3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of copper. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which

major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

3.2.1.1 Death

No studies were located regarding death of humans or animals following inhalation exposure to copper.

3.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, musculoskeletal, renal, dermal, or body weight effects in humans or animals following inhalation exposure to copper.

Respiratory, gastrointestinal, hematological, hepatic, endocrine, and ocular effects were observed in humans. Respiratory effects have also been observed in animals exposed to copper sulfate aerosols.

Respiratory Effects. In humans, copper is a respiratory irritant. Workers exposed to copper dust report a number of symptoms that are suggestive of respiratory irritation, including coughing, sneezing, thoracic pain, and runny nose (Askergren and Mellgren 1975; Suciu et al. 1981). In the Suciu et al. (1981) study of 75–100 workers involved in sieving copper, lung radiographs revealed linear pulmonary fibrosis, and in some cases, nodulation. During the first year of operation, the workers were exposed to 434 mg Cu/m^3; the exposure levels declined each year, and by year 3, the levels were 111 mg Cu/m^3. In

sheet metal workers exposed to patina dust (copper-hydroxide-nitrate, copper-hydroxide-sulfate, copper silicate, copper oxide), 6 of the 11 examined workers had increased vascularity and superficial epistatic vessels in the nasal mucosa (Askergren and Mellgren 1975); no exposure levels were reported.

Copper is considered the etiologic agent in the occupational disease referred to as "vineyard sprayer's lung". This disease, which is observed in vineyard workers spraying an antimildew agent containing 1–2.5% copper sulfate neutralized with hydrated lime, was first described in humans by Cortez Pimentel and Marques (1969). In most cases, published information on this disease comes from case reports (Cortez Pimentel and Marques 1969; Cortez Pimentel and Menezes 1975; Stark 1981; Villar 1974; Villar and Nogueira 1980) with no concentration-response information. Common findings (obtained by alveolar lavage and biopsy) include intraalveolar desquamation of macrophages, formation of histiocytic and noncaseating granulomas containing inclusions of copper, and healing of lesions in the form of fibrohyaline nodules, very similar to those found in silicosis (Cortez Pimentel and Marques 1969; Plamenac et al. 1985). Higher incidences of abnormal columnar cells, squamous metaplasia without atypia, copper containing macrophages, eosinophilia, and respiratory spirals were found in the sputa of smoking and nonsmoking vineyard sprayers, as compared to rural workers from the same geographic region who did not work in the vineyards (Plamenac et al. 1985).

The potential of copper to induce respiratory effects has been tested in mice, hamsters, and rabbits. Decreased cilia beating was observed in Syrian-Golden hamsters exposed to 3.3 mg Cu/m^3 as copper sulfate for 3 hours (Drummond et al. 1986); this effect was not observed in similarly exposed CD-1 mice. Repeated exposure resulted in alveolar thickening in CD-1 mice exposed to 0.12 mg Cu/m^3 as copper sulfate for 3 hours/day, 5 days/week for 1–2 weeks (Drummond et al. 1986); the severity of the effect increased with the duration of exposure. In rabbits (strain not reported) exposed to 0.6 mg Cu/m^3 as copper chloride for 6 hours/day, 5 days/week for 4–6 weeks, the only histological alteration in the lungs was a slight increase in alveolar type II cell volume density (Johansson et al. 1984); this effect was not considered adverse. No functional or morphological alterations were observed in the alveolar macrophages of similarly exposed rabbits (Johansson et al. 1983).

Gastrointestinal Effects. In workers involved in grinding and sieving copper dust, anorexia, nausea, and occasional diarrhea were reported (Suciu et al. 1981); exposure levels ranged from 111 to 434 mg Cu/m^3 over a 3-year period. It is likely that the observed gastrointestinal effects were due to oral exposure to copper. Ingestion probably resulted from mucocilliary clearance of copper particles deposited in the nasopharyngeal and tracheobronchial regions of the respiratory tract.

