes in indicators of copper status or liver function were observed during the study. When compared with low-copper tap-water, drinking the 5 mg/L copper water preparations collectively produced a four-fold increase in the incidence of total gastrointestinal symptoms. No significant differences in symptoms was observed for the different ratios of soluble:insoluble copper. There was some evidence for an adaptive response with respect to diarrhea, as six of twelve total episodes occurred during the first week, independent of copper dose.

Paragraph 3

Olivares et al. (1998) examined biomarkers of copper homeostasis and liver toxicity in a group of infants from three to 12 months of age . The infants all had birth weights greater than 2,000 grams; some were breast fed and others received formula preparations. Drinking water copper concentrations for the mother and child were either less than 0.1 mg/L (n=48) or 2 mg/L (n=80). Blood samples were collected from the infants after an overnight fast at 6, 9, and 12 months of age and analyzed for serum copper, ceruloplasmin, bilirubin, transaminases, and γ-glutamyl transferase, plus erythrocyte superoxide dismuase and erythrocyte metallothionein. A field worker collected information on water intake and clinical signs weekly. Minor differences in biomarkers of copper nutrition were noted but there were no consistent significant differences between groups for the biomarkers of liver toxicity. The incidence of diarrhea was higher in breast fed infants than in formula fed infants.

Paragraph 4

(1998)A 26-year-old male presented with symptoms of cirrhosis, liver failure, and Kayser-Fleischer rings after more than 2 years of self-prescribed use of copper supplements (O'Donohue et al., 1993). The patient ingested 30 mg of supplemental copper per day for 2 years and 60 mg/day for a poorly defined period of up to a year. Liver damage was extensive, and a transplant was required. The diseased liver had an average copper concentration of 3230 µg/g dry weight (normal 20–50 µg/g); tissue histopathology was similar to that seen in Indian childhood cirrhosis and Wilson's disease. Based on an

evaluation of the patient's family medical history and the copper excretion of his parents and sisters, he did not appear to carry the Wilson's disease gene. Liver damage apparently resulted from the prolonged daily exposure to three to six times the recommended upper limit for dietary copper.

Paragraph 5

Buchanan et al. (1999) reported on an epidemiologic investigation of the association of ingestion of drinking-water containing copper and rapid-onset gastrointestinal illness in Nebraska. In August 1994, a retrospective cohort study was conducted on a total of 451 individuals from 148 households determined in 1993 to have morning first-draw copper concentrations of either ≤1.3, 2-3, or > 3 mg/L. A standardized questionnaire was administered by telephone interview with the household adult "most knowledgeable" about family water use and health. There was no association between gastrointestinal problems during the two weeks prior to the interview and 1993 drinking-water copper levels.

Paragraph 6

From these participants, a nested case-control study frequency-matched 25 cases to 27 controls in three age categories (August 1994). In-person interviews were conducted, water samples were taken and a variety of potential risk factors in addition to drinking-water copper were assessed. Again, no associations were observed between gastrointestinal problems and copper concentration in drinking-water from samples collected in 1993 (tap or morning first-draw). However, water concentrations in the August 1994 samples were found to be inconsistent with those from 1993. Accordingly, a second cohort study (December 1994) was conducted on 442 individuals (145 households) from the original study. New water samples were taken and new questionnaires administered. Risks for gastrointestinal symptoms were recalculated using the December 1994 sampling results and the combined August-December case-status results. Once more, no increased relative risks were apparent for the 2-3 mg/L (0.25, 95% CI = 0.10, 0.64) or > 3 mg/L (0.36, 95% CI = 0.09, 1.49) households, nor was a dose-response found when households were redistributed evenly by exposure tertiles.

Paragraph 7

Dieter et al. (1999) and Eife et al. (1999) have examined in detail the clinical records from children in Germany who were affected with childhood cirrhosis. For those cases that could not be ascribed to a specific medical condition, a link could be established in most cases between the affected child and formula feeding, copper plumbing and/or corrosive water, most often from a private well.

Paragraph 8

For adults without genetic disorders of copper homeostasis, long-term intake of copper in the diet at concentrations of 1 to 10 mg/day has no apparent adverse effects (IOM, 2001). Long-term daily intakes of copper below recommended requirements can lead to anaemia, neutropenia, and bone demineralization in malnourished children (IOM, 2001). Adults are

more resistant than children to the symptoms of a copper deficiency. Copper intakes in the 1 to 10 mg/day range may have adverse effects for individuals that carry the Wilson's disease gene, and for infants or children genetically predisposed for one of the childhood copper cirrhosis syndromes (NRC, 2000).

6. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

6.1 Acute exposure

Paragraph 1

Acute responses to copper vary with species and copper compound. Ferrets, sheep, dogs, and cats are more sensitive to copper than rodents, pigs, and poultry (Andrews et al., 1990; Linder & Hazegh-Azam, 1996). Soluble copper salts are more toxic than insoluble compounds.

Paragraph 2

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.

Paragraph 3

In a more recent 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 in the ferret is in the stomach. Other studies in dogs and ferrets (Andrews et al., 1990; Bhandari & Andrews, 1991; Fukui et al., 1994) confirm the importance of gastrointestinal neural pathways and receptors in copper sulfate-induced emesis. In beagle dogs, the vomiting response to 100 mg/kg of body weight copper sulfate was reduced or eliminated by high doses of a chemical blocker of receptors for serotonin as well as severing the vagus and splanchnic nerves. Seratonin is a neuro-active compound that may activate or sensitize abdominal gastric nerves involved in the emetic response (Fukui et al., 1994).

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6.2 Short-term exposure

Paragraph 1

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). Accordingly, only the data from rats are reported below.

