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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1984; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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LIST OF ABBREVIATIONS

bw	Body weight
CNS	Central nervous system
DNA	Deoxyribonucleic acid
DWEL	Drinking water equivalent level
FEL	Frank-effect level
GI	Gastrointestinal
G-6-PD	Glucose-6-phosphate dehydrogenase
HA	Health advisory
i.p.	Intraperitoneal
IUD	Intrauterine device
i.v.	Intravenous
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
ppm	Parts per million
RfD	Reference dose
RNA	Ribonucleic acid

I. SUMMARY

In environmental waters, copper is present almost exclusively as the Cu(II) valence state, usually as the aquated ion. The ion is generally complexed with organic or inorganic ligands but sometimes is present as the aquo ion $[Cu(H_2O)_6]^{2+}$. Complexes of the carbonate appear to be most common, although many other complexes are present, particularly in organic rich water. Hydrous manganese and iron oxides appear to have dominating control of soluble copper, strongly sorbing Cu(II) complexes; however, in organically rich sediments copper sorbed to the oxides may become redissolved. Clay and particulate organic material also sorb copper.

Municipal water treatment plants that use iron chloride coagulation may concentrate copper present in the raw water onto the coagulate, based on the sorption properties of copper to hydrous iron oxides. The majority of copper present in drinking water appears to come from copper water pipes.

Copper is regarded as an essential element for proper nutrition, and it is widely distributed in the body. Copper compounds can be absorbed by all three environmental routes; dermal, oral and inhalation. Although not thoroughly understood, homeostatic mechanisms in normal humans provide a balance between copper intake and elimination. Normal daily intakes of copper are 2-5 mg/day, mostly from food and water, with a slight contribution from air. The total body content of normal adult humans is ~70-120 mg of copper, most of which is located in the liver.

Acute toxicity from ingestion of excess copper can result in GI disturbance, hemolysis, G-6-PD inhibition, kidney and renal necrosis, and death. There are few data to relate chronic ingestion of copper compounds to toxic effects in humans, although individuals with genetic metabolic error (Wilson's disease) show copper toxicosis. The chronic occupational use of copper aerosols has resulted in adverse effects on the lungs, kidney and liver of exposed persons. Domestic and laboratory animals exhibit toxicity upon short-term exposure to high doses of copper (>10 mg/kg/day in diet).

Copper plays an important role in reproduction. Deficiency or excess of the metal can have a teratogenic effect. The spermicidal properties of copper have long been recognized.

Pertinent data regarding the carcinogenicity of ingested copper compounds could not be located in the available literature. The equivocal mutagenic responses of microbial, mammalian cell cultures and in vivo systems do not permit a conclusion regarding the mutagenicity of copper.

The available human data indicate that GI distress occurred following ingestion of solutions that contained 5-7 mg of copper and other unknown chemicals as a single dose. These data were used to derive a 1-day health advisory (HA) of 1.3 mg/ℓ which, when spread over a day, should provide protection from these acute effects. Since the effects appear to be local irritation, this HA is recommended for both the child and the adult.

II. PHYSICAL AND CHEMICAL PROPERTIES

Structure and Identification

Copper and some of its important compounds are listed in Table II-1, with available Chemical Abstracts Service (CAS) numbers.

Physical and Chemical Properties

The physical and chemical properties of copper and some if its important organic and inorganic compounds are listed in Table II-2.

Stability in Water

The dominant removal processes for copper in water include the following: sorption and coprecipitation by hydrous iron and manganese oxides, adsorption to clays, minerals and organic solids and ion exchange in the crystal lattice of carbonates. Adsorption occurs primarily when copper is complexed, but uncomplexed copper will also adsorb. Copper is not expected to volatilize or photolyze (Callahan et al., 1979).

In environmental waters, copper in solution is present as the Cu^{2+} aquo ion $[Cu(H_2O)_6]^{2+}$; the lifetime of the Cu^{2+} aquo ion depends on the conditions, but it will last for a few hours, at most, if air is excluded (Cotton and Wilkinson, 1980). Other copper valance states are possible (Cu^{3+} and Cu^{1+}) but rare. Consequently, whenever copper is mentioned in this discussion, the Cu^{2+} ion is meant unless otherwise specified.

Many organic and inorganic copper complexes can be formed by treating aqueous copper solutions with ligands (Cotton and Wilkinson, 1980). Stiff

TABLE II-1

Copper Compounds*

Compound	CAS No.	RTECS No.
Copper	7440-50-8	GL5325000
Copper abietate	10248-55-2	
Copper(I) acetate	598-54-9	
Copper(II) acetate	142-71-2	
Copper(II) acetate, basic	52503-63-6	
Copper(II) acetate monohydrate	6046-93-1	
Copper acetate oxide	6533-47-7	
Copper(II) acetoarsenite	12002-03-8	
	1299-88-3	GL6475000
Copper(II) acetylacetonate	13395-16-9	
Copper(I) acetylide	1117-94-8	
Copper(II) amine sulfate	14283-05-7	
Copper amino acid chelate	-	
Copper(I) ammonium acetate	43043-77-2	
Copper(II) ammonium acetate	23087-46-9	
Copper ammonium arsenate	32680-29-8	
Copper ammonium carbonate	33113-08-5	
Copper(I) ammonium chloride	-	
Copper(II) ammonium chloride	-	
Copper ammonium hydroxide	17500-49-1	
Copper(II) ammonium sulfate	-	
Copper(I) antimonide	12054-25-0	
Copper(II) antimonide	12054-21-6	
Copper(II) arsenate	10103-61-4	
	7778-41-8	
Copper arsenate hydroxide	16102-91-4	
Copper(I) arsenic sulfide	-	
Copper(I) arsenide	12005-75-3	
Copper(II) arsenide	12254-86-3	
Copper(II) arsenite	10290-12-7	
	1302-97-2	CG3385000
Copper aspinnate	23642-01-5	
Copper(II) benzoate	533-01-7	
Copper(II) benzoyl acetonate	14128-84-8	
Copper bicarbonate	7492-68-4	
Copper bis(salicylaldehyde)	14523-25-2	
Copper(II) borate	39290-85-2	
Copper bromate	-	
Copper(I) bromide	7787-70-4	
Copper(II) bromide	7789-45-9	
Copper(I) tert-butoxide	-	
Copper butylphthalate	-	

TABLE II-1 (cont.)

Compound	CAS No.	RTECS No.
Copper butyrate	540-16-9	
Copper iso-butyrate	-	
Copper caprylate	-	
Copper(II) carbonate	1184-64-1	
	16211-10-2	
Copper(II) carbonate, basic	12069-69-1	
Copper chelate	-	
Copper(II) chlorate	14721-21-2	
Copper(II) chlorate, tetrahydrate	10294-45-8	
Copper(I) chloride	7758-89-6	
Copper(II) chloride	7447-39-4	
	12258-96-7	
Copper(II) chloride, basic	16004-08-3	
	1332-65-6	GL7020000
	12167-76-9	
	12356-86-4	
Copper(II) chromate(III)	12018-10-9	
Copper(II) chromate(VI)	13548-42-0	
	12617-87-7	
	13675-47-3	
Copper(II) chromate, basic	1308-09-4	
Copper chromite	11104-65-7	
Copper(II) chromite	12018-10-9	
Copper chromite barium	-	
Copper chromite zinc oxide	-	
Copper(II) citrate	866-82-0	GL7056000
Copper(II) citrate, hydrate	6020-30-0	
Copper cobalticyanide	-	
Copper(II) columbate	-	
Copper(I) cyanide	544-92-3	
Copper(II) cyanide	4367-08-2	GL7175000
Copper(II) cyclohexylbutyrate	2218-80-6	
Copper(II) dichromate	13675-47-3	
Copper(II) dichromate, dihydrate	12018-10-9	
Copper dihydrazinium sulfate	-	
Copper diiron tetraoxide	-	
Copper(II) dimethyldithiocarbamate	137-29-1	FA0175000
Copper disodium ethylenediamine tetracetate	4025-15-1	
Copper(II) ethylacetoacetate	-	
Copper(II) ethylacetyl acetonate	14284-35-3	
Copper(II) ethylenediamine	14552-35-3	
Copper ethylenediamine-N,N'-diacetic acid	32575-57-8	
Copper ethylenediamine gold cyanide	18974-18-0	

TABLE II-1 (cont.)

Compound	CAS No.	RTECS No.
Copper ethylenediamine nickel cyanide	63427-32-7	
Copper ethylenediamine silver cyanide	67859-40-9	
Copper ethylenediamine sulfate cyanide	-	
Copper ethylenediamine tetracetate	54452-03-1	
Copper ethylenediamine zinc cyanide	67859-43-2	
Copper(II) 2-ethylhexanoate	149-11-1	
	22221-10-9	
Copper ethyl xanthate	-	
Copper(II) ferrate (III)	12018-79-0	
Copper(II) ferricyanide	-	
Copper ferrite	-	
Copper(II) ferrocyanate	-	
Copper(II) ferrocyanide	13601-13-3	
Copper(II) ferrous sulfide	1308-56-1	
Copper(I) fluoborate	14708-11-3	
Copper(II) fluoborate	38465-60-0	
Copper(II) fluoride	7789-19-7	
Copper(II) fluosilicate	12062-24-7	
Copper(II) formate	544-19-4	
Copper(II) formate tetrahydrate	5893-61-8	
Copper gallium selenide	12018-83-6	
Copper gallium sulfide	12158-59-7	
Copper gallium telluride	12018-84-7	
Copper(II) gluconate	527-09-3	
Copper(II) gluconate monohydrate	6020-31-1	
Copper glutanate	-	
Copper(II) glycinate	13479-54-4	
Copper glycolate	-	
Copper guaiacolsulfonate	-	
Copper heptanoate	5128-10-9	
Copper(II) hexacyanoferrate(II)	13601-13-3	
Copper hexafluoroacetylacetone		
Copper(I) hydride	13517-00-5	
Copper hydroselenite	10031-38-6	
Copper hydroselenite monohydrate	10031-39-7	
Copper hydroselenite dihydrate	10031-40-0	
Copper hydroselenite trihydrate	10031-41-1	
Copper(II) hydroxide	20427-59-2	
	1344-69-0	
Copper(II) hydroxyfluoride	13867-72-6	
Copper(II) 8-hydroxyquinolate	10380-28-6	
Copper(II) hydroxysalicylate	62320-94-9	

TABLE II-1 (cont.)

Compound	CAS No.	RTECS No.
Copper indium selenide	-	
Copper indium sulfide	-	
Copper indium telluride	-	
Copper(II) iodate	7681-65-4	
Copper(I) iodide	13767-71-0	
Copper(II) iron(III) oxide	-	
Copper(II) lactate	-	
Copper(II) linoleate	7721-15-5	
Copper malonate	7268-92-0	
Copper(II) magnesium sulfate	-	
Copper mercaptobenzothiazolate	4162-43-0	
Coper(I) mercuric iodide	13876-85-2	
Copper methane arsenate	-	
Copper(II) methylate	-	
Copper(II) molybdate	13767-34-5	
Copper(II) naphthenate	1338-02-9	
Copper(II) neodecanoate	32276-75-8	
	50315-14-5	
	68084-48-0	
Copper(II) nickel formate	68134-59-8	
Copper(II) nitrate	3251-23-8	
Copper(II) nitrate trihydrate	10031-43-3	
Copper(II) nitrate hexahydrate	13478-38-1	
Copper(I) nitride	1308-80-1	
Copper(II) nitride	-	
Copper(II) nitroprusside	14709-56-9	
Copper cis-9-octadecenoate	1120-44-1	
Copper octanoate	20543-04-8	
Copper(II) oleate	1120-44-1	
	10402-16-1	
Copper(II) oxalate	814-91-5	
Copper(I) oxide	1317-39-1	
	1317-38-0	
Copper(II) oxychloridesulfate	8012-69-9	
Copper(II) palmitate	-	
Copper(II) pentamethylene dithiocarbamate	-	
Copper peponate	-	
Copper(II) perchlorate	13770-18-8	
Copper(II) perchlorate hexahydrate	10294-46-9	
Copper(II) permanganate	-	
Copper(II) phenolsulfonate	547-56-8	

TABLE II-1 (cont.)

Compound	CAS No.	RTECS No.
Copper(I) phenylacetylide	13146-23-1	
Copper 3-phenylsalicylate	5328-04-1	
Copper phorphine	13007-96-0	
Copper phosphate	7798-23-4	
	18718-12-2	
Copper(II) phosphate, ortho	10103-48-7	
Copper(II) phosphate, pyro	10102-90-6	
Copper(I) phosphide	12019-57-7	
Copper(II) phosphide	12134-35-9	
Copper phthalate	-	
Copper(II) phthalocyanine	147-14-8	
Copper(II) potassium chloride	-	
Copper(I) potassium cyanide	13682-73-0	
	14263-73-1	
Copper potassium hexacyanoferrate(II)	14481-39-1	
Copper potassium pyrophosphate	-	
Copper(II) potassium sulfate	-	
Copper potassium tartrate	-	
Copper powder	7440-50-8	
Copper(II) propionate	3112-74-1	
Copper iso-propoxide	-	
Copper proteinate	-	
Copper(II) resinate	9007-39-0	
Copper b-resorcylate	-	
Copper ricinoleate	-	
Copper rubidium sulfate	-	
Copper(II) salicylate	16048-96-7	
Copper(II) selenate	15123-69-0	
Copper(II) selenate pentahydrate	10031-45-5	
Copper(I) selenide	20405-64-5	
Copper(II) selenide	1317-41-5	
Copper(II) selenite	10214-40-1	
Copper(II) silicate	1344-72-5	
	16509-17-4	
Copper silicide	-	
Copper(II) sodium chloride	-	
Copper(I) sodium cyanide	-	
Copper sodium tartrate	-	
Copper(II) stannate	12109-07-7	
Copper(I) stearate	20563-00-2	
Copper(II) stearate	660-60-6	
Copper(II) sulfamate	-	
Copper(II) sulfate	7758-98-7	
Copper(II) pentahydrate	7758-99-8	

TABLE II-1 (cont.)

Compound	CAS No.	RTECS No.
Copper sulfate, basic	1332-14-5 1344-73-6	GL8930000
Copper(I) sulfate b-naphthol	-	
Copper(I) sulfide	22205-45-4	
Copper(II) sulfide	1317-40-4	
Copper(I) sulfite	13982-53-1	
Copper(I) sulfite, monohydrate	10294-49-2	
Copper tallate	61789-22-8	
Copper(II) tartrate	815-82-7	
Copper(I) telluride	12019-52-2	
Copper(II) telluride	12109-23-7	
Copper(II) tellurite	-	
Copper tetramine sulfate	14283-05-7	
Copper tetramine sulfate monohydrate	10380-29-7	
Copper meso-tetraphenylporphrine	-	
Copper(I) thiocyanate	1111-67-7	
Copper(II) thiocyanate	-	
Copper thiourea gold cyanide	68975-64-4	
Copper thiophenoxide	-	
Copper(II) titanate	12305-89-4	
Copper p-toluenesulfonate	-	
Copper triethanolamine	-	
Copper trifluoroacetylacetonate	-	
Copper 3,5,5-trimethylhexanoate	35206-70-3	
Copper(II) tungstate	13587-35-4	
Copper undecanoate	7491-40-9	
Copper undecylenate	1322-15-2 1328-71-8	
Copper(II) vanadate	12789-09-2	
Copper m-vanadate	-	
Copper zinc chromate	1336-14-7	
Copper zinc chromate(VI)	10279-64-8	
Copper zinc oxide	-	
Copper(II) zirconate	-	

*Source: U.S. EPA TSCA list and U.S. EPA (1980b); SRI International (1980); USITC, 1980; Windholz, 1976; Chem. Sources - USA, 1980

TABLE II-2

Selected Physical and Chemical Properties of Copper and Some Copper Compounds[a]

Chemical	Formula	CAS Registry No.	Atomic or Molecular Weight	Appearance	Density or Specific Gravity	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure	Aqueous Solubility
Copper	Cu	7440-50-8	63.55	reddish metal	8.92	1083.4	2567	1 mm Hg at 1628°C	Insoluble
Copper(II) acetate, monohydrate	$Cu(C_2H_3O_2)_2 \cdot H_2O$	142-71-2	199.65	dark green powder	1.882	115	decomposes at 240	NA	72 g/ℓ in cold water[b]
Copper(II) carbonate, basic	$CuCO_3Cu(OH)_2$	12069-69-1	221.11	dark green crystal	4.0	decomposes at 200°	NR	NA	Insoluble in cold water, but decomposes in hot water
Copper(I) chloride	CuCl (or Cu_2Cl_2)	7758-89-6	98.99	white crystal	4.14	430	1490	1 mm Hg at 546°C	0.062 g/ℓ in cold water
Copper(II) chloride	$CuCl_2$	7447-39-4	134.44	brown or yellow hygroscopic powder	3.386^{4}_{25}	620	decomposes at 993	NA	706 g/ℓ at 0°C
Copper(II) naphthenate	Cu-salt of naphthenic acid also called cuprinol	1338-02-9	variable	green-blue solid	NA	NA	NA	NA	NA
Copper(II) nitrate, trihydrate	$CuNO_3 \cdot 3H_2O$	10031-43-3	241.60	blue deliquescent crystal	2.32^{4}_{25}	114.5	decomposes at 170	NA	1378 g/ℓ at 0°C
Copper(II) oleate	$Cu(C_{18}H_{33}O_2)_2$	1120-44-1	626.47	brown powder or green-blue solid	NA	NA	NA	NA	Insoluble
Copper(I) oxide	Cu_2O	1317-39-1	143.08	reddish, crystal	6.0	1235	decomposes at 1800°C	NA	Insoluble
Copper(II) oxide	CuO	1317-38-0	79.54	black crystal	6.3-6.49	1326	NA	NA	Insoluble
Copper(II) sulfate, pentahydrate	$CuSO_4 \cdot 5H_2O$	7758-99-8	249.68	blue crystal	2.284	decomposes at 110°C	NR	NA	316 g/ℓ at 0°C

[a]Source: Weast, 1980; SRC, 1980

[b]The temperature of cold water was not specified.

NA = Not available; NR = not relevant

(1971a,b) reported that at environmental temperatures, pH and inorganic carbon content, copper in solution is present as complexes of copper carbonate rather than the aqua ion $[Cu(H_2O)_6]^{2+}$. In polluted waters or those with high organic content, copper is present as complexes with cyanide, amino acids and humic substances in addition to the carbonate complexes and the aqua ion. Stiff (1971b) also found that most of the copper present in rivers in England was associated with suspended solids and that dissolved copper was present as organic complexes. Stumm and Morgan (1981) reported that the activity ratio diagram reveals that Cu^{2+} predominates up to pH 6; $CuCO_3(aq)$ predominates in the pH range 6-9.3, and $Cu(CO_3)_2^{-2}$ (aq) in the pH range 9.3-10.7.

Sylva (1976) and Ramamoorthy and Kushner (1975) confirmed the importance of organic complexing in the speciation of copper in organic rich waters. In waters with low organic content, precipitation of malachite ($Cu_2(OH)_2CO_3$) appears to be dominant, although the rate appears to be very slow at low copper concentrations (Sylva, 1976).

Lopez and Lee (1977) found that copper speciated in the order $CuOH^+ > Cu^{2+} > CuCO_3$ in a copper polluted organic depleted lake. Soluble copper concentrations were controlled by sorption onto hydrous iron and manganese oxides.

The dominating effect of hydrous manganese and iron oxides in controlling soluble copper was also reported by Hem and Skougstad (1960), Jenne (1968), Brown et al. (1983), Lee (1975), Carpenter et al. (1975), Steele and Wagner (1975) and Collins (1973). These oxides appear to be most important

in water with low organic content and soils. In reducing or acidic environments (i.e., organically rich sediments), copper sorbed to hydrous iron and manganese oxides may become redissolved, allowing the copper to reenter the water.