No studies were located regarding gastrointestinal effects in animals following inhalation exposure to copper.

Hematological Effects. Decreased hemoglobin and erythrocyte levels have been observed in workers exposed to airborne copper levels of 0.64–1.05 mg/m^3. Results of hair analysis reveal that the workers were also exposed to iron, lead, and cadmium (Finelli et al. 1981).

No studies were located regarding hematological effects in animals following inhalation exposure to copper.

Hepatic Effects. Hepatomegaly was observed in workers involved in grinding and sieving copper dust (Suciu et al. 1981); the exposure levels ranged from 111 to 434 mg Cu/m^3.

No studies were located regarding hepatic effects in animals following inhalation exposure to copper.

Endocrine Effects. Seven cases of enlargement of the sella turcica, nonsecretive hypophyseal adenoma, accompanied by obesity, arterial hypertension, and "red facies" were observed in a group of 100 workers exposed to 111–434 mg Cu/m^3 as copper dust (Suciu et al. 1981). The study authors noted that there was a possibility that the clinical manifestations of hypophyseal adenoma or of Cushing's syndrome may have been the result of a disturbance of copper metabolism. The significance of this effect and its relationship to copper exposure cannot be determined.

Ocular Effects. Eye irritation has been reported by workers exposed to copper dust (Askergren and Mellgren 1975). The irritation is likely due to direct contact with the copper rather than a systemic effect resulting from inhalation exposure.

Other Systemic Effects. A few studies have reported metal fume fever, a 24–48-hour illness characterized by chills, fever, aching muscles, dryness in the mouth and throat, and headache, in workers exposed to copper dust or fumes (Armstrong et al. 1983; Gleason 1968). Gleason (1968) reported airborne copper dust concentrations of 0.075–0.12 mg/m^3. It has been suggested that other metals present in the workplace may have been the causative agent for the metal fume fever, rather than copper. This is supported by the small number of reports of metal fume fever despite the extensive use of copper in many industries (Borak et al. 2000).

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans following inhalation exposure to copper.

An acute exposure study in mice reported an impaired immune response following exposure to copper sulfate and a bacterial challenge (Drummond et al. 1986). Increased mortality and decreased survival time were observed in CD-1 mice challenged by an aerosol of *Streptococcus zooepidemicus* following 0.56 mg Cu/m^3 for 3 hours or 0.13 mg Cu/m^3 for 3 hours/day, 5 days/week for 2 weeks. Decreased bactericidal activity of alveolar macrophages was also observed in mice exposed to 3.3 mg Cu/m^3 for 3 hours or 0.12 mg Cu/m^3 for 3 hours/day, 5 days/week for 2 weeks following exposure to an aerosol of *Klebsiella pneumonia*.

These LOAEL values for immunological effects are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

Only one study examining neurological effects was located. Headache, vertigo, and drowsiness were reported in factory workers exposed to 111–434 mg/m^3 copper dust (Suciu et al. 1981).

3.2.1.5 Reproductive Effects

Sexual impotence was reported in 16% of workers (75–100 workers examined) exposed to 111–434 mg/m^3 copper dust during grinding and sieving operations (Suciu et al. 1981). The significance of this finding is difficult to assess because a control group was not used.

No studies were located regarding reproductive effects in animals following inhalation exposure to copper.

Table 3-1 Levels of Significant Exposure to Copper - Inhalation

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/m^3)	LOAEL Less Serious (mg/m^3)		LOAEL Serious (mg/m^3)		Reference Chemical Form
	ACUTE EXPOSURE								
	Systemic								
1	Mouse	3 hr	Resp	3.3					Drummond et al. 1986
2	Mouse	1-2 wk 5d/wk 3hr/d	Resp		0.12	(alveoli thickening)			Drummond et al. 1986
3	Hamster	3 hr	Resp	1.21	3.3	(decr cilia beating frequency)			Drummond et al. 1986
4	Hamster	1-2 wk 5d/wk 3hr/d	Resp	0.13					Drummond et al. 1986
	Immuno/ Lymphoret								
5	Mouse	1-2 wk 5d/wk 3hr/d			0.12	(decr bactericidal activity)	0.13	(decr mean survival time)	Drummond et al. 1986
6	Mouse	3 hr			3.3	(decr bactericidal activity)	0.56	(decr mean survival time)	Drummond et al. 1986