Paragraph 2

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 36 mg Cu/kg/day (Hébert et al., 1993). A LOAEL of 10 mg Cu/kg/day was observed in male rats 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 Cu/kg/day. However, only slightly higher doses (31 mg Cu/kg/day) were accompanied by signs of clinical toxicity.

Paragraph 3

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 Cu/kg/day in males and females for 2 weeks and 16 mg/kg/day in males and 17 mg/kg/day in females 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 (46mg/kg/day in males and 41 mg/kg/day in females for 2 weeks and 33 mg/kg/day in males and 34 mg/kg/day for females for 13 weeks) were associated with hyperplasia and hyperkeratosis of the squamous epithelium of the limiting ridge of the rat fore-stomach.

Paragraph 4

With the 13-week exposure and the 2000 mg/kg of feed dietary concentration (33 mg/kg/day in males and 34 mg/kg/day in females), 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 $a_{2\mu}$ globulin was negative. Dose-related decreases in haematological parameters at 4000 mg/kg of feed (66 mg/kg/day in males and 68 mg/kg/day in females) and 8000 mg/kg of feed (140 mg/kg/day in males and 134 mg/kg/day in females) were indicative of a microcytic anaemia, whereas increases in serum enzymes were indicative of liver damage. Iron stores in the spleen were depleted, especially for the highest exposure concentration.

Paragraph 5

Aflatoxin B_1 alone (0,05 mg resp. 0,037 mg/kg/d), copper alone (6,6 mg/kg/d or 200 mg/l drinking water) or a combination of both was administered orally for 6 months to young guinea pigs from the first/second day of life (Seffner et al., 1997). In the copper group there were no pathomorphological changes. For the aflatoxin B_1 group, liver damage was established. In the combined group, liver injury was more frequent and more severe

compared to the aflatoxin B_1 group and biliary copper excretion was diminished compared with the copper group. Histologically, only the livers of this group exhibited degeneration, atrophy and steatosis of liver cells, inflammatory processes and more or less prominent fibrosis. These results support the possibility that a combined etiology between enhanced copper uptake and a liver damaging agent is a plausible hypothesis for copper associated liver disease.

6.3 Long-term exposure

Paragraph 1

Male weanling Wistar rats (four per group) were given either a standard 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, or 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 exhibited 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 was greater in the exposed animals than in the controls (23 µg/g).

6.4 Reproductive toxicity, embryotoxicity, and teratogenicity

Paragraph 1

There are no standard studies of copper toxicity and reproductive function. Sperm morphology and motility analyses, testis and epididymis weight determination, and estrous cycle characterization were performed in rats and mice as part of a subchronic dietary study (Hébert et al., 1993). No significant differences from control values were found for any of the following reproductive parameters: testis, epididymis and cauda epididymis weights, spermatid count, spermatid number per testis or per gram testis, spermatozoal motility and concentration, estrous cycle length, or relative length of time spent in the various estrous stages. A NOAEL of 8000 mg per kg of diet (140 mg Cu/kg/day for males and 134 mg Cu/kg/day for female rats) was established for these parameters in this study.

Paragraph 2

There is some evidence that copper is a developmental toxicant. When mice (7–22 females per group) were fed diets supplemented with 0, 500, 1000, 1500, 2000, 3000 or 4000 mg

of copper sulfate per kg of diet for 1 month (0, 65, 130, 195, 260 or 390 mg/kg/day assuming mice consume 13% if their body weight as food), fetal mortality and decreased litter size were observed in the 3000 and 4000 mg/kg of diet groups. Various skeletal and soft-tissue malformations were seen in 2–9% of the surviving fetuses from the two highest dose groups (Lecyk, 1980). The low concentrations of supplemental copper (500 and 1000 mg/kg of diet) had a beneficial effect on development.

Paragraph 3

For 7 weeks prior to and then during pregnancy, rats were supplied with water supplemented in step-wise fashion (details not provided) with copper acetate, up to a level of 0.185 percent (588 mg/L of copper, assuming use of copper(II) acetate monohydrate and a dose of 82 mg/kg/day assuming a 0.14 L/kg/day water intake factor for Wistar rats) (Haddad et al., 1991). Maternal parameters were normal, except for liver and kidney effects typical of copper overload. In 11.5 day old embryos, mean yolk sac diameter, crown rump length, and somite number were reduced significantly, with only minor retardation observed for several other developmental parameters. The number of offspring/litter and mean fetal weight were similar to controls, but most ossification centers were markedly reduced in 21.5-day old fetuses. In newborn rats, only the numbers of cervical and caudal vertebrae and hindlimb phalanges were significantly reduced.

6.5 Mutagenicity and related end-points

Paragraph 1

Copper sulfate has been reported to induce reverse mutations in TA98 or TA100 strains of *Salmonella typhimurium* (Moriya et al., 1983). Copper(II) chloride has tested negatively in various strains of *Salmonella typhimurium* and *Escherichia coli (E. coli.)*; however, responses were positive responses in both the Mutatox™ and SOS Chromotest (*E. coli.*) assays (Wong, 1988; Codina et al., 1995).

Paragraph 2

In vitro exposure of rat hepatocytes to copper sulfate caused DNA strand breakage at 1.0 mM, but not at lower concentrations (Sina et al., 1983), and unscheduled DNA repair at concentrations of 0.008 to 0.079 mM (Denizeau & Marion, 1989). A dose-responsive increase in DNA-protein cross-links was found in Chinese hamster ovary (CHO) and nontransformed human fibroblast cell cultures when exposed to 0.5 to 2.0 mM copper sulfate (Olin et al., 1996). Copper sulfate pentahydrate induced bone marrow chromosomal aberrations in Swiss albino mice after oral, subcutaneous, and intraperitoneal exposures (Bhunya & Pati, 1987; Agarwal et al., 1990).