Brown et al. (1983), Baes and Sharp (1983), Payne and Pickering (1975) and Huang et al. (1977) reported that copper was strongly adsorbed to clay materials and that this sorption was strongly pH dependent. The addition of certain particulate organic materials increased the sorption. Soluble organic matter, however, appears to efficiently increase the solubility of copper, possibly by competing for clay active sites (Jackson and Skippen, 1978).

Sewage treatment plant sludge has long been known to concentrate many heavy metals including copper (Bradford et al., 1975; Sopper, 1973; Page, 1974; Lester et al., 1979). In addition, based on the above discussion, municipal drinking water plants that practice iron chloride coagulation may concentrate copper present in the raw water on the coagulate, thus reducing the dissolved copper present in the finished water. Nonetheless, water that is acidic, low in hardness and alkalinity, and consequently corrosive to piping, may leach copper from drinking water pipes and supply copper levels in >1.7 mg/ℓ (Karalekas et al., 1983). Sharrett et al. (1982) found that copper levels in drinking water were an order of magnitude lower when transported through galvanized pipe as opposed to copper pipe. Standing water gave levels about twice as high as did flowing water in copper pipe, but had little effect in galvanized pipe. The largest factor affecting copper concentrations in drinking water was found to be piping (Karalekas et al., 1983; Sharrett et al., 1982).

Summary

In environmental waters, copper is present, almost exclusively, as the Cu^{2+} ion (Cotton and Wilkinson, 1980). The ion is generally a complex of organic or inorganic ligands but sometimes is present as the aquo ion $[Cu(H_2O)_6]^{2+}$. Complexes of the carbonate appear to be most common (Stiff, 1971a,b), although many other complexes are present, particularly in organic rich water (Stiff, 1971a; Sylva, 1976; Ramamoorthy and Kushner, 1975). Hydrous manganese and iron oxides appear to have dominating control of soluble copper, strongly sorbing the complex ion (Hem and Skougstad, 1960; Jenne, 1968; Brown et al., 1983; Lee, 1975); however, in organically rich sediments copper sorbed to the oxides may become redissolved. Clay and particulate organic material also sorb copper (Brown et al., 1983; Baes and Sharp, 1983; Payne and Pickering, 1975).

Municipal water treatment plants that use iron chloride coagulation may concentrate copper present in the raw water onto the coagulate, based on the sorption properties of copper to hydrous iron oxides. The majority of copper present in drinking water appears to originate from copper water pipes (Karalekas et al., 1983; Sharrett et al., 1982).

III. TOXICOKINETICS

Absorption

Copper may be absorbed into the systemic circulation from oral administration, inhalation, or contact, and possibly from intrauterine contraceptive devices (IUDs).

Ingestion

Bearn and Kunkel (1954) characterized the uptake of ^{64}Cu by serum in rats and human subjects given an oral dose of (^{64}Cu) copper nitrate. Rats and human subjects were given 25.6 (2 mCi) and 12.8 (1 mCi) mg of ^{64}Cu, respectively. Blood samples were taken at intervals up to 24 hours and were assayed for radioactivity. Blood serum was subjected to electrophoretic separation and the fractions obtained were assayed for radioactivity. In both rats and men, the serum ^{64}Cu was localized in the albumin fraction 15 minutes after administration. After this period, serum ^{64}Cu began to localize in the ceruloplasmin fraction in both rats and man. By 24 hours after administration, at least 70% of the serum ^{64}Cu was localized in the ceruloplasmin fraction.

Weber et al. (1969) studied the GI absorption of radiocopper in human subjects given oral doses of ^{64}Cu as copper acetate. Seven patients who had been fasting and who had experienced no liver damage or related problems were given (^{95}Zr) zirconium oxalate (0.5-10 μCi, specific activity not reported) followed by (^{64}Cu) copper acetate (100-500 μCi, specific activity 0.3-1.3 μCi/μg Cu). Zirconium oxalate was used as a non-absorbed stool marker to compensate for the short half-life of ^{64}Cu (12.8 hours). The radionuclides were counted each day for 4 days in a whole body

scintillation counter using gamma ray spectroscopy. In addition, the movement of orally administered ^{64}Cu in the GI tract was monitored with scintiphotographs obtained with a scintillation camera. Urine and stools were collected each day, blood samples were taken hourly for 6 hours, and the ^{64}Cu content in plasma, urine and stools was counted by gamma ray spectroscopy. One week after the oral dosing, the subjects were again given similar oral doses of (^{95}Zr) zirconium oxalate and an i.v. dose of 500 μCi of (^{64}Cu) copper acetate that had been incubated with 10-20 mℓ of the subject's plasma for 30 minutes (to assure protein binding of copper). Radioactivity was monitored as before.

When ^{64}Cu was administered orally, maximal absorption of the metal from the stomach and duodenum by plasma was observed within 1 hour of administration. A second phase of absorption, which occurred at a lower rate, was observed for $\geq$3.5 hours after administration. At 2 hours after administration, the bolus of ^{64}Cu seen in scintiphotographs was present in the small intestine, and after 5 hours, in the ileocecal region and first portion of the large intestine. After i.v. administration, an average of 11.5% of the dose was excreted into the GI tract (range 0-23%). The average net absorption of ^{64}Cu was ~60% (range 15-97%). Obviously, a wide variation in copper absorption was observed in these normal subjects. A steady-state ^{64}Cu plasma concentration was observed after ~3 hours of oral or i.v. administration. The initial decline of ^{64}Cu plasma concentration after i.v. administration occurred rapidly and was observed to follow first order kinetics. Negligible urinary excretion of ^{64}Cu was observed.

In mammals, the sites of copper absorption following oral administration are limited to the upper GI tract (Evans, 1973). The absorption of copper from the GI tract is a complex process controlled by a number of mechanisms (Crampton et al., 1965). Copper absorption is facilitated by active transport of ionic and organic copper complexes with amino acids and proteins; it may be controlled by an intestinal protein called metallothionein. Metallothionein may control copper homeostasis by providing binding sites in intestinal mucosa for ionic copper, and by acting as a mucosal block to prevent absorption of toxic amounts of copper when present in excess quantities (Evans, 1973). Competition for intestinal binding sites by other metals (cadmium, zinc, mercury, silver) can alter the amount of copper absorbed.

The amount of dietary protein can affect copper absorption by forming protein complexes with the metal and thus inhibiting its absorption. Other factors that may affect copper absorption include the number and type of anions present (i.e., inhibition by sulfite ions), and ascorbic acid (Evans, 1973). It is believed that these mammalian homeostatic mechanisms control the copper balance in normal individuals despite large variations in dietary intake (Sternlieb, 1980). Studies with radiolabeled ^{64}Cu and ^{67}Cu showed that in adults ~60% of human dietary copper is not absorbed and is eliminated by the feces (Scheinberg and Sternlieb, 1969).

Inhalation

Data regarding the absorption of inhaled copper in humans are scarce. Villar (1974) reported observing copper-containing granulomas in the lung, liver and kidney of a deceased patient who had been occupationally exposed to Bordeaux mixture (an aqueous solution of lime and 1-2% copper sulfate)

during the spraying of vineyards. Pimental and Menezes (1975) reported finding copper-containing liver granulomas in three patients with a history of using Bordeaux mixture while involved in vineyard spraying. Gleason (1968) reported symptoms of metal fume fever (general discomfort, fever, sensations of chills or warmth and stuffiness of the head) in three workers exposed to fine copper dust at concentrations of 0.03-0.12 mg/m^3. Metal fume fever symptoms were absent in these workers after the installation of local exhaust ventilation, which reduced the airborne copper concentration to <0.008 mg/m^3.

Copper has been shown to permeate intact (Walker et al., 1977) and burned (Holtzman et al., 1966) human skin. Apparently, copper bracelets used for relief of arthritic complaints are slowly dissolved and copper is subsequently absorbed (Walker et al., 1977).

When included in plastic IUDs, copper results in more efficient contraception than plastic devices alone. The interaction of dissolved ions with blastocysts may prevent their implantation, and copper has spermicidal potential as well. Concern has been raised that systemic absorption of copper may occur in those women using IUDs (Oreke et al., 1972). Copper IUDs do have systemic effects but appear to be unrelated to the systemic accumulation of the metal (Oster and Salgo, 1977).

Batsura (1969) produced evidence of the pulmonary uptake of copper oxide in rats exposed to aerosols containing 50-80 mg/m^3. Animals were exposed for 15, 30, 45 or 60 minutes and killed immediately; another group was exposed for 180 minutes and killed at 0, 3, 6, 12, 18 or 24 hours after

exposure. Electron microscopic histologic examination showed that absorption of copper had occurred in animals exposed for 180 minutes. Copper oxide particles penetrated the epithelial cells of alveoli and were found in plasma 6 hours after exposure began. Copper oxide was also observed in the proximal convoluted tubules of the kidney.

A radiolabeled saline solution of copper complex, bis[glycinato]copper [II], topically applied to the skin of cats was observed in the blood ~6 hours after administration. By 24 hours after administration, it was shown that ~3.3% of the applied copper had completely penetrated the skin (Walker et al., 1977).

Highly localized deposits of hepatic and renal copper have been observed in monkeys with copper IUDs and in control monkeys. Both copper and inert material IUDs have been observed to increase plasma copper levels (Oster and Salgo, 1977). This may be explained by the observation that stress or inflammation alone can result in increased serum copper levels (Evans, 1973).

Oreke et al. (1972) studied the systemic absorption of copper from radiolabeled (^{64}Cu or ^{67}Cu) copper wire that had been implanted into the uteri of Holtzman rats. Radiolabeled copper was found in the serum, liver and kidney of rats as early as 18.5 hours after implantation.

Distribution

Copper is normally present in all human tissues. In humans, the liver, brain, heart, kidney and adrenal contain the highest concentrations of copper. Tissues with moderate copper concentrations include intestine, lung

and spleen. Low copper concentrations are found in endocrine glands, bone, muscle, larynx, trachea, aorta and testes (Schroeder et al., 1966; Evans, 1973). About 50% of the body burden of copper is found in muscle and bone because of the large masses involved. The liver contains ~10% of the body's copper stores (Evans, 1973).

Absorbed dietary copper is transported in the blood as complexes with plasma albumin (93% of that moving from gut to liver) and amino acids. These complexes include the immediate transport form of plasma copper, and albumin copper is in rapid equilibrium with tissue copper (Neumann and Sass-Kostsak, 1967; Evans, 1973). Copper is also contained in erythrocytes in the form of erythrocuprein (Shields et al., 1961) and other proteins (i.e., superoxide dismutase). Removal of copper from the serum takes place primarily in the liver, where it is either excreted into the bile, stored or used in synthesizing ceruloplasmin, which is released into the blood. Ceruloplasmin contains 90% of the copper found in the blood of a normal person.

Osborn et al. (1963) studied the hepatic uptake of radiolabeled copper in normal and Wilson's disease patients. Two normal subjects were given i.v. injections of 80-120 μCi of (^{64}Cu) copper chloride dissolved in saline. The livers of normal subjects had taken up 82-94% and 95-96% of the administered radioactivity within 24 and 30 hours of dosing, respectively. Patients with liver disease, heterozygous for Wilson's disease and other medical conditions, had reduced hepatic uptake of copper compared with normal subjects. Other observations that illustrate the puzzling aspects of

Wilson's disease include chronic hepatic storage of copper with reduced serum ceruloplasmin levels.

When radiolabeled copper is administered to humans either orally or by i.v. injection, a rapid rise and fall of serum radioactivity in a 4-hour period is followed by a second phase wherin serum radioactivity rises more slowly (Bearn and Kunkel, 1954; Adelstein and Vallee, 1961). This second phase reflects the appearance of newly synthesized ceruloplasmin, which is released from the liver. Meanwhile, the concentration of liver radioactivity rises continuously, and small increases in bone marrow and erythrocyte radioactivity also occur (Bush et al., 1955; 1956; Adelstein and Vallee, 1961).

Bioaccumulation/Retention

Normal mammalian homeostatic mechanisms are effective in maintaining proper copper balance despite a wide range of dietary intake. A normal adult man (70 kg) is believed to store 70-120 mg of copper (U.S. EPA, 1980a,b). The liver functions as a depot for copper that synthesizes ceruloplasmin, the most abundant copper protein in the blood (U.S. EPA, 1980a, b).

Hepatic copper is distributed in several subcellular fractions associated with copper-dependent enzymes and copper-bound proteins (Evans, 1973). The large granule fraction (containing lysosomes and mitochondria) and nuclear fractions of normal mammalian livers each contain ~20% of the total hepatic copper. About 10% of hepatic copper is contained in the microsomal fraction. The cytosol fraction from normal mammalian liver contains the major portion of the total hepatic copper (Evans, 1973).

Factors that influence the amount of copper distributed in various body tissues include age, sex, amount of dietary copper and overall health of the individual. Neonates contain high concentrations of liver copper, ~6-10 times the amount found in a normal adult man (U.S. EPA, 1980a,b). These levels quickly decline within the first year of life, and a normal copper balance is then observed. The copper concentrations normally found in various human tissues are presented in Table III-1.

Metabolism

Copper is essential to the proper functioning of many important enzyme systems. It is well established that copper is required in the formation of hemoglobin, in pigment formation, in carbohydrate metabolism, in tissue respiration (Van Ravensteyn, 1944) and in catecholamine biosynthesis (Ahmed et al., 1981). Copper forms stable complexes and chelates with organic molecules such as amino acids, purines, pyrimidines, nucleotides, RNA, DNA and proteins (Evans, 1973). Several human copper proteins have been isolated including ceruloplasmin, superoxide dismutase, cytochrome oxidase, tyrosinase, monoamine oxidase and metallothionein (NAS, 1977a).

It is difficult to prepare a diet adequate in all other respects that contains <2 mg of copper/day (Scheinberg and Sternlieb, 1969). However, some special diets or unusual dietary conditions may result in dietary deficiencies of copper (Williams, 1982). It is generally accepted that ingestion of 2.5-5 mg Cu/day will result in proper human copper balance. Many investigators have reported estimates of total body copper, and although subject to variation, a value of ~80 mg Cu in a 70 kg man is an apparent modal value (Williams, 1982).

TABLE III-1

Copper in Various Human Tissues (ppm ash)*

	TISSUE						
	Liver	Brain	Heart	Kidney	Pancreas	Lung	Spleen
U.S. Children	1300	290	340	250	160	140	100
U.S. Adults	680	370	350	270	150	130	93

*Source: Schroeder et al., 1966

The most important organ involved in copper metabolism is the liver. It functions as a storage depot, a site for ceruloplasmin synthesis (for copper mobilization) and a site for forming a complex of copper with substances of variable molecular weight for subsequent biliary excretion (Dowdy, 1969).

Several investigators have attempted to characterize the copper compounds that leave the liver by the bile. The particular components include low molecular weight proteins and polypeptides, macromolecular complexes and bile acids and pigments (Sternlieb, 1980).

In vitro experiments in isolated perfused rat livers indicate that the copper put into storage, incorporated into ceruloplasmin or released in bile may originate in separate intracellular pools (Hazelrig et al., 1966). A three-compartment model has been postulated to explain this phenomenon. Under normal conditions, the three liver compartments and body tissues are in equilibrium. When excess copper is absorbed, the storage compartment accumulates the metal and eventually moves it to the other compartments for biliary excretion and ceruloplasmin synthesis. Ceruloplasmin is released into serum and is distributed. The liver may catabolize ceruloplasmin that is returned to the liver (Dowdy, 1969).

Mechanistic and quantitative models for human copper balance are presented in Figures III-1 and III-2.

Excretion

Copper is excreted from the body in bile, feces, sweat, hair, menses and urine. Biliary excretion is a major pathway for excretion of copper in man

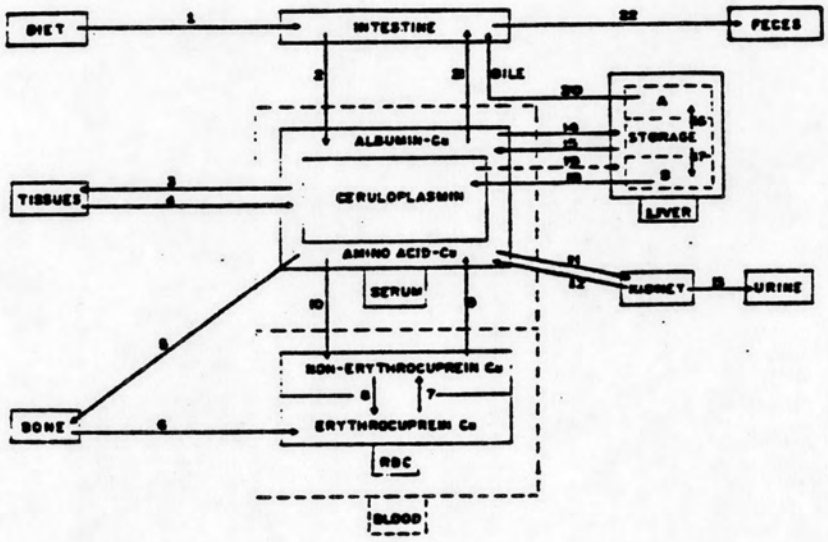

FIGURE III-1

Mechanisms of Human Copper Balance

Source: Dowdy, 1969

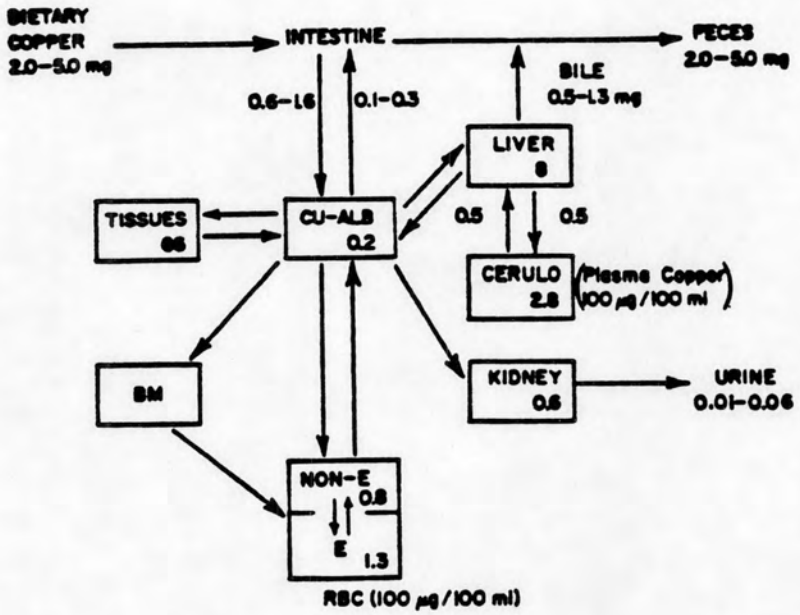

FIGURE III-2

Schematic Representation of Some Metabolic Pathways of Copper.

The numbers in the boxes refer to milligrams of copper in the pool. The numbers next to the arrows refer to milligrams of copper transversing the pathway each day. CU-ALB, direct-acting fraction; CERULO, ceruloplasmin; NON-E, nonerythrocuprein; E, erythrocuprein; BM, bone marrow; RBC, red blood cell.

Source: Herbert, 1970

(Gollan and Deller, 1973). Estimates of the amount of biliary copper excretion range from 0.5-1.2 mg/day (U.S. EPA, 1980a,b). Human bile was labeled in vivo by administering 500 μCi of ^{64}Cu-cupric acetate (specific activity = 2.5 m Ci/mg) by i.v. injection to seven patients. The bile was collected at scheduled cholecystectomy operations, dialyzed and subjected to gel filtration with subsequent counting of radioactivity. About 2-4% of the administered radioactivity was present in gall bladder bile after 4 hours. Most (62-99%) of the ^{64}Cu in bile was in nondiffusible form, as observed in dialysis experiments. Gel filtration revealed the presence of two heat stable radioactive protein fractions, with molecular weights between 20,000 and 200,000. A smaller amount of radioactivity was excreted in a readily diffusible compound with low molecular weight.