Table 3-1 Levels of Significant Exposure to Copper - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/m^3)	LOAEL			Reference Chemical Form
					Less Serious (mg/m^3)		Serious (mg/m^3)	
	INTERMEDIATE EXPOSURE							
	Systemic							
7	Rabbit (NS)	1 mo 5d/wk 6hr/d	Resp	0.6 M				Johansson et al. 1983 copper chloride
8	Rabbit (NS)	4-6 wk 5d/wk 6hr/d	Resp	0.6 M				Johansson et al. 1984 copper chloride
	CHRONIC EXPOSURE							
	Systemic							
9	Human	8 hr/d, 5 d/wk	Hemato		0.64	(decr hemoglobin and erythrocyte levels)		Finelli et al. 1981 NS

aThe number corresponds to entries in Figure 3-1.

d = day(s); decr = decreased; hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; (NS) = not specified; Resp = respiratory;wk = week(s); yr = year(s)

Figure 3-1. Levels of Significant Exposure to Copper- Inhalation

Acute (≤14 days)

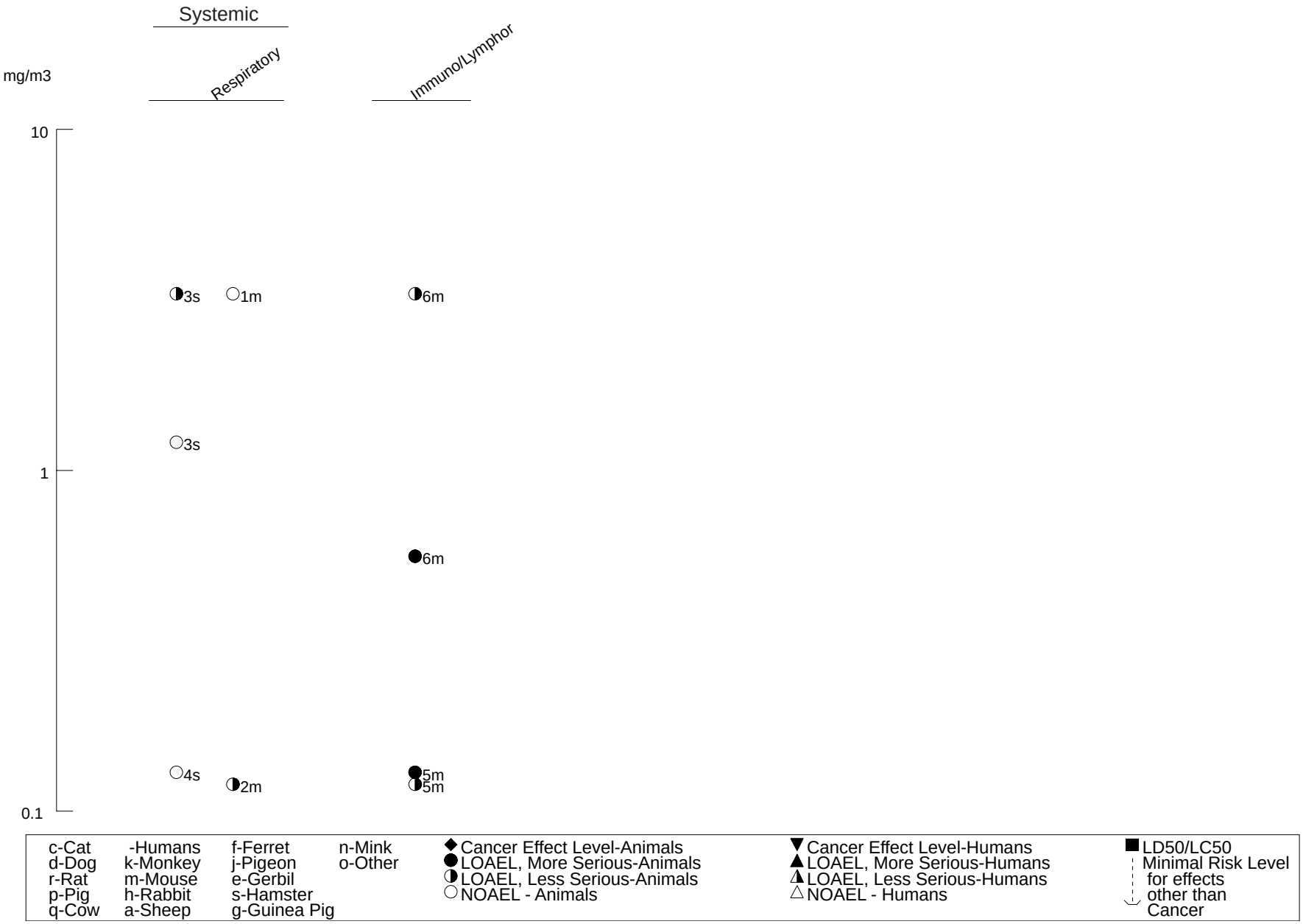


Figure 3-1. Levels of Significant Exposure to Copper- Inhalation (*Continued*)

Intermediate (15-364 days)