Biliary copper travels to the intestine where it can be eliminated in the feces. Enterohepatic cycling does not occur to a great extent, particularly if adequate protein is present in the diet (Evans, 1973).

In adults, fecal excretion of unabsorbed and biliary copper probably represents ~60% of that taken in, as estimated by the difference between oral intake and fecal excretion (U.S. EPA, 1980b). Some excreted copper from saliva and gastric and intestinal mucosa may be present in the feces, but may only account for ~0.4 and 1 mg/day, respectively. Menstrual fluid may contain 0.1 mg Cu/month (U.S. EPA, 1980a,b).

Of the daily turnover of copper in normal persons, only ~4% is excreted through the urine (Dowdy, 1969). What little copper does appear in the urine may originate from amino acid-bound metal or from the dissociation of

copper-albumin complexes. Little of the copper that is bound to ceruloplasmin or contained in erythrocytes will permeate the glomerulus, thus preventing excretion (Evans, 1973). Schroeder et al. (1966) estimated that daily copper excretion in normal humans from feces, urine and sweat is ~3640, 60 and 2 μg, respectively.

Summary

Administration of radiocopper to human subjects has been helpful in elucidating the mechanisms of copper disposition. Absorption of ingested copper occurs primarily in the upper portion of the GI tract, with rapid appearance of copper bound to albumin and amino acids in the blood 1-2 hours after administration. Absorbed copper is transported by the blood to the liver, where it accumulates and results in decreased levels of blood copper. Blood copper levels then begin to rise slowly, reflecting the hepatic production and release of copper-bound ceruloplasmin. Copper is also incorporated into several other proteins, including cytochrome oxidase, monoamine oxidase, tyrosinase and erythrocyte superoxide dismutase. Biliary excretion of low molecular weight copper complexes occurs rapidly after administration, with increasing amounts of high molecular weight copper complexes being formed over time. Copper elimination occurs primarily by the fecal route. Negligible enterohepatic recycling of protein bound copper occurs in well-fed individuals. Relatively little copper is excreted through urine, sweat or menstrual fluid.

IV. HUMAN EXPOSURE

Detailed information concerning the occurrence of and exposure to copper in the environment is presented in a draft document entitled "Estimated National Occurrence and Exposure to Copper in Public Drinking Water Supplies" (U.S. EPA, 1987). This section summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food and air.

Exposure Estimation

The sources of intake considered in this analysis are limited to drinking water, food and air, since these media are considered to be general sources common to all individuals. Some individuals may be exposed to copper from sources other than the three considered here. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary from person to person based on individual lifestyles, physiologic characteristics. Individuals living in the same neighborhood or even in the same household can experience very different levels of exposure.

The following sections summarize the available information on the occurrence of copper in drinking water, diet and air. To the extent possible the relative significance of the three routes for total intake of copper is evaluated.

Drinking Water. Information on both the occurrence of copper in public water supplies, and estimates of resulting human exposure from drinking water are presented in U.S. EPA (1987).

VI. HEALTH EFFECTS IN HUMANS

The essentiality of copper in human nutrition has long been recognized. Copper deficiency is associated with reduced hemoglobin formation, reduced elastin formation, teratogenesis, abnormal amino oxidase activity and many other problems. The literature is replete with studies regarding the nutritional essentiality of copper, but copper toxicity from chronic exposure has not been as well investigated. Chronic toxicity has been characterized from study of individuals with Wilson's disease, an inborn error of metabolism that results in accumulation of copper.

The NAS (1980a) has a recommended daily allowance (RDA) for copper of 1.5-2.5 mg/day for a child aged 0-10 years and 2.0-3.0 mg/day for children >11 years and adults. Copper sulfate historically was used as an emetic at a dose of 300 mg (76.2 mg Cu), but this use has been discontinued. Copper sulfate is included in many vitamin supplement tablets (0.5-2 mg Cu) and has been used as a hematinic at adult doses of 15-30 mg/day (3.8-7.6 mg Cu/day) (Hoover, 1970).

Clinical Case Studies

Reports of single cases or a series of cases after exposure is believed to have occurred and are useful for obtaining clinical details of adverse effects. Evidence of exposure, such as serum copper levels, support the qualitative association and help to obtain typical symptomology. However, these cases often offer limited ability to measure the exposure level of the causative agent, duration of exposure to the agent, previous health status or possible exposures to other agents. Rates of response can only be obtained from planned or epidemiologic studies.

Copper occurs in drinking water largely as the result of corrosion of plumbing materials. Naturally occurring levels are expected to be small, <50 µg/ℓ. The level of copper in drinking water is not constant for any drinking water system. Levels will vary for each tap as a function of the specific materials used in the connection, the corrosivity of the water, and the length of time that the water is in contact with the materials. Because of the high degree of variation in copper levels, the U.S. EPA is unable to accurately estimate the number of individuals who consume water that, on average, exceeds the copper MCL. The best available source of occurrence data for the distribution of copper levels at consumers' taps is provided by a survey of drinking water samples by Patterson (1981). This survey found that ~10% of the taps sampled had 500 µg/ℓ of copper and ~2% of the nation's taps had levels of copper >1300 µg/ℓ. While the Agency has some reservations concerning the use of this data in estimating national occurrence, it strongly suggests that a significant number of individuals consume water with levels of copper >1300 µg/ℓ.

Adults consuming 2 ℓ/day of water with 500 or 1300 µg/ℓ copper will have daily intakes of 1 and 2.6 mg, respectively.

Diet. The FDA provided mean daily intakes of copper reflecting levels of copper in 12 Total Diet Studies conducted from mid-1982 to mid-1984 (Pennington et al., 1986). These values, shown in Table IV-1, represent average daily intakes of copper from the diet.

TABLE IV-1

Summary of FDA Total Diet Study
Estimates of Copper Intake for 1982-1985*

Age Group	Sex	Intake (mg/day)
14-16 year old	Female	0.77
14-16 year old	Male	1.18
25-30 year old	Female	0.93
25-30 year old	Male	1.24
60-65 year old	Female	0.86
60-65 year old	Male	1.17

*Source: Adapted from Pennington et al., 1986

Air. Limited information is available on the presence of copper in ambient air. The U.S. EPA's Environmental Monitoring Systems Laboratory provided information from a nationwide study on copper concentrations in ambient air for the years 1977-1983 (U.S. EPA, 1984). Concentrations of copper in the 23,814 air samples analyzed ranged from 0.003-7.32 $\mu g/m^3$, with a range of median values for different cities and years of 0.004-1.79 $\mu g/m^3$ and mean values of 0.0043-1.96 $\mu g/m^3$.

Using the high value of the range of median levels and assuming a ventilation rate of 20 m^3/day for the average adult male, the intake of copper from air would be 35.8 μg/day.

Summary

Although data are available on the intake of copper from drinking water, food and air, there is not sufficient information available to describe in detail the relative contributions of those sources to total intake. However, some general observations can be made. Food and water are the major sources of exposure for all individuals. Air concentration levels are low and thus constitute a minor contribution to the total intake. For the majority of adults intake from diet will be larger than intake from drinking water. However, drinking water intake will be greater than dietary intake in those individuals who have high levels of copper in their tap water (>500 $\mu g/\ell$).

V. HEALTH EFFECTS IN ANIMALS

Introduction

Copper is an essential element because it is a component of enzymes that are vital in hemopoiesis, maintenance of vascular and skeletal integrity and structure and function of the central nervous system (O'Dell, 1976). As is usual for elements that play a key physiological role, homeostatic mechanisms exist to resist toxicity. There are abundant data on the effects of copper deficiency in mammalian species, leading to information on copper metabolism.

Chronic copper poisoning in domestic animals such as pigs, sheep and cattle occurs as a result of contamination of feeds or even under natural grazing conditions. The continued ingestion of excess copper results in accumulation in tissues, especially of the liver although hepatic storage capacity varies among species. In sheep and cattle high liver copper levels lead to liberation of copper into the blood followed by hemolysis and jaundice. Hemolytic jaundice has not been reported in rats or rabbits, and rats are extremely tolerant of high copper intakes (Underwood, 1971). Target organs of copper toxicity also include the central nervous system, kidney and eye. In pigs the toxicity is determined by the ratio of level of copper to that of other dietary components such as zinc, iron and calcium (Scheinberg and Sternlieb, 1976).

Copper plays an important role in mammalian reproduction, as deficiency of this metal leads to reproductive abnormalities. Some evidence exists to implicate copper as an animal teratogen when present in excess quantities.

Short-term Toxicity. Boyden et al. (1938) studied the effects of feeding high levels of copper to albino rats for 4 weeks. Groups of 3-5 mixed male and female 28-day-old albino rats were maintained on diets supplemented with 0, 500, 1000 or 2000 ppm copper (as copper sulfate) for 4 weeks. (Diets of 4000 ppm resulted in death within 1 week.) The authors stated that these dosage levels resulted in average copper intakes of 0, 5.1, 8.2, and, for 2000 ppm, 9.8 and 11.8 mg/rat/day based on observed food consumption. These intakes are not proportional to the copper in the food because of the decreased food intake with higher doses. Animal body weight was recorded weekly. Reduced body weight gain, reduced food intake, and jaundice were observed in rats maintained on diets containing 2000 ppm copper. Slightly reduced body weight gain and food intake were observed in rats maintained on diets containing 500 ppm copper; greater reductions occurred at 1000 ppm. At the end of the 4th week of exposure, blood samples were taken, the rats were killed and their gross pathology was examined. The livers and spleens were removed for analysis of copper content. Copper concentrations in the liver increased with each increase in copper dosage, while the blood showed the most pronounced increase between 500 and 1000 ppm. Greater copper accumulation was observed in the blood, spleen and especially liver of all treated rats in comparison with the control group. The authors stated that slight toxicity was observed in rats fed 500 ppm and toxicity increased as the dietary copper level was increased (Table V-1).

Rana and Kumar (1980) reported liver and kidney necrosis in rats given copper sulfate by gavage for 20 days. Groups of ten 90-day-old male albino rats were given control diets or 100 mg copper sulfate/kg bw/day by gavage (vehicle not reported) for 20 days. Based on the molecular weight of copper/copper sulfate, the amount of copper given each day was 25.4 mg/kg or

TABLE V-1

Short-term Oral Toxicity Data in Rats

Species/Strain	Sex/No.	Weight/Age	Compound	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/NR	M/4/group 12 controls	NR/weanling	$CuSO_4$	oral	diet	0 (control)	1-15 weeks	No observed effect	Haywood, 1980
						500 mg Cu/kg diet (2000 mg $CuSO_4$/kg diet)	1 week	No observed effect	
							2 weeks	Hypertrophy of liver	
							6 weeks	Liver and kidney necrosis	
							9 weeks	Regeneration	
							15 weeks	Regeneration	
Rat/albino	M/10	90-110 g/ 90 days	$CuSO_4$	oral	gavage (vehicle NR)	0 (control)	20 days	No observed liver and kidney necrosis, although body weight, tail length and hematocrit were decreased	Rana and Kumar, 1980
						100 mg $CuSO_4$/ kg bw/day (~2.5 mg Cu/day)	20 days		
Rat/white	M/4, F/4	NR/21 days	$CuSO_4$	oral	diet	0 (control)	27 days	No observed effect	Boyden et al., 1938
	M/4, F/4					(500 ppm diet)	27 days	Slight growth reduction, increased blood, spleen and liver Cu concentration	
	M/1, F/2					(1000 ppm diet)	27 days	Reduced growth and food intake, increased blood, spleen and liver Cu concentration	
	M/5, F/3					(2000 ppm diet)	27 days	No growth. Increased blood, spleen and liver Cu concentration	
	M/1, F/2					(4000 ppm diet)	7 days	Rapid weight loss and death, probably from starvation	
Rat/Wistar	M/4	90-100 g/ "young"	$CuSO_4$	oral	diet	none added (control)	35 days	4.3 µg Cu/g wet liver,	Miranda et al., 1981
						50 ppm added		67.1 µg Cu/g wet liver, significant at $p<0.05$	

TABLE V-1 (cont.)

Species/Strain	Sex/No.	Weight/Age	Compound	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/Albino	M/12	180-200 g/NR	$CuCl_2$	i.p.	saline	0 (0.5 ml saline)	21 days	Baseline enzymatic activities	Malhotra et al., 1982
						2 mg Cu/kg/day		Brain ATPase, SDH, cytochrome C-oxidase, diaphorase, MDH, and 5-hydroxytryptamine not significantly different from controls	
								Levels of brain Dopamine and norepinephrine significantly ($p<0.05$) higher than controls	
Rat	NR	NR	copper acetate	oral	diet	250 ppm Cu acetate diet	16 months	Renal and hepatic copper accumulation	Howell, 1959

NR = Not reported

2.5 mg/day. Several changes in treated rats, absent in control rats, were observed upon gross, histopathologic and hematologic examination. The paws of treated rats had changed from pink to white. Significant decreases in the tail length, body weight gain, hemoglobin concentration, hematocrit percentage and red blood cell count of treated rats were observed as compared with controls. Upon histologic examination, the livers and kidneys of control rats appeared normal. The livers of treated rats showed centrilobular necrosis, perilobular sclerosis and heavy deposition of copper in centrilobular parenchyma. Necrosis, tubular engorgement and copper retention were observed in the kidneys of treated rats.

Miranda et al. (1981) added copper to the feed of rats to determine effects of copper on toxicity of tansy ragwort (Senecio jacbaea). They reported that 50 μg/g copper supplement to the diet enhanced the effect of the plant on hepatotoxicity. Supplementary copper alone at 50 ppm resulted in significantly increased liver copper concentrations but did not affect liver enzymes.

Malhotra et al. (1982) reported that i.p. injection of cupric chloride (2 mg Cu/kg) produced neurologic changes in rats. Groups of 12 ITRC bred male albino rats received daily injections of saline or copper for 21 days. Brain mitochondrial enzyme levels were determined on these rats, and no effect of copper was observed on the levels of cytochrome-c-oxidase, malate dehydrogenase, diaphorase, succinic dehydrogenase, adenosine triphosphatase or 5-hydroxytryptamine. Statistically significant ($p<0.05$) increases were observed in brain dopamine and norepinephrine levels in treated rats when compared with controls.

Subchronic Toxicity

In a series of studies on pigs, Suttle and Mills (1966a,b) demonstrated that the effects of copper salts in the diet can be modified by supplementary iron and zinc. Groups of six Large White female pigs were maintained on maize meal diets containing 0 and 750 ppm of basic copper carbonate [$(CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$] alone or with supplemental zinc (500 ppm $ZnCO_3$) or supplemental iron (750 ppm $FeSO_4 \cdot 7H_2O$). This experiment was a 2 by 2 factorial design that lasted for 42 days (zinc) and 49 days (iron). The copper doses to the pigs were ~22 mg Cu^{+2}/kg/day, as calculated from weight and food intake data. Toxic effects at 750 ppm Cu included gross degenerative changes in the liver, anemia, accumulation of copper in the serum and decreased food consumption, but the effects were reduced with the additional iron or zinc (see Table V-2).

In a second experiment to test the effect of dietary protein, Suttle and Mills (1966b) maintained groups of six female Large White pigs on maize meal diets supplemented with 600 ppm basic copper carbonate to which soybean meal, dried skim milk or white fish meal protein supplements were added. The treatment lasted for 48 days and the pigs in the soya, milk and fish fed groups received 6.4, 11.0 and 15.4 mg Cu^{+2}/kg/day, respectively. Gradual development of anemia was observed in all treatment groups, but the group fed fish showed signs of toxicity (see Table V-2). It was presumed that the high calcium levels in the fish meal diet adversely affected zinc availability, and thus led to toxicity.

TABLE V-2

Subchronic Oral Toxicity Data in Pigs

Species/Strain	Sex/No.	Age	Compound	Vehicle	Dose	Duration (days)	Effects	Comments	Reference
Pig/Large White	F/6/group	weanling	copper carbonate	maize diet	750 ppm diet, 750 ppm Cu plus 500 ppm Zn or Fe	49	Increased serum copper and aspartate transaminase (AST) in 9/12, and jaundice in 7/12. Reduced hemoglobin. Centrilobular necrosis and bile canaliculi disruption	Addition of iron (500 ppm) and zinc reduced these effects compared with controls with no copper.	Suttle and Mills, 1966
Pig/Large White	F/6/group	weanling	copper carbonate	maize and soya diet	600 ppm supplementary Cu	48	Gradual development of anemia		Suttle and Mills, 1966
			copper carbonate	maize and skim milk diet	600 ppm Cu in diet	48	Gradual development of anemia		
			copper carbonate	maize and fish meal diet	600 ppm Cu in diet	48	Increased serum copper, increased AST growth rate reduction. Gradual development of anemia and jaundice	High calcium in fish may have affected Zn availability	
			copper sulfate·$5H_2O$	maize and fish meal diet	250 ppm Cu in diet	79	Slight weight gain, increased liver copper concentrations, increased AST, jaundice	High calcium in fish may have affected Zn availability	
			copper sulfate·$5H_2O$	maize and fish meal diet	425 ppm Cu in diet	47-60	Severe growth depression and toxicosis after 14 days. Gastrointestinal hemorrhages	High calcium in fish may have affected Zn availability	
Pig/Hampshire and Yorkshire	NR/8	NR	copper sulfate·$5H_2O$	corn-soy diet	250 ppm Cu in diet	61	Accelerated growth with less feed		Kline et al., 197

TABLE V-2 (cont.)

Species/Strain	Sex/No.	Age	Compound	Vehicle	Dose	Duration (days)	Effects	Comments	Reference
Pig/Hampshire and Yorkshire	NR/8	NR	copper sulfate·$5H_2O$	corn-soy diet	500 ppm diet	61	Reduced growth and hemoglobin levels, increased liver copper concentrations		Kline et al., 1971
Pig/Hampshire and Yorkshire	NR/12	NR	copper sulfate·$5H_2O$	corn-soy diet	0 supplemental Cu	88	Normal hemoglobin hematocrit and liver copper levels		Kline et al., 1971
					150 ppm diet supplement:	88	Accelerated weight gain		
					200 ppm diet supplement:	88	Accelerated weight gain		
					250 ppm diet supplement:	88	Accelerated weight gain. No significant influence on hemoglobin, hematocrit or plasma copper levels. Increased liver copper stores.		

NR = Not reported

Suttle and Mills (1966b) then maintained groups of six female Large White pigs on maize meal/white fish diets containing 0, 250 or 425 ppm of copper sulfate ($CuSO_4 \cdot 5H_2O$) for 60-79 days, plus a group consuming a 425 ppm diet with supplementary iron and zinc (150 ppm each). The group treated with 425 ppm copper sulfate became so intoxicated that euthanasia was performed on 4/6 by the 60th day. The 250 ppm (2.6 mg Cu^{+2}/kg/day) group had slight weight gain for the first 30 days of treatment, but 3/6 had jaundice by the 46th day. Serum copper and aspartate transaminase levels were significantly increased compared with controls. On the 79th day, the hepatic copper concentration of treated pigs was significantly increased compared with controls. The highest growth curve was obtained for the group consuming 425 ppm copper plus iron and zinc.

Kline et al. (1971) maintained groups of eight Hamshire, Yorkshire or Hamshire-Yorkshire pigs on a corn-soy diet supplemented with 250 (3.2 mg Cu^{+2}/kg/day) or 500 (5.5 mg Cu^{+2}/kg/day) ppm copper for 61 days. In a second experiment, groups of 12 pigs were fed a corn-soy diet supplemented with 0, 150 (1.8 mg Cu^{+2}/kg/day), 200 (2.5 mg Cu^{+2}/kg/day) or 250 (2.9 mg $Cu^{+}2$/kg/day) ppm copper sulfate for 88 days. Animals were weighed and food consumption was recorded. Blood samples were drawn and representative animals were slaughtered at the end of treatment. The control group was normal and had normal blood parameters. Pigs fed 150-250 ppm copper sulfate (1.8-3.2 mg Cu^{+2}/kg/day) all had accelerated weight gain and no visible signs of copper toxicity but had increases in liver copper that indicated a significant ($p<0.05$) linear correlation with dose. Reduced growth and hemoglobin levels, and increased liver copper were observed in the 500 ppm treatment group.

Chronic Toxicity

Oral. Limited data were available regarding the chronic toxicity of copper in laboratory animals. One study (Howell, 1959) emphasized copper distribution rather than examination of toxicologic endpoints.

Howell (1959) maintained rats on diets supplemented with 5000 ppm copper acetate for 16 months. This dose is equivalent to 250 mg Cu acetate/kg/day (79.6 mg Cu/kg/day), assuming that rats consume food at a rate of 5% of their body weight per day. No copper deposition was found in the brain or cornea. Liver and kidney tissues were observed to accumulate copper heavily.

Haywood (1980) reported that effects of dietary copper on kidney and liver of rats are reversible over time. Groups of four male weanling rats (strain not stated) were maintained on control diets or supplemented with 2000 mg Cu (as copper sulfate)/kg and killed at intervals of 1, 2, 3, 6, 9 and 15 weeks. Upon sacrifice, the livers and kidneys were removed, examined histologically, and were assayed for copper content. Animals maintained on the test diet for 1 week had normal livers and kidneys, and there was no evidence of copper accumulation in these organs. After 2 weeks of exposure, the livers of all rats on the test diet showed hypertrophy of liver parenchymal cells and accumulation of copper in the liver. Kidneys were normal and no copper accumulation was evident. By 3 weeks on the test diet, the livers of treated rats had clearly established areas of hypertrophied hyperchromatic parenchymal cells, some of which were necrotic. Marked liver deposition of copper was observed. After 6 weeks on the test diet, all livers had marked changes, consisting of widespread and sometimes severe necrosis and bile duct hyperplasia. Some necrotic areas had macrophages

present, and regenerative activity in surviving cells was observed. Kidneys had extensive desquamation of the epithelial cells in the proximal convoluted tubules. Some regenerative activity was observed in surviving epithelial cells. By 9 weeks the livers of the treated rats showed necrosis in the periportal zone; bile duct hyperplasia and bands of fibroblastic connective tissues and kidneys were reported to be undergoing regeneration of the proximal convoluted tubules; most appeared reconstituted. After 15 weeks on the test diet, advanced healing of the liver was observed, but bile duct hyperplasia and necrotic remnants were still present. In the kidney, reconstitution was mostly complete, but a low level of regenerative activity was observed. The authors discussed the triphasic nature of renal copper toxicity in the rat. The first phase was characterized by rising copper content with little initial effect, but increased cellular disruption with increasing concentration. When copper content reached a maximum, a second phase characterized by severe necrosis was observed. A third regenerative phase was then observed and was characterized by declining liver copper content and repair of damaged tissue.

These results are difficult to understand in view of the toxicity at similar dose levels reported by Boyden et al. (1938). If galvanized cages were used, the ingestion of zinc may have contributed to the unusual results.

Summary. Studies of copper toxicity serve to characterize the nature of the toxicity qualitatively, but the design and goal of studies do not permit characterization of nontoxic dose levels. The liver is the main storage depot for copper, and the damage done in this organ is associated with accumulation of the metal. The specific etiology of copper-induced

liver damage has not been completely determined (Bremner, 1979). Administration of copper compounds to laboratory animals has resulted in hepatocellular necrosis, regenerative activity, cirrhosis, Kupffer cell mobilization and hepatocellular pigment formation (Barka et al., 1964).

Excessive ingestion of copper was reported to cause kidney necrosis in rats upon subchronic administration (Rana and Kumar, 1980; Haywood, 1980). Haywood (1980) reported that the onset of kidney necrosis in rats occurred only after the liver began to accumulate high levels of copper.

Serum levels of copper rise after copper accumulates in the liver. Several investigators have described a sudden hemolytic crisis in sheep poisoned by copper. Ruminants have been observed to be sensitive to the effects of high levels of copper.

Few chronic data exist and rat studies presented all have serious flaws such as high dose levels and associated weight loss, reduced dietary intake, and short duration (Boyden et al., 1938). Subchronic studies in pigs do not clearly indicate levels free of adverse effects without dietary supplements.

Inhalation

Pimental and Marques (1969) reproduced lesions characteristic of vineyard sprayers' lung in guinea pigs exposed to saturated atmospheres of Bordeaux Mixture. Groups of six guinea pigs were exposed to air or the

smoke from burning sulfur wicks (3 times/day) for 6.5 months. A group of 12 guinea pigs were exposed 3 times/day to an atmosphere saturated with Bordeaux Mixture for 6.5 months. Copper-containing micronodular lesions and small histiocytic granulomas were observed in exposed guinea pigs.

Other Effects

Carcinogenicity. BRL (1968) studied the carcinogenicity of a copper-containing compound, copper hydroxyquinoline, in two strains of mice (B6C3F1 and B6AKF1) fed a diet that provided sufficient copper (i.e., 5.7 mg Cu/kg feed). The copper complex was administered orally and by subcutaneous injection. Groups of 18 male and 18 female 28-day-old mice of both strains were given a single s.c. injection of gelatin or 1000 mg copper hydroxyquinoline/kg bw (180.6 mg Cu/kg) suspended in 0.5% gelatin. The animals were observed until they were 78 weeks old, and then killed. Similar groups of 7-day-old mice were treated daily by gavage with 1000 mg copper hydroxyquinoline/kg bw (180.6 mg Cu/kg) suspended in 0.5% gelatin until they were 28 days old, whereupon the compound was administered in the feed at a concentration of 2800 ppm (505.6 ppm Cu). Animals were fed the treated diet until they were 78 weeks old, at which time they were killed. All animals killed or found dead were subjected to routine macroand microscopic histological analysis to identify tumor-bearing tissues. No statistically significant increases (with respect to controls) in the incidence of lymphatic leukemias, reticulum cell sarcomas, pulmonary adenomas or carcinomas, hepatomas, hepatic carcinomas, mammary carcinomas, skin carcinomas or cavernous angiomas were observed in orally-treated mice.

In the portion of the study using subcutaneous exposure, male B6C3F1 mice had an increased incidence of reticulum cell sarcomas compared with controls (e.g., 6/17 treated; 8/141 control; $p<0.001$). No tumors were observed in treated male B6AKF1 mice. Female mice of either strain had low incidences of reticulum cell sarcomas. Treated and control B6C3F1 females had incidences of reticulum cell sarcoma of 1/18 and 1/154, respectively. Treated and control B6AKF1 females had incidences of reticulum cell sarcoma of 3/18 and 5/157, respectively (BRL, 1968).

Gilman (1962) studied the carcinogenicity of several metallic compounds including cupric oxide, cupric sulfide and cuprous sulfide in 2- to 3-month-old Wistar rats. Groups of 30-32 rats were given single intramuscular injections containing 20 mg of cupric oxide (16 mg Cu), cupric sulfide (13.3 mg Cu) and cuprous sulfide (16 mg Cu) into the left and right thigh of each rat. All animals were observed for up to 20 months, after which histopathological evaluation was conducted. Controls were not reported. Of the animals receiving cupric oxide, cupric sulfide and cuprous sulfide, the ratios of animals surviving the experiment/animals dosed were 10/32, 19/30 and 20/30, respectively. No injection-site tumors were observed, and the groups of animals receiving cupric oxide, cupric sulfide and cuprous sulfide had 0, 2 and 1 tumors, respectively.

The sulfides of other metals tested, nickel and cobalt, induced more tumors than the oxide. However, intramuscular injection is not considered the route of choice for evaluating carcinogenic potential.

Haddow and Horning (1960) published a table of bioassay results on various copper compounds from which Table V-3 was prepared. No other experimental detail was provided. Thus there are few data, none suggesting carcinogenicity of copper.

Mutagenicity. The available data obtained from in vitro mutagenicity assays are summarized in Table V-4.

A reverse mutation assay reported dose-related mutation in E. Escherichia coli with 2-10 ppm copper sulfate (Demerec et al., 1951). More recently, Moriya et al. (1983) reported the absence of mutation in E. coli incubated with up to 5 mg copper quinolinolate/plate and in Salmonella typhimurium strains TA98, TA1535, TA1537 and TA1538. Copper 8-quinolinolate was mutagenic to S. typhimurium strain TA100, but only when a source of mammalian metabolic activation was included (Moriya et al., 1983). Up to 5 mg of copper sulfate/plate did not induce reverse mutations in S. typhimurium TA98 and TA100 either with or without metabolic activation.

Other investigators have obtained negative mutagenic results with copper sulfate or copper chloride in other microbial assays. These include Saccharomyces cerevisiae D-7 (Singh, 1983) and Bacillus subtilis (Nishioka, 1975; Matsui, 1980; Kanematsu et al., 1980).

Several isolated cell mutagenicity assays have produced positive results with copper compounds. Errors in DNA synthesis from poly(c)templates have been induced in viruses (Sirover and Loeb, 1976) and chromosome aberrations

TABLE V-3

Tumorigenicity of Some Copper Compounds*

Agent Under Test	Number and Strain of Mice	Number of Weekly Subcutaneous Injections/Dose	Months of Experiment to Date and Survivors	Tumors Recorded
Copper-dextran	20 stock	6/0.1 cc of 1 in 4 dilution	10 (13)	None
8-hydroxyquinoline copper complex	20 stock	39/0.1 mg	10 (14)	1 pleomorphic sarcoma
Cross-conjugated macrocycle copper porphyrin	20 stock	4/0.5 mg	10 (14)	None
Copper phthalocyanine	20 stock	34/0.5 mg	8 (17)	None
Copper phthalocyanine tetra-3-sulfonic acid	20 stock	36/0.5 mg	8 (20)	None
Copper phthalocyanine tetra-4-sulfonic acid	20 stock	25/0.5 mg	8 (11)	None

*Source: Haddow and Horning, 1960

TABLE V-4

Mutagenicity Data for Copper Compounds

Assay	Indicator/ Organism	Application	Concentration or Dose	Activating System	Response*	Comment	Reference
Reverse mutation	Salmonella typhimurium TA98, TA100	plate incorporation	≤5000 µg copper sulfate/plate	+ rat liver S-9	-	NC	Moriya et al., 1983
Reverse mutation	S. typhimurium TA100	plate incorporation	copper 8-quinolinolate 5-10 µg/plate	+ rat liver S-9	+	NC	Moriya et al., 1983
Reverse mutation	S. typhimurium TA100	plate incorporation	0.5-10 µg copper 8-quinolinolate/plate	- rat liver S-9	-	NC	Moriya et al., 1983
Reverse mutation	S. typhimurium TA98, TA1535, TA1537, TA1538	plate incorporation	≤5000 µg copper 8-quinolinolate/plate	+ rat liver S-9	-	NC	Moriya et al., 1983
Reverse mutation	S. typhimurium TA100, LT2	spot test (paper disc)	10 µl of 10^{-6} to 10^{-1} M aqueous solution of $CuCl_2 \cdot 2H_2O$	NA	-	NC	Tso and Fung, 1981
Reverse mutation	Saccharomyces cerevisiae D-7	spot test (center wall)	0.1 M copper sulfate	NA	-	NC	Singh, 1983
Reverse mutation	Escherichia coli WP2 hcr	plate incorporation	≤5000 µg copper 8-quinolinolate/plate	NA	-	NC	Moriya et al., 1983
Reverse mutation	E. coli Sd-4	plate incorporation	2-10 ppm copper sulfate	NA	+	Dose related at ≥2 ppm	Demerec et al., 1951
Growth inhibition (rec)	Bacillus subtilis H17, M45	spot test (paper disc)	0.05 ml of 0.05 M CuCl or $CuCl_2$ solution	NA	-	NC	Nishioka, 1975
Growth inhibition (rec)	B. subtilis H16 17, H16 45	liquid cultivation	16.5-18 mg copper sulfate/l	NA	-	NC	Matsui, 1980
Growth inhibition (rec)	B. subtilis H17, M45	spot test (paper disc)	0.05 ml of 0.001-10 M CuCl or $CuCl_2$ solution	NA	-	NC	Kanematsu et al., 1980
Chromosome aberrations	Avian myeloblastosis virus, DNA polymerase	liquid holding	20-150 mM $CuCl_2$ or $Cu(C_2H_3O_2)_2$	NA	+	NC	Sirover and Loeb, 1976

TABLE V-4 (cont.)

Assay	Indicator/ Organism	Application	Concentration or Dose	Activating System	Response*	Comment	Reference
Chromosome aberrations	rat hepatocytes	plate incorporation, then alkaline elution	0.03-0.3 mM cupric sulfate	NA	-	Elution rate ≤3 times the control rates	Sina et al., 1983
Chromosome aberrations	rat hepatocytes	plate incorporation, then alkaline elution	1.0 mM cupric sulfate	NA	+	Elution rate ≥3 times controls rates	Sina et al., 1983
Cell transformation	Syrian hamster embryo cells by simian adenovirus SA7	plate incorporation	≥0.38 mM Cu_2S	NA	+	Enhancement ratio: Transformation frequency treated / Transformation frequency control = 16.2	Casto et al., 1979
Cell transformation	Syrian hamster embryo cells by simian adenovirus SA7	plate incorporation	0.08-0.64 mM $CuSO_4$	NA	+	Enhancement ratio = 2.2	Casto et al., 1979
Recessive lethals	Drosophila melanogaster Oregon-R	microinjection into larvae	0.1% $CuSO_4$	NA	+	1.06% lethals (0% in controls)	Law, 1938
Recessive lethals	D. melanogaster Oregon-R	immersion of eggs for 10 minutes	concentrated aqueous solution of $CuSO_4$	NA	+	1.25% lethals (0% in controls)	Law, 1938
Mitotic abnormalities	MTK-sarcoma III rat ascites	in vivo	150 mg copper sulfide/kg i.p.	NA	+	Chromatic aggregation, stickiness, contraction, scattering, lagging and clumping of chromosomes	Kimura and Makino, 1963
Mitotic abnormalities	MTK-sarcoma III rat ascites	in vivo	300 mg copper sulfate/kg i.p.	NA	+	Reversible events: lobated nuclei, karyorrhexis and multipolar spindle formation	Kimura and Makino, 1963

NA = Not applicable; NC = No comment

have resulted in isolated rat hepatocytes (Sina et al., 1983) when incubated in 20-150 mM $CuCl_2$ or $Cu(C_2H_3O_2)_2$ and 1.0 mM $CuSO_4$, respectively. Casto et al. (1979) induced enhanced simian adenovirus cell transformation in Syrian hamster embryonic cells with the addition of 0.38 mM Cu_2S and to a lesser extent with 0.08 mM of $CuSO_4$.

High concentrations of copper compounds have been reported to induce abnormalities at mitosis in rat ascites cells and recessive lethals in Drosophila melanogaster. Law (1938) reported increases in the percent lethals observed in Drosophila larvae and eggs when exposed to copper by microinjection (0.1% $CuSO_4$) or immersion (concentrated aqueous $CuSO_4$), respectively.

The available data for mutagenicity are largely negative. There is insufficient evidence to assess the mutagenicity of copper.

Teratogenicity and Reproductive Toxicity. Copper deficiency has been observed to produce teratogenic response in lambs, goats, rats, guinea pigs, dogs and chicks. Terata include neural degeneration, reduced growth, skeletal malformations and cardiovascular lesions (Hurley and Keen, 1979).

The spermicidal properties of copper are well known and were first demonstrated in the 19th century (Holland and White, 1982). Prevention of mammalian embryogenesis because of the small amounts of copper absorbed from intrauterine loops or wires fashioned from copper has been demonstrated (Oster and Salgo, 1977; Hurley and Keen, 1979).

The embryotoxicity and teratogenicity of i.v. injected copper salts was first demonstrated in hamsters by Ferm and Hanlon (1974). Copper sulfate and copper citrate dissolved in demineralized water were both observed to reduce embryonic viability and produce abnormal offspring when injected into the lingual vein of pregnant dams on the 8th day of gestation. Day 1 of gestation was considered the day after which mating occurred. Administration of demineralized water alone produced no abnormal embryos (Table V-5). Administration of copper sulfate (2.13 mg Cu/kg) to 16 dams caused 12 abnormal formations of 155 live embryos (five thoracic wall hernias, four encephalocoeles, two spina bifida and one microphthalmia). Similar administration of copper sulfate (at 4.25 mg Cu/kg) to three dams caused 4 of 7 live embryos to be abnormal (one exencephaly, one hydrocephalus, one abdominal hernia and one abnormal spinal curvature). Administration of higher doses of copper sulfate (7.5 and 10 mg/kg) resulted in 100% mortality of embryos and dams, respectively.

Copper administered in a chelated form (copper citrate) was observed to be a more potent teratogen than the uncomplexed form (copper sulfate). When 0.25-1.5 mg Cu/kg (as citrate) was administered to 13 dams, 4 of the 172 live embryos were abnormal (two tail defects, one microphthalmia and one craniorachischisis). Similar administration of 1.8 mg Cu/kg to six dams produced 14 abnormal embryos of the 81 live embryos (13 tail defects and 1 meningocoele). Administration of 2.2 mg Cu/kg to eight dams produced 35 abnormal embryos (25 tail defects, 6 thoracic wall defects, 2 microphthalmias, 1 abdominal wall defect and 1 facial cleft). Administration of 4.0 mg Cu/kg to two dams resulted in the death of both.

TABLE V-5

Teratogenicity Data for Copper Compounds

Compound, Route	Species/ Strain	No. Dams at Start	Vehicle	Daily Dose or Exposure	Treatment Days[a]	Observation Day	Maternal Response	Fetal Response			Reference
								Avg. Litter Size	Avg. Weight (g)	Malformations	
$CuSO_4$, oral	mouse/ C57BL	21	diet	0	30 to 0	19	NR	3.1	1.1	0	Lecyk, 1980
		10		25.9 mg Cu/kg/day[b]				4.6	1.3	0	
		18		51.7 mg Cu/kg/day[b]				4.5	1.2	0	
		7		77.6 mg Cu/kg/day[b]				4.4	1.1	0	
		10		103.5 mg Cu/kg/day[b]				4.2	1.2	0	
		22		155.3 mg Cu/kg/day[b]				2.5	1.0	1 skeletal	
		18		207.1 mg Cu/kg/day[b]				1.9	1.0	3 hernia, hydrocephalus, skeletal	
$CuSO_4$, oral	mouse/DBA	17	diet	0	30 to 0	19	NR	4.5	1.0	0	Lecyk, 1980
		10		25.9 mg Cu/kg/day[b]				5.4	1.2	0	
		10		51.7 mg Cu/kg/day[b]				5.1	1.2	0	
		14		77.6 mg Cu/kg/day[b]				4.1	1.2	0	
		10		103.5 mg Cu/kg/day[b]				4.1	1.1	0	
		18		155.3 mg Cu/kg/day[b]				3.1	1.1	2 skeletal	
		20		207.1 mg Cu/kg/day[b]				2.7	1.1	4 encephaloceles, skeletal	
Copper citrate, i.p.	hamsters/ Golden	37	D.I. H_2O	0	8	12-13	NR	Free of gross teratogenic effects (0/455), 2/37 had abnormalities, 2/68 heart defects			DiCarlo, 1980
		89	D.I. H_2O	2.7 mg/kg			No effect on survival, but a 10% reduction in body weight gain.	45/855 fetuses with gross defects (limb, tail, edema) 19/89 litters had abnormalities, 58 ventricular septal defects in 49 fetuses.			
$CuSO_4$, i.v.	hamsters/ Golden	10	D.I. H_2O	0	8	12-13	NR	92% viable embryos, 8% resorption, 0% abnormal			Ferm and Hanlon, 1974
		16		2.13 mg Cu/kg			NR	74% viable embryos, 26% resorption, 6% abnormal			
		3		4.25 mg Cu/kg			NR	14% viable embyros, 86% resorption, 8% abnormal			
		3		7.50 mg Cu/kg			NR	0% viable embryos, 74% resorption, 8% abnormal			
		2		10.0 mg Cu/kg			Death				

TABLE V-5 (cont.)