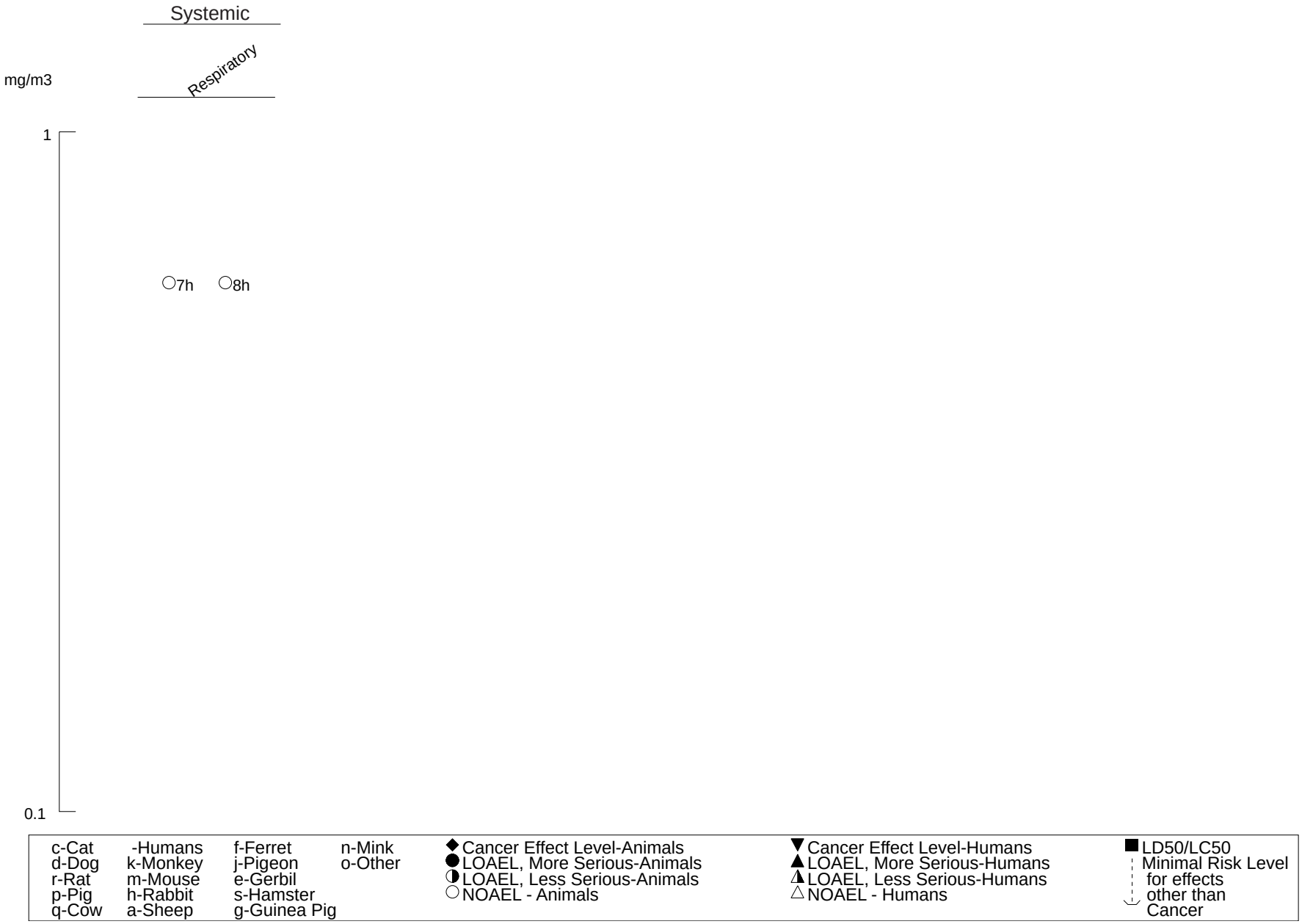