Compound, Route	Species/ Strain	No. Dams at Start	Vehicle	Daily Dose or Exposure	Treatment Days[a]	Observation Day	Maternal Response	Fetal Response	Reference
Copper citrate, i.v.	hamsters/ Golden	13	D.I. H_2O	0.25-1.5 mg Cu/kg	8	12-13	NR	83% viable embryos, 16% resorptions, 2% abnormal	Ferm and Hanlon, 1974
		6		1.8 mg Cu/kg			NR	59% viable embryos, 41% resorptions, 17% abnormal	
		8		2.2 mg Cu/kg			NR	66% viable embryos, 34% resorptions, 35% abnormal	
		2		4.0 mg Cu/kg			Death		

[a]Relative to day of conception (day 0)

[b]Assume mice consume 13% of bw/day

NR = Not reported

Experiments with ^{64}Cu nitrate injected into pregnant dams showed that with 0.55, 12.8, 0.53, 1.47 and 0.81 μg ^{64}Cu/g tissue in the maternal blood, maternal liver, uterus, placenta and embryo, respectively, copper permeates the hamster placenta (Ferm and Hanlon, 1974).

DiCarlo (1980) produced terata in hamsters by i.p. injection of copper citrate. Pregnant female Golden hamsters were given an i.p. injection of demineralized water either alone or containing 2.7 mg copper citrate/kg on the 8th day of gestation. The day after mating was considered day 1 of gestation. The control and dosed groups consisted of 37 and 89 animals, respectively. All dams were killed on the 12th or 13th day of gestation, whereupon all viable embryos were removed for histopathologic analysis. Copper was not observed to affect maternal survival, but did reduce maternal body weight gain, possibly by inducing a high resorption rate. Control embryos were observed to be free of gross teratologic effects, but on histopathologic examination, 2 of 68 randomly-chosen embryos were observed to have cardiac muscular ventricular septal defects. Of the treated embryos, 45 of 855 embryos examined had gross malformations (e.g., limb and tail defects and edema). On histopathological examination, 58 cardiac defects (various ventricular septal malformations) were observed in 49 embryos with gross malformations. The author stated that copper's role as a prosthetic group in oxidative enzymes could lead to teratogenesis when present in excessive amounts by interfering with these metabolic reactions during organogenesis. However, the relevance of these studies is questionable because i.p. ingestion of a high level of copper does not duplicate any of the means whereby toxic levels might enter the body.

Lecyk (1980) oberved teratogenic effects in two strains of mice fed diets supplemented with copper sulfate before mating. Various numbers (see Table V-5) of C57Bl and DBA mice were maintained for 1 month on diets supplemented with 0, 500, 1000, 1500, 2000, 3000 and 4000 ppm copper sulfate. These concentrations are equivalent to 0, 199, 398, 597, 796, 1195 and 1593 ppm Cu, respectively. Assuming that mice consume food at a rate of 13% of their body weight per day, doses at 199 and 398 ppm copper are equivalent to 25.9 and 51.7 mg Cu/kg/day, respectively. After 30 days of treatment, the females were mated with males of respective strains and the day on which a vaginal plug was observed was determined as day 0 of gestation. Pregnant mice were allowed to gestate until the 19th day, at which time they were killed and the fetuses were examined for morphologic defects. Low doses (500-1000 ppm) of copper were observed to stimulate embryonic development; increased litter size and increased fetal weight resulted. Higher copper doses (>1000 ppm) increased fetal mortality and decreased litter size. When supplemented in the diet at 3000 and 4000 ppm, copper sulfate caused a level (2-8% of living fetuses) of various skeletal and other malformation that was absent at lower doses and controls. No abnormal fetuses were observed in control groups. However, the observations by Boyden et al. (1938) imply that food intake decreases at high concentrations of copper. Therefore, the actual food intake may have been seriously reduced, therefore adversely affecting fetal development.

Summary

Efficient homeostatic mechanisms generally protect mammals from the adverse effects of dietary copper excess. With the exception of ruminant animals, the chronic toxicity of orally-administered copper has not been

well investigated. An inborn error in copper metabolism in humans (Wilson's disease) results in chronic copper toxicosis in man. The information this has yielded is reviewed in the next chapter.

Ingestion of 150 mg Cu/kg/day (as 500 ppm Cu in diet) by rats for 1 week resulted in no observable effects (e.g., no liver accumulation and no adverse renal or hepatic morphology). Administration of this dose for up to 6 weeks caused severe renal and hepatic effects in rats. Further administration of copper at this dose to rats for up to 15 weeks resulted in no further damage; rather, the authors reported regeneration of hepatic and renal tissues (Haywood, 1980). Rana and Kumar (1980) observed liver and kidney necrosis in rats fed 25.4 mg Cu/kg/day for 20 days.

Increased liver copper concentrations were observed in rats fed 500 ppm copper in diet for 27 days (Boyden et al., 1938) and 50 ppm copper in diet for 35 days (Miranda et al., 1981). Administration of higher levels of dietary copper resulted in elevated liver and splenic copper levels, growth reduction and reduced dietary intake resulting in death (Boyden et al., 1938). Therefore, it is not appropriate to convert intake to mg/kg/day.

Pigs appear to be more sensitive than rats to the acute toxicity of copper. Suttle and Mills (1966a,b) reported adverse effects in pigs given copper supplements in doses as low as 600 ppm diet for 48 days and 250 ppm for 79 days. Kline et al. (1971) reported beneficial effects of copper supplementation in pigs at doses of 150-200 ppm diet for 61-88 days. Administration of 500 ppm in diet for 61 days caused adverse effects such as

growth reduction, reduced hemoglobin and increased hepatic copper (Kline et al., 1971). No adverse effects were observed at 200 ppm (8.2 mg/kg/day).

Equivocal results have been obtained from experiments designed to evaluate the carcinogenicity and mutagenicity of copper compounds. Administration of copper compounds to mice by subcutaneous injection has been reported to induce tumor formation (BRL, 1968; Haddow and Horning, 1960). The only tumorigenicity studies for orally-administered copper were negative (BRL, 1968).

Microbial mutation assays using copper compounds have generally provided negative results. Some mutagenic activity by copper compounds at low concentrations has been observed in cell culture assays. Copper sulfate was observed to increase the frequency of recessive lethal mutations in D. melanogaster at high concentrations (Law, 1938).

Copper compounds have been observed to elicit a teratogenic response at ~2 mg Cu/kg when injected into female hamsters on the 8th day of pregnancy (DiCarlo, 1980; Ferm and Hanlon, 1974). Lecyk (1980) reported a teratogenic response of orally-administered copper sulfate in mice for the 30-day dietary exposures at 103.5 mg Cu/kg/day.

Ingestion. The potential for acute poisoning from ingestion of copper compounds has long been recognized. Several references in the pre-20th century literature describe cases of accidental human poisoning by copper compounds (Owen, 1981). The unwitting ingestion of many types of food and nonfood items containing copper has produced typical symptomatology of copper poisoning, ranging from GI disturbances, headache, dizziness and metallic taste in the mouth, to death. In several instances the exposure is to copper sulfate; therefore, some of the toxicity may be due to the sulfate component. Dose data can be found in some of the reports on humans. These dose estimates are summarized in Table VI-1. (Reports of single cases are not included.)

Spitalny et al. (1984) and Sargent and Jean (1983) report on a family whose recurrent episodes of emesis and abdominal pain were linked to copper in their drinking water. Early morning Cu concentrations on four different days were <2, 4.8, 2.8 and 7.8 mg/ℓ. During the 1.5 year period when exposure may have been occurring, 3 of the 4 family members reported recurrent episodes of emesis and abdominal pain. Although these levels are lower that those reported in other studies of toxicity, the continuous nature of the exposure suggests that tissue Cu levels may already have been high in the family. Further investigation showed the following: 1) aggressive water (Langelier index -3.5) was delivered through a copper main; 2) other families receiving the same water showed no symptoms and had lower copper levels in the tap water because of a different main or location; 3) the family with symptoms had higher copper levels in the hair than other families and these levels were greater than the normal range; 4) concentrations of arsenic, iron, lead, manganese, zinc and nitrate in the water were

TABLE VI-1

Human Dose-Effect Data for Copper

Sex/Age/ Number	Chemical	Route	Dose	Whole Blood Copper µg/100 mℓ	Serum Copper µg/100 mℓ	Effect	Comment	Reference
Male 32/1 Female 7,5/2	copper	oral	2-8 mg/ℓ Cu in drinking water	NR	NR	episodic emesis and abdominal pain in mornings	elevated copper in hair symptoms stopped when water source changed	Spitalny et al., 1984
Male and female/ 14-60/48	copper sulfate	oral	1-30 g copper sulfate (0.25- >7.6 g Cu^{+2})	383.4- 605.5	264.6- 346.2	diarrhea in 14/48 jaundice in 11/48 hemoglobinuria or hematuria or both in 14/48 anuria in 13/48 oliguria in 5/48 hypotension in 4/48 coma in 4/48 death in 5/53	NC	Chuttani et al., 1965
			6-15 g copper sulfate (1.5- 3.8 g Cu^{+2})	519.0	332.4	metallic taste, nausea, vomiting and burning in epigastrium		
			16-30 g copper sulfate (4.1- 7.6 g Cu^{+2})	684.0	346.2	metallic taste, nausea, vomiting and burning in epigastrium		
Male and female/ 14-60/NR	copper sulfate	oral	1-5 g copper sulfate (0.25- 1.3 g Cu^{+2})	383.4	264.6	metallic taste, nausea, vomiting and burning in epigastrium	normal whole blood copper ~217.0 µg/ 100 mℓ; normal serum copper = 151.6 µg/100 mℓ	Chuttani et al., 1965

TABLE VI-1 (cont.)

Sex/Age/ Number	Chemical	Route	Dose	Whole Blood Copper µg/100 mℓ	Serum Copper µg/100 mℓ	Effect	Comment	Reference
Male and female/ 14-60/NR	copper sulfate	oral	>30 g copper sulfate (>7.6 g Cu^{+2})	605.5	239.5	metallic taste, nausea, vomiting and burning in epigastrium	normal whole blood copper ~217.0 µg/ 100 mℓ; normal serum copper = 151.6 µg/100 mℓ	Chuttani et al., 1965
Male/NR/20	copper sulfate	oral	30 ppm Cu^{+2} in tea (≥6.9 mg Cu^{+2}) (≥0.1 mg Cu/kg/day x 1 day)[a]	NR	NR	diarrhea in 9/20 nausea in 9/20 vomiting in 6/20	NC	Nicholas and Brist, 1968
NR/NR/18	copper sulfate	oral	44 ppm Cu^{+2} in tea (≥10 mg Cu^{+2}) (~0.14 mg Cu/kg/day x 1 day)[a]	NR	NR	abdominal pain, vomiting, diarrhea, headache, dizziness	NC	Semple et al., 1960
Female/NR/15	copper	oral	5.3-32 mg Cu (0.09-0.55 mg Cu/kg/day x 1 day)[b]	NR	NR	nausea, vomiting, diarrhea, abdominal cramps, headache in 10/15	patients ingested copper on empty stomach	Wyllie, 1957
Male/NR/3	copper metal	inhalation	<0.008 mg/m³	NR	NR	no metal fume fever	NC	Gleason, 1968
			0.12 mg/m³ (0.84 mg/day or 0.012 mg/kg/day)	NR	NR	metal fume fever	NC	

[a] assume adult 70 kg men, one 8 oz cup (i.e., 0.23 ℓ) of tea

[b] assume adult 58 kg women

NR = Not reported

NC = No comment

below EPA standards; and 5) all symptoms were resolved when the family stopped drinking the water. According to a standard approach for identifying agents that cause infectious disease, this approach helps to link copper to the illness episode. Reconstruction of quantitation exposure is more difficult, but several measures exist to suggest the range of copper concentrations associated with the episodes of illness.

Chuttani et al. (1965) studied the clinical data from 53 patients poisoned with copper sulfate in suicide attempts. The amounts of copper sulfate ingested ranged from 1 to >30 g (i.e., 0.25-7.6 g Cu). Five of the patients studied died of acute copper sulfate poisoning (specific causes unknown), and the remaining 48 exhibited a variety of symptoms (see Table VI-1). All the patients studied had a metallic taste in their mouths, nausea, vomiting and epigastric pain. Levels of serum total copper, serum ionic copper and whole blood copper were observed to be much higher than normal. Whole blood copper levels appeared to correlate weakly with the estimated doses. Autopsy of the deceased patients showed ulceration of gastric and intestinal mucosa, liver cell necrosis and renal tubular cell necrosis.

An outbreak of gastroenteritis in 18 workmen following ingestion of tea made from copper sulfate-contaminated water was reported by Semple et al. (1960). Symptoms ranged from dizziness, headache, diarrhea, vomiting and abdominal pain to complete collapse. Water from a corroded geyser (heater) was later observed to contain $\geq$44 ppm total copper. Assuming each 70 kg man drank one cup (0.23 ℓ), the estimated dose was 0.143 mg Cu/kg. Nicholas and Brist (1968) reported a similar outbreak of toxicity from contaminated

tea in factory workmen. Twenty workmen felt sick after ingesting morning tea made in a copper contaminated geyser. It was not stated how many men were exposed to the tea. Diarrhea occurred in 9/20, vomiting in 6/18 and nausea in 9/18. A sample of tea taken later contained 30 ppm Cu. It was stated that the copper concentration was probably higher. Assuming each man (70 kg) drank 1 cup (0.23 ℓ), the estimated dose was $\geq$0.1 mg/kg. There was no analysis for other metals or other contaminants.

Wyllie (1957) reported an outbreak of acute copper poisoning caused by the dissolution of copper contained in a cocktail shaker. Analysis of cocktail fluid prepared in the shaker allowed an estimate of the amounts of copper ingested (5.3-32 mg Cu). Fifteen adult females were exposed, and only five were asymptomatic. Weakness, abdominal cramps, headaches, nausea, dizziness and vomiting were experienced by the other 10 patients. Assuming these females weighed 58 kg, the estimated doses were 0.09-0.57 mg/kg/day. No measures or tests for other contaminants were reported. There are no values for serum copper or ceruloplasmin.

Walsh et al. (1977) reported a case of acute copper intoxication in an 18-month-old boy. After drinking a solution estimated to contain 3 g cupric sulfate (0.76 g Cu), the boy began to vomit and was admitted to a hospital. High copper levels were observed in serum (1.65 mg/100 mℓ) and urine (50 μg/100 mℓ) upon admittance. Acute hemolytic anemia developed on the day after admittance, at which time reduced G-6-PD activity, hematuria, glycosuria and proteinuria were also noted.

Roberts (1956) reported that a well-nourished 24-year-old Negro male, after self-treatment with copper sulfate (~600 g over 4 months) was hospitalized with complaints of vomiting and GI pain. The patient developed hemolytic anemia, but neither blood nor serum copper concentration was measured. The patient survived and was released after 2 weeks.

Acute copper poisoning in a 27-year-old man, resulting from the ingestion of at least 50 g of copper sulfate (12.7 g Cu) was reported by Chugh et al. (1975). In addition to emesis of his stomach contents, the patient was cyanotic, oliguric and anemic. Analysis of blood samples indicated a copper concentration of 8267 μg/100 mℓ, severe intravascular hemolysis and methemoglobinemia. A G-6-PD assay of erythrocytes showed reduced activity of this enzyme. The patient suffered circulatory collapse and hypotensive shock and coma, and was reported to have died 16 hours after taking the chemical.

Sanghvi et al. (1957) reported two fatal cases of copper sulfate poisoning. Sulfhemoglobinenia and acute renal failure were observed in both patients, one of whom ingested about an ounce of copper sulfate and the other, an unknown amount. Both patients had sulfhemoglobin in their blood by 72 hours after ingestion of copper sulfate. Acute tubular necrosis and renal failure were observed in both patients, presumably caused by anemic anoxia.

Stein et al. (1976) reported a case wherein a patient died after she was treated with an emetic dose of copper sulfate. A 44-year-old female ingested both alcohol and diazepam and was given 20 mℓ of a 10% solution of

copper sulfate (508 mg Cu). The patient failed to vomit and stomach contents were removed by lavage. Respiratory collapse, hemolytic anemia, hemoglobinuria, hepatic failure, renal failure and massive GI bleeding were observed, and the patient died. Rapid intestinal absorption of copper undoubtedly occurred, as she had a three-quarter gastrectomy. On autopsy, acute renal tubular necrosis and a liver copper level of 7.46 mg/100 g were observed.

Children may be particularly susceptible to copper toxicosis. Hepatic copper concentrations in normal newborns can be up to 8 times the levels in adults (Sternlieb, 1980). Two cases of copper poisoning in children have been reported. Salmon and Wright (1971) studied a nonfatal case of copper poisoning that initially appeared to be mercury poisoning. A 15-month-old boy was admitted to the hospital with prostration, vomiting, red extremities, hypotonia, photophobia and peripheral edema, but no mercury could be demonstrated. The outstanding abnormal finding was the elevated serum copper level (286 μg/100 mℓ); the plasma ceruloplasmin level was not measured directly but was estimated at 22.5 mg/100 mℓ. The copper content of the patient's drinking water was 0.35 and 0.8 mg Cu/ℓ in the cold and hot water taps, respectively. This water had been available to the patient for a period of 3 months, as this was the period of time that the family had occupied the residence. Apparently, the hot water system of the dwelling was entirely fabricated from copper, and the family used hot water for all cooking and beverages. The patient's ceruloplasmin level was normal, indicating that he was not suffering from Wilson's disease. The overall nutritional state of the patient and the presence of other contaminants in the water were not reported.

Walker-Smith and Blomfield (1973) reported a fatal case of a 14-month-old boy who may have been poisoned by excessive copper in drinking water both in utero and postpartum. Because Wilson's disease rarely manifests itself at such an early age, and there were no Kayser-Fleisher rings present (a diagnostic criterion for Wilson's disease), the authors were uncertain if this patient actually had Wilson's disease. However, the patient had a reduced ceruloplasmin level, raised urinary copper level and raised free plasma copper. These indicate the presence of Wilson's disease. The patient's household water supply had low pH and high copper levels. The cold water tap had copper concentrations of 6.8 and 0.3 mg/ℓ when first turned on and after running for 5 minutes, respectively. The hot water tap had copper concentrations of 9.7 and 6.9 mg/ℓ when first turned on and after running for 5 minutes, respectively. High copper concentrations were observed at autopsy in the liver, kidney, heart and brain of the patient. The authors suggested that the high environmental copper levels alone may have caused this death or the copper may have acted by exacerbating an existing liver infection or by overwhelming the patient's ability to remove copper because of Wilson's disease. The final diagnosis remained uncertain.

The Centers for Disease Control have reported 112 cases of copper intoxication from drinking water sources from the years 1977-1982. The majority of cases involved the leaching of copper from plumbing into drinking water with reported copper levels ranging from 4.0-70 mg/ℓ (CDC, 1977-1982). This is consistent with copper concentrations in the studies summarized in Table VI-1. Unpublished data from a school in the United Kingdom indicated that copper toxicity (GI problems) were observed in school children.

Samples taken following complaints of immediate illness after drinking showed 18.7 mg/ℓ in classroom and 381 mg/ℓ at a drinking fountain. (The latter level was rechecked and confirmed.)

Samples taken on a Monday morning after water had been standing in the system over the weekend measured 3.38 mg copper/ℓ and after running to waste for 20 minutes measured 0.01 mg copper/ℓ. The system had oversized plumbing with copper piping; therefore, the level associated with toxicity cannot be determined.