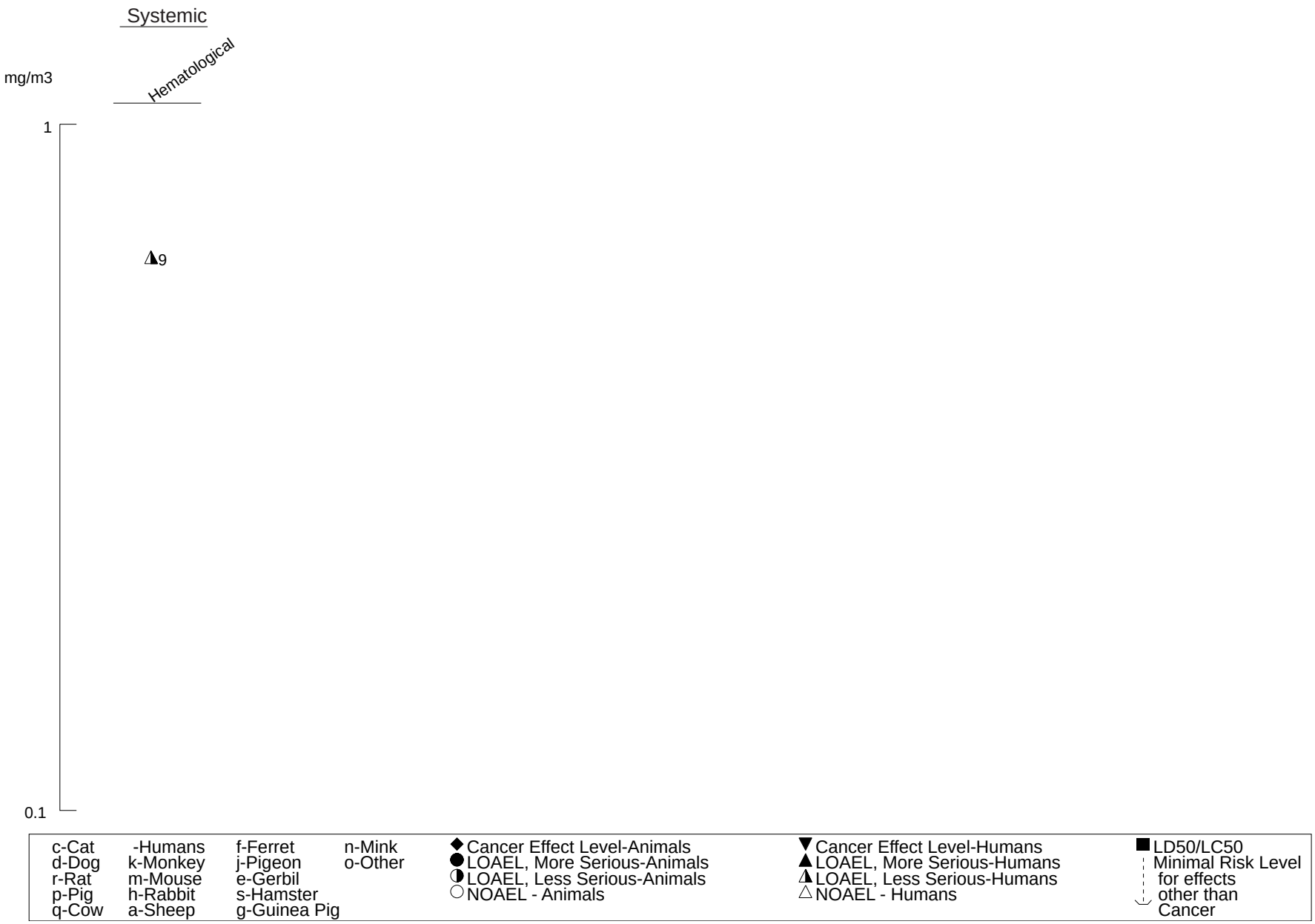


Figure 3-1. Levels of Significant Exposure to Copper- Inhalation (*Continued*)

Chronic (≥365 days)



3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans and animals following inhalation exposure to copper.

3.2.1.7 Cancer

There are limited data for humans and no data for animals on the carcinogenicity of inhaled copper. Although a number of studies have examined cancer risk among copper smelters, these papers are not discussed because the cancer risk has been attributed to exposure to arsenic rather than to copper. In a study of over 6,700 male workers at a Chinese copper mine, significant increases in the risk of cancer (all sites combined) (standardized mortality ratio [SMR] =123; 95% confidence interval [CI] =109–139), stomach cancer (SMR=131; 95% CI=105–161), and lung cancer (SMR=147; 95% CI=112–189) were observed (Chen et al. 1993). The cancer risk increased with increasing duration of employment and time since first exposure and was also higher in workers employed in the 1950s when there was a dramatic increase in production, dry drilling methods were used, and there was poor underground ventilation. Radon and radon daughters were detected in the underground mines; between 1960 and 1990, radioactivity levels of 1.29×10^{-11} Ci/L were measured. To assess the relative contribution of radon and radon daughters to the lung cancer risk, the workers were divided into two groups: underground miners and workers involved in drilling (presumably above ground). Increases in lung cancer risk were observed in both groups, thus suggesting that exposure to radioactivity was not the primary source of increased cancer risk. The copper ore also contained silica, iron, manganese, arsenic, titanium, and sulfur. The study authors noted that the arsenic level in the copper was relatively low (0.061%) and did not likely contribute to the lung cancer risk; however, the lung cancer risk from exposure to silica and iron could not be ruled out. A significant increase in the risk of silicosis was observed in the miners. In a 7-year follow-up of this cohort (Chen et al. 1995), the risk of all sites of cancer (SMR=129; 95% CI=117–142), stomach cancer (SMR=141; 95% CI=116–169), and lung cancer (SMR=152; 95% CI=123–187) were still significantly elevated. This study also conducted a smoking survey and found that a higher percentage of the miners were smokers (71.7%) than in the control population of local residents (64.3%); this increased smoking rate, along with exposure to radioactivity, silica, iron, and arsenic may have contributed to the increased cancer risk.