Inhalation. There are several reports of local and systemic effects caused by the inhalation of copper sulfate (in Bordeaux mixture) by vineyard sprayers. Pimental and Marques (1969) were the first to recognize the disease they named "vineyard sprayer's lung." They reported their observations of two vineyard sprayers who had been occupationally exposed to Bordeaux mixture, a 1-2% solution of copper sulfate in hydrated lime used to control mildew on grapes. Two male vineyard sprayers were examined and found to have altered lung pathology similar to that seen in silicosis, although polarized light microscopy revealed no silica. The lungs of these patients had a well-defined histological morphology including the presence of desquamation, intra-alveolar macrophages, interalveolar septal histiocytic granulomas and the scars of these lesions. Guinea pigs exposed to Bordeaux mixture by inhalation had similar lesions (see Chapter V).

Villar (1974) further defined the clinical aspects of vineyard sprayers' lung by analyzing 15 cases of the disease. Fourteen male patients and one female patient with a history of intermittent (3 months/year) exposure to Bordeaux mixture were admitted to a hospital for the diagnosis of a variety

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of pulmonary maladies, including cancer. Dyspnea, weakness, appetite loss, weight loss, radiographic opacities and the presence of copper in the lungs were observed to be common among these patients. In some cases that were followed for 3 years, retraction and calcification of lesions previously described as tumor-like opacities were observed. The authors were able to classify the various manifestations of the disease into three separate groups: subclinical, active and chronic.

Pimental and Menezes (1975) reported an additional adverse effect of occupational exposure to aerosols of Bordeaux mixture. Copper-containing liver granulomas were observed in three cases of vineyard sprayers' lung, along with the characteristic pulmonary lesions of the disease. Exposure to Bordeaux mixture ranged from 3-15 years duration. Liver specimens were collected at autopsy in two cases and by percutaneous biopsy in the third. Well-defined nodules composed of histiocytic cells, granulomas organized in sarcoid-type follicles and neoformation of reticulin fibers were observed in the livers of these patients. Tests for copper showed that the metal was present in granulomas and fibro-hyaline nodular scars, and this finding differentiates the observed lesions with those observed in other conditions.

Three workmen involved in polishing copper plates were exposed to fine copper dust and complained of stuffiness of the head, sensations of chills or warmth and a general feeling of discomfort, similar to the onset of a cold (Gleason, 1968). These symptoms, when present after exposure to metal fumes, are generally known as metal fume fever. While the copper dust in question was not fume per se (i.e., airborne particles condensed from the molten state), they were observed to be of "extreme fineness." General air

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samples in the workplace contained 0.030-0.075 mg Cu/m³. A breathing zone air sample of the polishing wheel operator contained 0.120 mg Cu/m³. These concentrations were associated with metal fume fever. After the installation of engineering controls (water spray and local exhaust ventilation), copper concentrations were reduced to <0.008 mg/m³ and no further symptoms of metal fume fever were observed.

Earlier reports of acute poisoning from inhalation of copper-containing dust are summarized in Browning (1961). Dusts of metallic oxides and Cu were reported to have caused symptoms of heavy metal poisoning, pain in the chest, dyspnea, nausea and vomiting.

Dermal Absorption. Holtzman et al. (1966) reported the death of a burn patient who absorbed large amounts of copper through her skin during debridement with copper sulfate crystals. The patient (5.5 years old) had suffered second and third degree burns over 30-40% of her body. She underwent seven separate debridement procedures ~1 week apart. Following the debridement procedures, the patient was pale, anemic, icteric, oliguric and jaundiced. Copper levels in the patient's urine and serum were 220 and 540 µg/100 mℓ, respectively. Erythrocyte glucose-6-phosphate dehydrogenase was not reduced and serum ceruloplasmin was elevated to 86 mg/100 mℓ.

Epidemiological Studies

Several epidemiological studies of workers involved in copper smelting have reported that the incidence of lung cancer is increased in this population. However, it is believed that the etiologic agents do not include copper; rather, arsenicals such as arsenic trioxide may be responsible.

Some areas of North Wales, Cheshire and Devonshire have a high prevalence of gastric carcinoma. It was observed that these areas also have increased ratios of zinc to copper in the soil. Copper intake (also zinc, cadmium and chromium) was observed to be correlated with the incidence of leukemia and other cancers (Schrauzer et al., 1977). These studies, as all such geographical correlation studies, are designed to generate testable hypotheses. They are inconclusive regarding the role of copper in human carcinogenicity because of the grouped exposure and response data.

High Risk Subpopulations

Wilson's Disease Patients. Hepatolenticular degeneration (also known as Wilson's disease) was described as a clinical syndrome by Wilson in 1912 (Williams, 1982). Wilson's disease is probably the best studied form of copper toxicity in man, and this effort has provided much information regarding human copper metabolism (Scheinberg and Sternlieb, 1969). This disease is an inherited autosomal recessive disorder of copper metabolism characterized by abnormally low levels of ceruloplasmin, increased plasma copper levels, hypercupuria and increased copper deposition in liver, brain, kidneys and cornea (Schroeder et al., 1966; Evans, 1973). A rare disorder, Wilson's disease occurs in perhaps 1 of 200,000 individuals (Scheinberg and Sternlieb, 1969). The prevalence of persons heterozygous for Wilson's disease may be ~1 in 200 (Sternlieb, 1980). Wilson's disease patients may be particularly susceptible to the toxic effects of copper from environmental sources.

In Wilson's disease, the liver may concentrate up to 20 times the normal level of copper. This eventually destroys parenchymal cells, and their

copper content is thus released into the blood. Once released, copper may affect many organs and systems, including erythrocytes, kidneys, cornea and the CNS (Scheinberg and Sternlieb, 1969).

Untreated Wilson's disease patients may suffer from tremors, drooling, incoordination, seizures, behavioral abnormalities, anemia, jaundice and, ultimately, death. Chelating agents, such as d-penicillimine and British Anti Lewisite (BAL) have been used to control the disease. However, the efficiency of penicillimine for increasing urine copper is low. Limitation of copper intake and administration of potassium sulfide are also used to reduce copper absorption (Schroeder et al., 1966).

Wilson's patients should measure the copper level in their drinking water because soft, acidic or basic water may dissolve copper from copper tubing in residential plumbing.

Glucose-6-Phosphate Dehydrogenase (G-6-PD) Deficiency. Excessive copper has been demonstrated to reduce the activity of the hexose monophosphate shunt, of which G-6-PD apparently plays a role. This has been demonstrated in Wilson's disease patients (Diess et al., 1970), in *in vitro* incubations with human erythrocytes (Boulard et al., 1975; Calabrese et al., 1980) and in copper sulfate acute poisoning cases in humans (Walsh et al., 1977; Chugh et al., 1975). The concentrations of Cu in the *in vitro* experiments are much higher than those encountered *in vivo*.

It is reported that 13% of the American Negro male population have G-6-PD deficiency and may be at increased risk to the toxic effects of

copper (Calabrese et al., 1980; Calabrese and Moore, 1979). Boulard et al. (1975) observed that the G-6-PD activity of human erythrocytes is inhibited when incubated with 0.1 mM copper.

Kidney Dialysis Patients. Several reports of acute hemolytic anemia in patients undergoing hemodialysis have been attributed to excess copper in the dialysis fluid (Williams, 1982). Leaching of copper from copper tubing in home units and from copper filters and tubing within units have both been reported. Increased copper concentrations are associated with soft water and acidic water. This effect has been observed at copper concentrations from 22-50 µg/ℓ in the dialysate (Owen, 1981). The problem can be controlled by removing copper from the system and monitoring the pH and conductivity of the dialysate (Williams, 1982).

Summary

Severe effects of acute copper intoxication reported include hemolytic anemia, G-6-PD inhibition, renal failure, hematuria and death (Walsh et al., 1977; Roberts, 1956; Chugh et al., 1975). These effects were reported to occur at doses between 3 and 600 g of copper sulfate (0.76-152.4 g Cu). Although acute poisoning episodes in children are reported, there are no data sufficient to identify differences in dose-response data between adults and children.

The symptomatology and effects of acute copper poisoning have been characterized and include jaundice and liver effects similar to those seen in animals (Chuttani et al., 1965). Subjective effects such as a metallic taste, vomiting, diarrhea and epigastric pain have been reported at acute

exposures to copper in concentrations as low as 5.3-32 mg of copper (Semple et al., 1960; Wyllie, 1957; Spitalny et al., 1984). Of these, only Spitalny et al. (1984) describes additional exposure to chemicals other than copper sulfate.

Chronic copper toxicity is rarely encountered in humans. Long-term exposure to aerosols of Bordeaux mixture ($CuSO_4$ and lime) has been reported to cause adverse effects on pulmonary and liver morphology and may be associated with tumor formation (Pimental and Marques, 1969; Pimental and Menezes, 1975; Villar, 1974). Inhalation of copper fumes and fine aerosols may result in metal fume fever (Gleason, 1968).

Epidemiological investigations of populations with high copper exposure by inhalation are equivocal with respect to the carcinogenicity of the metal, because the exposures encountered by copper miners and smelters usually involve other metals including arsenic known to contribute to lung cancer.

Wilson's disease (hepatolenticular degeneration), a rare inborn error of copper metabolism, appears to be the only manifested form of chronic copper toxicity by ingestion in humans. Elevated environmental copper levels may enhance the toxic effects of the metal but no quantitative data are available. Copper is accumulated in the liver, brain, kidney and cornea of Wilson's disease patients (Evans, 1973). Cirrhosis of the liver, hemolytic anemia, neurologic abnormalities and corneal opacities (Kayser-Fleischer rings) are some of the marked adverse effects of copper observed in Wilson's disease.

It has been suggested that persons with Wilson's disease, deficiencies in G-6-PD activity, and those having occupational exposure to copper may be at additional risk from the toxic effects of copper (U.S. EPA, 1980b).

It is entirely possible to prepare an adequate diet containing >10 mg copper that has satisfactory caloric value (Schroeder et al., 1966). This undoubtedly occurs frequently in normal individuals without adverse effects.

VII. MECHANISMS OF TOXICITY

The metabolism and toxicity of copper and other heavy metals are controlled by many complex biochemical reactions. In addition to copper's affinity to become bound to proteins, the metal in both its cupric (+1) or cuprous (+2) form is essential for the proper functioning of many enzyme systems. Copper levels in various human tissues are affected by age, sex, nutritional status, disease state and a wide variety of other factors.

Transition metals (such as copper) are known to catalyze lipid peroxidation, possibly forming free radicals (Dougherty and Hoekstra, 1982). However, copper is usually incorporated into stable complexes within cells or vascular fluids (Evans, 1973).

The wide ranging effects of copper in normal metabolism have been known for some time. Fluctuations in serum copper levels in pregnancy were first observed in 1928; and since that time, changes in the various copper proteins and enzymes with various biological states have been documented. Copper homeostasis is affected by a variety of hormones. Several pituitary, adrenal, thyroid and sex gland hormones have been observed to cause effects in copper metabolism (Evans, 1973).

The daily balance of copper in normal humans is dependent upon a variety of variables, including age and dietary components such as type of protein or carbohydrate, and nutritional balance. Some metals, cadmium, zinc, silver and molybdenum, may influence the metabolism of copper by altering its absorption or cellular utilization (Evans, 1973).

Hepatotoxicity

When homeostatic mechanisms are overwhelmed by excess copper exposure, accumulation of the metal in the liver results. Chronic copper toxicity in sheep, rats and man is associated with an absence of outward symptoms during a phase of constant accumulation of hepatic copper (Helman et al., 1983). Lal and Sourkes (1971) observed that during the asymptomatic phase of copper toxicity in rats, copper accumulated in all subcellular hepatic fractions.

Animal Model for Hepatotoxicity. The beige and conventional strains of C57Bl/6 mice were used to study the effects of copper administration on the intracellular compartmentalization of copper and hepatotoxicity. Beige mice possess hepatocytes with altered phagocytic capacity to scavenge metal proteins and sequester them in lysosomes. Both strains received i.p. injections of 8 mg Cu/kg/day for up to 4 weeks. After exposure, livers were removed, homogenized and fractionated by centrifugation. Analysis of the various fractions showed that copper initially accumulated in the cytosol fraction of both strains, but only the conventional mice had subsequent binding of copper to the lysosomal fractions. On histological examination, beige mice were observed to have more severe hepatic lesions than conventional mice. It was concluded that sequestration of copper into lysosomes by thiol rich macromolecules (metallothioeins) is a normal protective mechanism. In beige mice, copper accumulated in cytosol, saturated the available binding sites, was not sequestered into lysosomes and caused cellular injury (Helman et al., 1983).

Blood Toxicity

Cellular membranes can be adversely affected by copper. The metal has the ability to catalyze lipid peroxidation, to inhibit membrane ATPase

(Evans, 1973) and to increase the osmotic fragility of erythrocytes (Moroff et al., 1974). The exact mechanism of copper's effect on cell membranes is not known, but it is believed to function directly, by binding to exterior sites, or indirectly by causing intracellular changes that affect membrane integrity.

Erythrocytes are susceptible to the toxic effects of copper. It is known that copper can produce homolysis, reduce blood glutathione, increase methemoglobin, induce formation of Heinz bodies, reduce G-6-PD activity and possibly catalyze the autooxidation of glutathione (Chuttani et al., 1965; Bremner, 1979).

Glucose 6-Phosphate Dehydrogenase Deficiency (G-6-PD). Calabrese and Moore (1979) postulated a mechanism describing copper-induced hemolysis. Copper can inhibit both glutathione reductase and G-6-PD. Decreased levels of reduced glutathione in erythrocytes have been associated with their susceptibility to hemolysis. Boulard et al. (1975) demonstrated that a variety of erythrocyte enzymes are inhibited when incubated with low levels of copper (0.1 mM). It is not known whether the "so called acquired enzymatic defects" found in various chronic disorders are a cause or effect of the simultaneous high levels of serum copper.

Dorset strain sheep (Calabrese et al., 1980) have erythrocytes with low G-6-PD activity and sensitivity to primaquine. These traits are possessed by human erythrocytes deficient in G-6-PD. Increased methemoglobin, decreased GSH and decreased acetylcholinesterase (ACH) activity have been observed in both types of cells when incubated with 2 mM copper.

Wilson's Disease

Medical study of the nature of Wilson's disease has afforded a unique understanding of the pathogenesis of chronic copper intoxication. Patients with this disease represent probably the only form of chronic copper toxicosis in humans. While the exact mechanisms of Wilson's disease have not been elucidated, these studies illustrate the complexity of the copper homeostatic mechanisms in normal man.

Wilson's disease is characterized by decreased plasma copper and ceruloplasmin, increased amounts of amino acid and albumin bound copper, increased urinary excretion of copper, and deposition of copper in the liver, brain and cornea. The effects of chronic copper poisoning in Wilson's disease patients include hepatic cirrhosis, kidney defects, hemolysis, brain damage and nerve demyelination (Stokinger, 1980). The available evidence suggests that copper deposition in tissues is related to the developmental mechanism of the disease (Narasaki, 1980).

Animal Models. Bedlington terriers have been suggested as an animal model for Wilson's disease patients because the abnormal copper metabolism in both is transmitted by an autosomal recessive inheritance (Sternlieb, 1980). Accumulation of tissue copper leading to hepatitis, cirrhosis and anemia is common to both. Bedlington terriers are a good model to study liver toxicosis, but do not emulate Wilson's disease patients in other respects. For example, the dogs do not have decreased ceruloplasmin levels, and do not develop KayserFleisher rings (Sternlieb, 1980).

Cattle and sheep with chronic copper poisoning (especially those with enzootic jaundice) undergo hemolysis in a manner quite similar to patients with Wilson's disease. For example, hemolysis occurs after large tissue stores and high urine copper are observed. The quick release of copper from the liver at this time may induce the hemolytic effect (Diess et al., 1970).

Menkes Syndrome

Menkes syndrome is an X-linked inherited defect in copper absorption, leading to severe deficiency states (Danks et al., 1972). Insufficient intestinal absorption of copper was proposed by these authors as the basic biochemical defect of the disease.

Animal Model. The mottled mutant mouse provides an excellent animal model for studying Menkes disease. Hemizygous males with the X-linked allele, brindle (Mo^{br}/y), possess some of the severe defects observed in Menkes disease. These include hypopigmentation, skeletal and vascular defects, abnormal hair, and early death (Williams, 1982).

Summary

The human homeostatic mechanisms controlling copper balance can store copper, prevent its excess absorption from the gut, utilize the metal in cellular function, and excrete the metal through the bile or urine. Copper can bind to proteins and is essential for many enzyme functions. The metabolism of copper as well as serum copper levels vary by age, sex and diet. Homeostatic control prevents toxicity from the wide normal variations in copper. In the cases of excess, copper accumulates in the liver, producing no outward symptoms of toxicity. When copper levels increase in the

blood, toxic effects on erythrocytes and cell metabolism are seen. A form of chronic toxicosis (Wilson's disease) is a rare genetic error of metabolism that leads to accumulation of copper in tissues, hemolysis cirrhosis, neurologic abnormalities and renal damage.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Introduction

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{[Uncertainty Factor(s) x Modifying Factor]}} = \_\_\_\_ \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicological effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner,

the U.S. EPA (1986a) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977b, 1980b) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]

- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]

- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]

- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$DWEL = \frac{(RfD) \times (\text{Body weight in kg})}{\text{Drinking Water Volume in } \ell/\text{day}} = \_\_\_\_ \text{ mg/}\ell$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 ℓ/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$HA = \frac{(\text{NOAEL or LOAEL}) \times (bw)}{(UF) \times (\_\_\_ \ell/\text{day})} = \_\_\_\_ \text{ mg/}\ell$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 ℓ water per day.
2. 10-day HA for a 10 kg child ingesting 1 ℓ water per day.
3. Longer-term HA for a 10 kg child ingesting 1 ℓ water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 ℓ water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

> Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.
>
> Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
>
> Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
>
> Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.
>
> Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these

estimates usually come from lifetime exposure studies using animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 ℓ of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is

uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Noncarcinogenic Effects

Animals. Short-term oral administration of copper to rats results in accumulation of the metal in hepatic and renal tissue and necrosis of these tissues (Boyden et al., 1938; Haywood, 1980; Rana and Kumar, 1980). Boyden et al. (1938) studied the effects of feeding high levels of copper to albino rats for 4 weeks. Groups of 3-5 mixed male and female 28-day-old albino rats were maintained on diets supplemented with 0, 500, 1000, 2000 or 4000 ppm copper (as copper sulfate) for 4 weeks. The authors stated that these dosage levels resulted in average excess copper intakes of 0, 5.1, 8.2, 10.8 and 7.6 mg/rat/day based on observed food consumption, but because of inanition these doses are not proportional to supplementary copper. Reduced body weight gain and reduced food intake were observed in rats maintained on diets containing 1000 or 500 ppm copper. Greater copper accumulation in the blood, spleen and especially liver in comparison with the control group was

observed in all treated rats. The authors stated that slight toxicity was observed in rats fed 500 ppm and increased toxicity was observed as the dietary copper level was increased (see Table V-1).

Haywood (1980) maintained rats (4 male weanling rats/group) for 1, 2, 3, 6, 9 or 15 weeks on diets containing copper at levels of 2000 mg copper/kg (as copper sulfate; 200 mg copper/kg/day). Histologic descriptions of the liver and kidneys were provided for each week of exposure. The authors report regeneration by week 9 that limited the necrotic area, and copper deposition was less pronounced. Although almost complete recovery was observed after 15 weeks of exposure, these results are puzzling because other experiments suggest severe toxicity at 2000 mg/kg. It has been proposed that galvanized cages may provide access to zinc and cadmium which affect copper for availability and toxicity (Petering, 1987).

Rana and Kumar (1980) administered lower exposure levels of 100 mg/kg bw copper sulfate by gavage to 20 male rats (90 days old). During the 21 days of treatment, the copper-fed rats had decreased skeletal development and decreased body, liver and kidney weights; however, no histopathologic changes were observed in the liver or kidneys. In studies using slightly lower exposures, Miranda et al. (1981) observed no changes in serum enzymes indicative of liver damage in rats (number not specified) exposed to copper at a level of 55 mg/kg diet (6 mg/kg/day) for 5 weeks. The only effect of treatment was an increase in liver copper content.

In a study of the effects of copper on reproduction, Lecyk (1980) fed two groups of 7-22 female mice (either C57BL or DBA mice) diets containing copper sulfate at levels of 0, 0.5, 1.0, 1.5, 2.0, 3.0 or 4.0 g/kg diet. The two lower doses resulted in stimulation of embryonic development (fetal weight and litter size); however, at 1.5 g/kg fetal development was decreased. Although low levels of copper sulfate (0.5-1.0 g/kg diet) appeared to be beneficial to fetal development, higher levels produced adverse effects.

When pigs (groups of 8-12) were given diets containing copper at levels of 150, 200 or 250 ppm for 61-88 days (6.2, 8.2 or 10.3 mg/kg/day), there was accelerated weight gain (Kline et al., 1971). At the next higher dose level of 500 ppm (20.5 mg/kg/day), weight gain was decreased compared with controls. Also at the higher dose level, hemoglobin and hematocrit levels were reduced. In another study using pigs (Suttle and Mills, 1966a,b), however, exposure of female weanling pigs (6/group) to diets containing 250 ppm (10.3 mg/kg/day) of copper for 79 days resulted in increased aspartate transaminase activity and jaundice. Higher levels of 600 ppm (24.6 mg/kg/day) for 48 days resulted in anemia. The results in the lower exposure group may have been partially caused by high levels of calcium in the diet.

Humans. The Centers for Disease Control have reported 112 cases of copper intoxication from various drinking water sources from the years 1977-1982. The majority of cases involved the leaching of copper from plumbing into drinking water with reported copper levels ranging from 4.0-70 mg/ℓ (CDC, 1977-1982). Thus, exposures of humans to copper in the

drinking water is associated with disease. This is important in view of the numerous reports of effects of copper sulfate, although these solutions may in some cases have contained other contaminants.

Chuttani et al. (1965) evaluated case reports of acute copper sulfate poisoning survivors (48 cases) and deaths (5 cases). They observed that 48/48 patients ingesting from 250 mg to >7.6 g of Cu had common symptoms: metallic taste, nausea, vomiting and GI disturbance. Diarrhea, nausea and vomiting were experienced in human populations of 150, 20 and 15 persons who ingested between 5.3 and $\geq$10 mg Cu. Semple et al. (1960) reported that 18 factory workers ingesting tea containing at least 44 ppm (44 mg Cu/ℓ) experienced nausea, vomiting and diarrhea. Assuming these were 70 kg adult male workers and that they ingested ~8 ounces of tea (0.23 ℓ), the approximate dose was 10 mg for one exposure. Using the same assumptions, Nicholas and Brist (1968) reported a similar outbreak in tea drunk by at least 20 workmen. Tea water was observed to contain 25-30 ppm (25-30 mg Cu/ℓ) and the estimated single dose, $\geq$7.0 mg, caused diarrhea in 9/20, nausea in 9/20 and vomiting in 6/20. Wyllie (1957) reported that 10/15 "fasted" adult females ingested alcoholic drinks resulting in between 5.3 and 32 mg Cu/person had nausea, vomiting and diarrhea.

Spitalny et al. (1984) reported that three members of a family suffered acute copper intoxication characterized by nausea and stomach cramps, after ingesting water with copper levels of 2.8-7.8 mg/ℓ. This occurred on several mornings after ingestion of an unspecified amount of drinking water and juice. The water consumed was quite corrosive having a Langelier Index of -3.92 and an Aggressive Index of 8.93.

Quantification of Noncarcinogenic Effects

Derivation of 1-Day HA. At high dietary levels of 2000 mg Cu/kg, Haywood (1980) observed the beginning of copper accumulation in the liver and kidneys of rats by 14 days. Jaundice was observed in rats fed diets containing 2000 ppm copper and at 4000 ppm the rats died in 1 week. Lower exposures of 500 or 1000 ppm reduced body weight gain and food intake (Boyden et al., 1938). However, because there are various flaws in these studies including reduced food intake that would make calculations unreliable, human data should be used.

The available human studies of Chuttani et al. (1965), Semple et al. (1960) and Wyllie (1957) all suggest that ingestion of between 5.3 and 32 mg copper/person results in GI disorders, vomiting, nausea and diarrhea. Since no lasting adverse effects were reported and the symptoms observed could be the result of local GI irritation, the single oral dose to an adult of 5.3 mg which is equivalent to 0.75 mg/kg/day for a 70 kg adult, may be considered a LOAEL. The human data describe lower exposure levels than the animal data and do not require species-to-species extrapolation. Thus, these human data would be the most appropriate for deriving the 1-day HA.

The derivation of a 1-day HA for a 10 kg child based on this human LOAEL should be the same as that for the adult since the progression of symptoms and vomiting occurring soon after exposure, suggesting that systemic absorption had not occurred. If effects observed are considered local in origin, then it is not appropriate to distribute the dose over the body weight. The 1-day HA for the child is the same as that calculated for an adult.

The calculation of the 1-day HA for an adult from this human LOAEL is as follows:

$$\text{1-day HA} = 5.3 \text{ mg/day} \div 2 \text{ ℓ/day} \div 2$$
$$= 1.3 \text{ mg/ℓ}$$

where

5.3 mg = the total dose ingested at one time

2 ℓ = amount of water consumed in ℓ/day by an adult

2 = uncertainty factor

According to the NAS guidelines, an uncertainty factor of 10 should be applied when using calculations based upon a human study with a NOAEL. In this situation, an uncertainty factor of 2 was applied because of the following: 1) the symptom reported was a temporary local GI irritation and no long-term effects were reported; 2) 5.3 mg/day was the lowest value determined in the literature based upon a number of studies using fasted subjects; 3) copper is an essential element and the use of a larger safety factor would bring the level below that considered necessary for human nutrition; and 4) copper absorption is controlled by a homeostatic mechanism and the chemical does not tend to accumulate in the body. Further support for this number as conservative is obtained from the incident reported by Spitalny et al. (1984) in which acute toxicity occurred after water was ingested that had >2 mg/ℓ and in which exposure may have been occurring over a period of several months.

Derivation of 10-Day HA. As described previously for the 1-day HA, Haywood (1980) observed transient liver damage, which was at maximal severity at 6 weeks, in rats exposed for 15 weeks to copper at doses of 100 mg/kg

bw. In 90-day studies using pigs, dietary exposure to copper at doses of ~3.2 mg Cu/kg bw/day was reported to accelerate body weight gain (Kline et al., 1971), while a similar dose of 2.6 mg Cu/kg bw/day administered to pigs was reported to result in jaundice and increases in serum aspartate transaminase levels (Suttle and Mills, 1966a,b). The results of this study were possibly affected by high calcium levels in the diet and interactions between metals and other natural dietary components are not uncommon. Also, pigs are more sensitive to copper than rats.

A 10-day HA will not be calculated for copper because of inadequate data. The available animal data do not consist of dose-response information that adequately defines a NOAEL or a LOAEL. The human data consists solely of one-time exposure information.

Derivation of Longer-term HA. A NOEL was ascertained in a study in pigs exposing the animals to 8.2 mg copper/kg/day (200 ppm) as a dietary supplement for 88 days (Kline et al., 1971). However, this study is not appropriate for the purpose of calculating a longer-term HA because the exposure period is too short (10% of lifespan would be 584 days).

Assessment of Lifetime Exposure and Derivation. Howell (1959) observed that rats maintained on diets containing 5000 mg Cu acetate/kg/day for up to 16 months had copper deposition in renal and hepatic tissue. However, no other toxicologic effects were investigated, and it is not possible to state whether 5000 mg Cu acetate/kg/day represents a LOAEL. Additional data for chronic oral toxicity in nonruminant laboratory mammals were not

not found in the available literature. Pigs exposed for 88 days to 8.2 mg copper/kg/day (200 ppm copper) as a dietary supplement showed no toxic effects but this duration does not constitute chronic exposure in pigs (Kline et al., 1971).

There are no data indicating that human exposure to copper results in chronic toxic effects to normal individuals. The available information on human copper toxicity consists of data on GI effects resulting from acute copper toxicity. These data were used to calculate the 1-day HA. Additional data for chronic oral toxicity in nonruminant laboratory mammals were not found in the available literature, and there are no data for chronic human exposure to copper. Thus, inadequate data precludes calculation of an RfD; consequently, no DWEL can be calculated.

The 1-day HA, however, used a LOAEL of 5.3 mg, based upon the lowest single oral dose of copper resulting in adverse effects as reported in the published literature. A number of studies have shown the ingestion of copper at levels in this general range to result in adverse effects. Sargent and Jean (1983) reported acute copper intoxication after ingestion of water with copper levels of 5.6-7.8 mg/ℓ. This occurred in the morning hours after ingestion of drinking water and juice. The exact amount of water consumed was not reported; however, the age of two of the affected family members (5 and 7) makes it improbable that a large quantity was consumed. It appears likely that <1 ℓ was ingested and thus it may be assumed that a dose <5.6 mg resulted in acute copper toxicity. The CDC have also reported a number of cases of acute copper intoxication from drinking water sources. The exact level of copper in the water was not reported for

many of the incidents; however, levels ranging from 4.0-70 mg/ℓ were reported for a number of cases (CDC, 1977-1982).

The average adult copper intake through the diet is variable, although it appears to be <2 mg/day (NAS, 1980b). Absorption of inhaled copper probably contributes little to the overall daily intake of copper (U.S. EPA, 1980b). Schroeder (1970) estimated that inhalation of ambient air would contribute <1% of the total normal daily intake. Other sources (copper bracelets, IUDs) are also expected to contribute little to total intake (U.S. EPA, 1980b).

In addition, the taste and odor of copper in drinking water serves to limit excess intake. Cohen et al. (1960) determined the copper concentrations added to distilled water required to elicit taste sensations in a population (15-20) of human volunteers. The concentrations of Cu^{+2} ion in distilled water necessary to cause a threshold taste sensation in 5 and 95% of a panel were 2.6 and 15.8 ppm (2.6 and 15.8 mg/ℓ), respectively. Copper was generally described as having a bitter taste (Cohen et al., 1960). In setting standards for copper levels in ambient water, the Federal Water Quality Administration (FWQA, 1968) based their acceptable limit of 1 mg/ℓ on these organoleptic properties of copper dissolved in water. The U.S. EPA (1980b) also recommended an ambient water quality criterion for copper of 1 mg/ℓ based on organoleptic considerations, and the U.S. EPA (1979) set a secondary drinking water standard of 1 mg/ℓ based on the taste and odor considerations. The World Health Organization (WHO, 1984) also recommends 1.0 mg/ℓ as a guideline for drinking water quality.

A level of 1.3 mg/ℓ is recommended to be the basis for the drinking water standard for the following reasons: 1) this level would satisfy the nutritional requirements for copper; the National Academy of Sciences (NAS, 1980b) estimated that "an adequate and safe" intake of 2-3 mg copper in a 70 kg adult and 1.5-2.5 mg/day for children will satisfy nutritional requirements and be protective of human health; and 2) assuming consumption of 2 ℓ of water per day, 1.3 mg/ℓ copper in the drinking water would result in a daily intake of less than the lowest levels that were seen to result in GI effects in humans (5.3 mg/day, 3-8 mg/ℓ). This value would thus be protective against acute toxic effects in humans. This value is not protective against copper toxicity in sensitive members of the population, such as those rare individuals with Wilson's disease. These individuals would have to further limit their intake of copper from all sources.

Carcinogenic Effects

A special complex of copper, copper 8-hydroxyquinoline, when administered by a single subcutaneous injection gave significantly increased incidence of reticulum cell sarcomas only in male B6C3F1 mice (BRL, 1968). This route is not considered indicative of carcinogenic potential by ingestion. In the same study groups of 18 male and female mice were administered copper 8-hydroxyquinoline by gavage from days 7-28. No statistically significant increase in neoplasms was observed.

Gilman (1962) studied the carcinogenicity of cupric oxide, cupric sulfide and cuprous sulfide in Wester rats by administering 13-16 mg copper by intramuscular injections. No injection site tumors and so increases over controls were observed.

There are no human data and inadequate animal evidence of carcinogenicity; therefore, according to EPA guidelines for Risk Assessment (U.S. EPA, 1986b), copper is designated as group D - not classified as to human carcinogenicity.

Mutagenicity studies have reported mixed results. No evaluation of the mutagenicity of copper or copper compounds can be made on the basis of the available data.

Quantification of Carcinogenic Effect

Since copper is categorized as Group D, excess carcinogenic risk values have not been calculated for copper.

Special Considerations

Copper tubing is widely used in home plumbing systems. The variables that may affect copper levels in drinking water are degrees of water pH, softeners, temperature, duration of contact with copper tubing and grounding of electrical systems to copper pipes.

Synergistic Effects. The mechanistic nature of the metabolic interactions between copper and molybdenum, calcium, sulfate, iron and zinc in animals and man is not thoroughly understood. Copper toxicity in swine may be reduced with the concurrent administration of zinc and iron, and copper has a prophylactic effect on rats concurrently exposed to toxic levels of zinc (NAS, 1977).

Special Groups at Risk. Untreated Wilson's disease will result in chronic toxicity even at normal levels of copper intake (2-3 mg/day). Walker-Smith and Blomfield (1973) reported a case wherein a child with suspected (undiagnosed) Wilson's disease died from exposure to 6.9-9.7 mg/ℓ of drinking water for 14 months.

Kidney dialysis patients may be susceptible to acute toxicity from dialysis fluid containing excess copper. Such exposure may have an adverse effect on erythrocyte membranes and enzyme systems.

Persons with G-6-PD deficiency are likely to be susceptible to toxic effects of oxidative stressors (Calabrese and Moore, 1979) such as copper. Inasmuch as ~13% of the American Black male population (along with other populations) may have this deficiency, elevated copper in their drinking water may place these persons at special risk.

Summary

Copper is recognized as an essential element. About 2-3 mg/day are required for proper nutrition. Human exposure to copper has been demonstrated to cause acute toxicity. Chronic effects are not likely because of the acute toxicity limits intake and because copper is an essential element controlled by a homeostatic mechanism and does not tend to bioaccumulate. The lowest level of copper exposure reported to cause this toxicity was a single oral dose of 5.3 mg. A 1-day HA of 1.3 mg/ℓ is associated with this dose and is appropriate for both children and adults. The data base for copper was insufficient to derive a 10-day HA, longer-term HA or a DWEL. Given that there are nutritional requirements for copper of 2-3 mg/kg/day, the recommendation is to use 1.3 mg/ℓ as the basis of the drinking water standard to protect against acute toxic effects.

IX. REFERENCES

Adelstein, S.J. and B.L. Vallee. 1961. Copper. In: Mineral Metabolism, Vol. II: The Elements, Part B, C.L. Comar and F. Bronner, Ed. Academic Press, NY. 32: 371-401.

Ahmed, T., A. Januszkiewicz, P. Eyre, M.J. Robinson and M.A. Sackner. 1981. Acute pulmonary hemodynamic effects of intravenous copper sulfate: role of alpha-adrenergic system. J. Appl. Physiol. Respir., Environ. Exercise Physiol. 51(5): 1204-1213.

Baes, C.F. III and R.D. Sharp. 1983. A proposal for estimation of soil leaching and leaching constants for use in assessment models. J. Environ. Qual. 12: 17-28.

Barka, T., P.T. Scheuer, F. Schaffner and H. Popper. 1964. Structural changes of liver cells in copper intoxication. Arch. Pathol. 78: 331.

Batsura, Y. 1969. Electron-microscopic study of the penetration of copper aerosol from the lungs into the blood and internal organs. Bryull. Eksp. Biol. Med. 68(10): 105. (Rus.)

Bearn, A.G. and H.G. Kunkel. 1954. Localization of Cu^{64} in serum fractions following oral administration: An alteration in Wilson's disease. Proc. Soc. Exp. Biol. Med. 85: 44-48.

Boulard, M., K.G. Blume and E. Beutler. 1975. The effect of copper on red cell enzyme activities. J. Clin. Investigations. 51: 456-461.

Boyden, R., V.R. Potter and C.A. Elvehjem. 1938. Effect of feeding high levels of copper to albino rats. J. Nutr. 15: 397-402.

Bradford, G.R., A.L. Page, I.J. Lund and W. Olmstead. 1975. Trace element concentrations of sewage treatment plant effluents and sludges, their interactions with soils and uptake by plants. J. Environ. Qual. 4: 123.

Bremner, I. 1979. Copper toxicity studies using domestic and laboratory animals. Copper Environ. 2: 285-306.

Bremner, I. and N.T. Davies (n.d.). Unpublished observations.

BRL (Bionetics Research Labs). 1968. Evaluation of Carcinogenic, Teratogenic and Mutagenic Activities of Selected Pesticides and Industrial Chemicals. Vol. I. Carcinogenic Study Prepared for National Cancer Institute. NCI-DCCP-CG-1973-1-1.

Brown, K.W., J.C. Thomas and J.F. Slowey. 1983. The movement of metals applied to soils in sewage effluents. Water, Air, Soil Pollut. 19: 43-54.

Browning, E. 1961. Toxicity of Industrial Metals. Butterworths, London.

Bush, J.A., J.P. Mahoney, H. Markowitz, C.J. Gubler, G.E. Cartwright and M.M. Wintrobe. 1955. Studies on copper metabolism. XVI. Radioactive copper studies in normal subjects and in patients with hepatolenticular degeneration. J. Clin. Invest. 34: 1766-1778.

Bush, J.A., J.P. Mahoney, C.J. Gubler, G.E. Cartwright and M.M. Wintrobe. 1956. Studies on copper metabolism. XXI. The transfer of radiocopper between erythrocytes and plasma. J. Lab. Clin. Med. 47: 898-906.

Calabrese, E.J. and G.S. Moore. 1979. Can elevated levels of copper in drinking water precipitate acute hemolysis in G-6-PD deficient individuals? Med. Hypotheses. 5: 493.

Calabrese, E.J., G.S. Moore and S.C. Ho. 1980. Low glucose-6-phosphate dehydrogenase (G-6-PD) activity in red blood cells and susceptibility to copper-induced oxidative damage. Environ. Res. 21: 366-372.

Callahan, M.A., M.W. Slimak, N.W. Gabel, et al. 1979. Water-Related Environmental Fate of 129 Priority Pollutants, Vol. 1, U.S. EPA Report No. EPA 440/4-79-029a, Office of Water Planning and Standards, Office of Water and Wastewater Management, U.S. Environmental Protection Agency, Washington, DC.

Carpenter, R.H., T.A. Pope and R.L. Smith. 1975. Fe-Mn oxide coatings in stream sediment geochemical surveys. J. Geochem. Explor. 4: 349-363.

Casto, B.C., J. Meyers and J.A. DiPaolo. 1979. Enhancement of vital transformation for evaluation of the carcinogenic or mutagenic potential of inorganic metal salts. Cancer Res. 39: 193.

CDC (Centers for Disease Control). 1977-1982. Centers for Disease Control: Water-Related Disease Outbreaks. Annual Summaries.

Chem. Sources - USA, 1980 Ed. 1980. Directories Publishing Company, Inc., Ormand Beach, FL. p. 167-169.

Chugh, K.S., P.C. Singhal and B.K. Sharma. 1975. Methemoglobinemia in acute copper-sulfate poisoning. Ann. Intern. Med. 82(2): 226-227.

Chuttani, H.K., P.S. Gupta, S. Gulati and D.N. Gupta. 1965. Acute copper sulphate poisoning. Am. J. Med. 39: 849-854.

Cohen, J.M., L.J. Kamphake, E.K. Harris and R.L. Woodward. 1960. Taste threshold concentrations of metal in drinking water. J. Am. Water Works Assoc. 52: 660.

Collins, B.I. 1973. The concentration control of soluble copper in a mine tailings stream. Geochim. Cosmochim. Acta. 37: 69-75.

Cotton, F.A. and G. Wilkinson. 1980. Advanced inorganic chemistry: A comprehensive text. John Wiley and Sons, Inc., NY. p. 798-821.

Crampton, R.F., D.M. Matthews and R. Poisner. 1965. Observations on the mechanism of absorption of copper by the small intestine. J. Physiol. 178: 111-126.

Danks, D.M., M.B. Campbell, B.J. Stevens, V. Mayne and E. Cartwright. 1972. Menkes' kinky hair syndrome. An inherited defect in copper absorption with widespread effects. Pediatrics. 50: 188-201.

Demerec, M., G. Bertani and Flint, J. 1951. A survey of chemicals for mutagenic action on E. coli. Am. Natur. 85: 119.

DiCarlo, F.J. 1980. Syndromes of cardiovascular malformations induced by copper citrate in hamsters. Teratology. 21: 89-101.

Diess, A., G.R. Lee and G.E. Cartwright. 1970. Hemolytic anemia in Wilson disease. Ann. Intern. Med. 73: 413.

Dougherty, J.J. and W.G. Hoekstra. 1982. Effects of vitamin E and selenium on copper-induced lipid peroxidation in vivo and on acute copper toxicity. Proc. Soc. Exp. Biol. Med. 169(2): 201-208.

Dowdy, R.P. 1969. Copper metabolism. In: Comments in Biochemistry. Am. J. Clin. Nutr. 22: 887.

Evans, G.W. 1973. Copper homeostasis in the mammalian system. Physiol. Rev. 53: 535-569.

Ferm, V.H. and D.P. Hanlon. 1974. Toxicity of copper salts in hamster embryonic development. Biol. Reprod. 11: 97-101.

FWQA (Federal Water Quality Administration). 1968. Water Quality Criteria. Rep. Natl. Tech. Adv. Comm. to the Secretary of the Interior.

Gilman, J.P.W. 1962. Metal carcinogenesis. II. A study on the carcinogenic activity of cobalt, copper, iron and nickel compounds. Cancer Res. 22: 158-166.

Gleason, R.P. 1968. Exposure to copper dust. Am. Ind. Hyg. Assoc. J. 29: 461-462.

Gollan, J.L. and D.J. Deller. 1973. Studies on the nature and excretion of biliary copper in man. Clin. Sci. 44: 9-15.

Haddow, A. and E.S. Horning. 1960. On the carcinogenicity of an iron-dextran complex. J. Nat. Cancer Inst. 24: 109-127.

Haywood, S. 1980. The effect of excess dietary copper on the liver and kidney of the male rat. J. Comp. Pathol. 90(2): 217-232.

Hazelrig, J.B., C.A. Owen and E. Ackerman. 1966. A mathematical model for copper metabolism and its relation to Wilson's disease. Am. J. Physiol. 211: 1075.

Helman, R.G., L.G. Adams, K.R. Pierce, C.H. Bridges and E.M. Bailey. 1983. The role of lysosomes in the pathogenesis of copper-induced hepatotoxicity. II. Intracellular distribution of copper in hepatocytes. Toxicol. Appl. Pharmacol. 67(2): 238-245.

Hem, J.D. and M.W. Skougstad. 1960. Co-precipitation effects in solutions containing ferrous, ferric and cupric ions. p. 104. U.S. Geological Survey Water Supply Paper 1459-E, Washington, DC. p. 110.

Herbert, V. 1970. Drugs Effective in Iron-Deficiency and Other Hypochronic Anemias. In: The Pharmacological Basis of Therapeutics, Chapter 63, 4th ed., L.S. Goodman, and A. Gilman, Ed. The Macmillan Co., London. p. 1410.

Holland, M.K. and I.G. White. 1982. Heavy metals and human spermatozoa. II. The effect of seminal plasma on the toxicity of copper metal for spermatozoa. Int. J. Fertil. 27(2): 95-99.

Holtzman, N.A., D.A. Elliot and R.H. Heller. 1966. Copper intoxication. Report of a case with observations on ceruloplasmin. New Engl. J. Med. p. 275-347.

Hoover, J.E. Ed. 1970. Cupric Sulfate NF. In: Emetics. Gastrointestinal Drugs. Chapter 44. Remington's Pharmaceutical Services, 14th ed. Mack Publishing Co., Easton, PA. p. 806.

Howell, J.S. 1959. Histochemical demonstration of copper in copper-fed rats and in hepatolenticular degeneration. J. Pathol. Bacteriol. 77: 473-484.

Huang, C.P., H.A. Elliott and R.M. Ashmead. 1977. Interfacial reactions and the fate of heavy metals in soil-water systems. J. Water Pollut. Control Fed. 49(5): 745-756.

Hurley, L.S. and C.L. Keen. 1979. Teratogenic effects of copper. Copper Environ. 2: 33-56.

Jackson, K.S. and G.B. Skippen. 1978. Geochemical dispersion of heavy metals via organic complexing: A laboratory study of copper, lead, zinc, and nickel behavior at a simulated sediment-water boundary. J. Geochem. Explor. 10: 117-138.

Jenne, E.A. 1968. Controls on Mn, Fe, Co, Ni, Cu and Zn concentrations in soils and waters: The significant role of hydrous Mn and Fe oxides. p. 337-387. In: Trace Inorganics in Water, R.F. Gould, Ed. Advances in Chem. Series 73. American Chemical Society, Washington, DC. p. 396.

Kanematsu, N., M. Hara and T. Kada. 1980. Rec assay and mutagenicity studies on metal compounds. Mutat. Res. 77: 109-116.

Karalekas, P.C. Jr., C.R. Ryan and F.B. Taylor. 1983. Control of lead, copper, and iron pipe corrosion in Boston. J. Am. Water Works Assoc. 75: 92-95.

Kimura, Y. and S. Makino. 1963. Cytological effects of chemicals on tumors. XVI. Effects of some inorganic compounds on the MTK-sarcoma III. in vivo. Gann (Jap. J. Cancer Res.). 54: 155-162.

Kline, R.D., V.W. Hays and G.L. Cromwell. 1971. Effects of copper, molybdenum and sulfate on performance, hematology and copper stores of pigs and lambs. J. Anim. Sci. 33: 771-779.

Lal, S. and T.L. Sourkes. 1971. Intracellular distribution of copper in the liver during administration of copper sulfate to the rat. Toxicol. Appl. Pharmacol. 18(3): 562-572.

Law, L.W. 1938. The effects of chemicals on the lethal mutation rates in Drosophila melanogaster. Proc. Nat. Acad. Sci. 24: 546-550.

Lecyk, M. 1980. Toxicity of cupric sulfate in mice embryonic development. Zool. Pol. 28(2): 101-105.

Lee, G.F. 1975. Transport of heavy metals in the environment. p. 137-147. In: Heavy metals in the aquatic environment, P.A. Krendel, Ed. Pergamon Press, Oxford, England. p. 352.

Lester, J.N., R.M. Harrison and R. Perry. 1979. The balance of heavy metals through a sewage treatment works. I. Lead, cadmium and copper. Sci. Total Environ. 12: 13.

Lopez, J.M. and G.F. Lee. 1977. Environmental chemistry of copper in Torch Lake, Michigan. Water Air Pollut. 8: 373-385.

Malhotra, K.M., G.S. Shukla and S.V. Chandra. 1982. Neurochemical changes in rats coexposed to lead and copper. Arch. Toxicol. 49(3-4): 331-336.

Matsui, S. 1980. Evaluation of a Bacillus subtilis rec-assay for the detection of mutagens which may occur in water environments. Water Res. 14(11): 1613-1619.

Miranda, C.L., M.C. Henderson and D.R. Buhler. 1981. Dietary copper enhances the hepatotoxicity of Senecio jacobaea in rats. Toxicol. Appl. Pharmacol. 60(3): 418-423.

Moriya, M., T. Ohta, K. Watanabe, T. Miyazawa, K. Kato and Y. Shirasu. 1983. Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat. Res. 116(3-4): 185-216.

Moroff, G., J. Poster and G. Oster. 1974. Enhancement of the copper-induced hemolysis by progesterone. J. Steroid Biochem. 5: 601.

Narasaki, M. 1980. Laboratory and histological similarities between Wilson's disease and rats with copper toxicity. Acta Med. Okayama. 34(2): 81-90.

NAS (National Academy of Sciences). 1977a. Copper: Medicinal and Biologic Effects of Environmental Pollutants. National Research Council. NAS, Washington, DC.

NAS (National Academy of Sciences). 1977b. Drinking Water and Health. Vol. 1, p. 19-63.

NAS (National Academy of Sciences). 1980a. Recommended Daily Allowances, 9th Rev. ed. Food and Nutrition Board, NAS, Washington, DC.

NAS (National Academy of Sciences). 1980b. Drinking Water and Health. Vol. 3. p. 25-67, 312-320.

Neumann, P.Z. and A. Sass-Kostsak. 1967. The state of copper in human serum: Evidence for an amino acid-bound fraction. J. Clin. Invest. 46(4): 646-658.

Nicholas, P.O. and M.B. Brist. 1968. Food poisoning due to copper in the morning tea. Lancet. 2: 40-42.

Nishioka, H. 1975. Mutagenic activities of metal compounds in bacteria. Mutat. Res. 31: 185-189.

O'Dell, B.L. 1976. Biochemistry and physiology of copper in vertebrates. In: Trace Elements in Human Health and Disease. Vol. I. Zinc and Copper, A.S. Prasad, Ed. Academic Press, NY.

Oreke, T., I. Sternlieb, A.G. Morell and I.H. Scheinberg. 1972. Systemic absorption of intrauterine copper. Science. 177: 358-360.

Osborn, S.B., C.N. Roberts and J.M. Walshe. 1963. Uptake of radioactive copper by the liver. A study of patients with Wilson's disease and various control groups. Clin. Sci. 24: 13-22.

Oster, G. and M.P. Salgo. 1977. Copper in mammalian reproduction. Adv. Pharmacol. Chemother. 14: 327-409.

Owen, C.A., Jr. 1981. Copper in Biology and Medicine Series: Copper Deficiency and Toxicity. Noyes Data Corp., Park Ridge, NJ. p. 190.

Page, A.L. 1974. Fate and Effects of Trace Elements in Sewage Sludge When Applied to Agricultural Lands. National Environ. Research Center, Cincinnati, OH. EPA 670/2-74-005. NTIS PB 231-171/OBA.

Patterson, J.W.. 1981. Corrosion in Water Distribution Systems. Office of Drinking Water, U.S. EPA, Washington, DC.

Payne, K. and W.F. Pickering. 1975. Influence of clay-solute intractions on aqueous copper ion levels. Water Air Soil Pollut. 5: 63-69.

Pennington J.A.T., B.E. Young, D.B. Wilson, R.D. Johnson and R.D. Vanderzveen. 1986. Mineral content of foods and total diets: The selected minerals in foods survey, 1982 to 1984.

Petering, H.G. 1987. Personnel communication to Environmental Criteria Assessment Office, U.S. EPA, Cincinnati, OH. Review comments from University of Cincinnati.

Pimental, J.C. and F. Marques. 1969. 'Vineyard sprayer's lung': A new occupational disease. Thora. 24: 678-688.

Pimental, J.C. and A.P. Menezes. 1975. Liver granulomas containing copper in vineyards sprayer's lung. A new etiology of hepatic granulomatosis. Am. Rev. Respir. Dis. 111: 189-195.

Ramamoorthy, S. and D.J. Kushner. 1975. Heavy metal binding components of river water. J. Fish Res. Bd. Can. 32: 1755-1766.

Rana, S.V.S. and A. Kumar. 1980. Biological, hematological and histological observations in copper-poisoned rats. Ind. Health. 18(1): 9-17.

Roberts, R.H. 1956. Hemolytic anemia associated with copper sulfate poisoning. Miss. Doctor. 33: 292-294.

Salmon, M.A. and T. Wright. 1971. Chronic copper poisoning presenting as 'pink' disease. Arch. Dis. Child. 46: 108-110.

Sanghvi, L.M., R. Sharma, S.N. Misra and K.C. Samuel. 1957. Sulfhemoglobinemia and acute renal failure after copper sulfate poisoning: Report of two fatal cases. Arch. Pathol. 63: 172.

Sargent, H.E. and H. Jean. 1983. An Environmental Health Investigation of Copper Intoxication from a Public Water Supply. Unpublished manuscript.

Scheinberg, I.H. and I. Sternlieb. 1969. Metabolism of trace metals. In: Duncan's Diseases of Metabolism, Vol. 2, Endocrinology and Nutrition, 6th ed., B.K. Bondy, Ed. W.D. Saunders Co., Philadelphia, PA.

Scheinberg, I.H. and I. Sternlieb. 1976. Copper toxicity and Wilson's disease. In: Trace Elements in Human Health and Disease. Vol. I. Zinc and Copper, A.S. Prasad, Ed. Academic Press, NY.

Schrauzer, G.N., D.A. White and C.J. Schneider. 1977. Cancer mortality correlation studies. IV. Association with dietary intakes and blood levels, notably Se-antagonists. Bioinorg. Chem. 7: 35-56.

Schroeder, H.A. 1970. A sensible look at air pollution by metals. Arch. Environ. Health. 21: 798-806.

Schroeder, H.A., A.P. Nason, I.H. Tipton and J.J. Balassa. 1966. Essential trace metals in man. Copper. J. Chronic Dis. 19: 1007-1034.

Semple, A.B., W.H. Parry and D.E. Phillips. 1960. Acute copper poisoning: An outbreak traced to contaminated water from a corroded geyser. Lancet. 2: 700-701.

Sharrett, A.R., A.P. Carter, R.M. Orheim and M. Feinleib. 1982. Daily intake of lead, cadmium, copper and zinc from drinking water: The Seattle study of trace metal exposure. Environ. Res. 28: 456-457.

Shields, G.S., H. Markowitz, W.H. Klassen, G.E. Cartwright and M.M. Wintrobe. 1961. Studies on copper metabolism. XXXI. Erythrocyte copper. J. Clin. Invest. 40: 2007-2015.

Sina, J.F., C.L. Bean, G.R. Dysart, V.I. Taylor and M.O. Bradley. 1983. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat. Res. 113(5): 357-391.

Singh, I. 1983. Induction of reverse mutation and mitotic gene conversion by some metal compounds in Saccharomyces cerevisiae. Mutat. Res. 117(1-2): 149-152.

Sirover, M.A. and L.A. Loeb. 1976. Infidelity of DNA synthesis in vitro: Screening for potential metal mutagens or carcinogens. Science. 194: 1434-1436.

Sopper, W.E. 1973. Water Recycling on Forest Lands. In: Proceedings of the Fourth North American Forest Soils Conference. Laval University, Quebec. p. 227-242.

Spitalny, K.C., J. Brondum, R.L. Vogt, H.E. Sargent and S. Kappel. 1984. Drinking-water-induced copper intoxication in a Vermont family. Pediatrics. 74(6): 1103-1106.

SRI (Stanford Research Institute) International. 1980. 1980 Directory of Chemical Producers: United States of America Menlo Park, CA, SRI International. p. 520-521.

Steele, K.F. and G.H. Wagner. 1975. Trace metal relationships in bottom sediments of a fresh water stream - The Buffalo River, Arkansas. J. Sed. Pet. 45(1): 310-319.

Stein, R.S., D. Jenkins and M.E. Korns. 1976. Death after use of cupric sulfate as emetic. J. Am. Med. Assoc. 235: 801.

Sternlieb, I. 1980. Copper and the liver. Gastroenterology. 78: 1615-1628.

Stiff, M.J. 1971a. Copper/bicarbonate equilibria in solutions of bicarbonate ion at concentrations similar to those found in natural waters. Water Res. 5: 171-176.

Stiff, M.J. 1971b. The chemical states of copper in polluted fresh water and a scheme of analysis to differentiate them. Water Res. 5: 585-599.

Stokinger, H.E. 1980. The metals: Copper. In: Patty's Industrial Hygiene and Toxicology. Vol. 2A. Toxicology, G.D. Clayton and F.E. Clayton., Ed. John Wiley and Sons, Inc., NY. p. 1620-1630.

Suttle, N.F. and C.F. Mills. 1966a. Studies of the toxicity of copper to pigs. I. Effects of oral supplements of zinc and iron salts on the development of copper toxicosis. Br. J. Nutr. 20: 135-148.

Suttle, N.F. and C.F. Mills. 1966b. Studies of the toxicity of copper to pigs. 2. Effect of protein source and other dietary components on the response to high and moderate intakes of copper. Br. J. Nutr. 20: 149-161.

Sylva, R.N. 1976. The environmental chemistry of copper(II) in aquatic systems. Water Res. 10: 798-792.

Tso, W.W. and W.P. Fung. 1981. Mutagenicity of metallic cations. Toxicol. Lett. 8: 195-200.

Underwood, E.J. 1971. Trace Elements in Human and Animal Nutrition, 3rd ed. Chapter 3. Copper. Academic Press, New York and London.

U.S. EPA. 1979. National Secondary Drinking Water Regulations. EPA 570-9-76-000, Washington, DC.

U.S. EPA. 1980a. Computer print-out of nonconfidential data from TSCA Inventory. U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances, Chemical Information Division, Washington, DC.

U.S. EPA. 1980b. Ambient Water Quality Criteria Document for Copper. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Water Regulations and Standards, Washington, DC. EPA 440/5-80-036. NTIS PB81-117475.

U.S. EPA. 1984. Computer printout of frequency distribution listing or copper in air, 1977-1983. Environmental Monitoring Systems Laboratory, Research Triangle Park, NC.

U.S. EPA. 1986a. Appendix A. Reference Dose (RfD): Description and Use in Health Risk Assessment. Integrated Risk Information System (IRIS). Online. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH.

U.S. EPA. 1986b. Guidelines for Carcinogen Risk Assessment. Federal Register. 51(185): 33992-34003.

U.S. EPA. 1987. Estimated National Occurrence and Exposure to Copper in Public Drinking Water Supplies. Prepared by SAIC (Science Application International Corporation) for the Office of Drinking Water, U.S. EPA.

USITC (U.S. International Trade Commission). 1980. Synthetic Organic Chemicals: U.S. Production and Sales, 1979. USITC Publ. No. 1099. USITC, Washington, DC. p. 284, 235.

Van Ravensteyn, A.H. 1944. Metabolism of copper in man. Acta Med. Scand. 118: 163-196.

Villar, T.G. 1974. Vineyard sprayer's lung: Clinical aspects. Am. Rev. Respir. Dis. 110: 545.

Walker, W.R., R.R. Reeves, M. Brosnan and G.D. Coleman. 1977. Perfusion of intact skin by a saline solution of bis (glycinato) copper (II). Bioinorganic Chem. 7: 271-276.

Walker-Smith, J. and J. Blomfield. 1973. Wilson's disease or chronic copper poisoning? Arch. Dis. Child. 48: 476.

Walsh, F.M., F.J. Crosson, M. Bayley, J. McReynolds, B.J. Pearson. 1977. Acute copper intoxication. Pathophysiology and therapy with a case report. Am. J. Dis. Child. 131: 149-151.

Weber, P.M., S. O'Reilly, M. Pollycove and L. Shipley. 1969. Gastrointestinal absorption of copper: Studies with ^{64}Cu, ^{95}Zr, a whole body counter and the scintillation camera. J. Nucl. Med. 10: 591-596.

WHO (World Health Organization). 1984. Guidelines for Drinking Water Quality, Vol. I. Recommendations. WHO, Geneva. p. 7.

Williams, D.M. 1982. Clinical Significance of Copper Deficiency and Toxicity in the World Population. In: Clinical, Biochemical and Nutritional Aspects of Trace Elements. Alan R. Liss, Inc., NY. p. 277-299.

Windholz, M., Ed. 1976. Merck Index, 9th ed. Merck and Co., Inc., Rahway, NJ. p. 342-347.

Wyllie, J. 1957. Copper poisoning at a cocktail party. Am. J. Publ. Health. 47: 617.