3.2.2 Oral Exposure

3.2.2.1 Death

A number of deaths have been reported in individuals intentionally ingesting large doses of copper sulfate (Chuttani et al. 1965). Thirteen of 53 individuals died after ingesting 6–637 mg/kg copper; because the amount of copper sulfate was self-reported, the estimated doses may be inaccurate. The deaths were attributed to shock and hepatic and/or renal complications. Deaths, probably due to central nervous system depression and hepatic and renal failure, have also been reported in individuals ingesting "spiritual green water", which contains ≥100 mg copper sulfate/L (Akintonwa et al. 1989).

Increased mortality was observed in rats fed a diet containing 4,000 ppm of copper (133 mg Cu/kg/day) for 1 week. Anorexia, possibly the result of taste aversion, contributed to the deaths (Boyden et al. 1938). Weanling rats exposed to 300 mg Cu/kg/day as Cu(II) in the diet (6,000 ppm) died after 2 weeks (Haywood 1985). The deaths were attributed to extensive centrilobular necrosis.

Lifetime exposure to 42.5 mg Cu/kg/day as copper gluconate in drinking water resulted in a 12.8% reduction of the maximal lifespan (from 986 to 874 days) in mice (Massie and Aiello 1984).

The doses associated with deaths in the Haywood (1985) and Massie and Aiello (1984) studies are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding endocrine, dermal, ocular, or metabolic effects in humans or animals following oral exposure to copper.

The highest NOAEL values and all reliable LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. Data on the potential of copper to induce respiratory effects are limited to the NTP (1993) study that found no histological alterations in the lungs of rats exposed to 285 or 134 mg Cu/kg/day as copper sulfate in the diet for 14 or 90 days, respectively, or in mice exposed to 717 or 814 mg Cu/kg/day for 14 or 90 days.

Table 3-2 Levels of Significant Exposure to Copper - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Death						
1	Rat (Wistar)	2-15 wk (F)				550 M (increased mortality)	Haywood 1985 NS
2	Rat (Fischer- 344)	14 d (W)				31 F (100% mortality)	NTP 1993 copper sulfate
3	Mouse (B6C3F1)	14 d (W)				62 M (increased mortality)	NTP 1993 copper sulfate
	Systemic						
4	Human	once (W)	Gastro	0.011	0.017 (nausea, vomiting, diarrhea, or abdominal pain)		Araya et al. 2001 copper sulfate
5	Human	once (W)	Gastro	0.012 F	0.018 F (nausea)		Araya et al. 2003a copper sulfate
6	Human	once (W)	Gastro		0.046 (nausea, delayed gastric emptying)		Araya et al. 2003c copper sulfate
7	Human	once (W)	Gastro		0.03 (nausea and vomiting)		Gotteland et al. 2001 copper sulfate

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
8	Human	once (W)	Gastro		6 (vomiting)		Karlsson and Noren 1965 copper sulfate
9	Human	once (W)	Gastro		0.08 M (vomiting, diarrhea)		Nicholas and Brist 1968 NS
10	Human	once (W)	Gastro	0.0057	0.011 (nausea)		Olivares et al. 2001 copper sulfate
11	Human	2 wks (W)	Gastro	0.0272[b] F	0.0731 F (abdominal pain, nausea, and/or vomiting)		Pizarro et al. 1999 copper sulfate
12	Human	1 wk (W)	Gastro		0.096 F (nausea, vomiting, and/or abdominal pain)		Pizarro et al. 2001 copper sulfate and copper oxide
13	Rat (NS)	1-2 wk (F)	Hepatic		300 M (parenchymal cell hypertrophy)		Haywood 1980 copper sulfate
			Renal	300 M			

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
14	Rat (NS)	1-2 wk (F)	Hepatic		300 M (increased alanine aminotransferase activity)		Haywood and Comerford 1980 copper sulfate
15	Rat (Wistar)	1-2 wk (F)	Hepatic		450 M (hepatocellular necrosis)		Haywood et al. 1985a NS
			Renal		450 M (copper-containing droplets and granules in proximal tubule cells)		
16	Rat (Wistar)	2 wk (F)	Renal		200 M (droplets in proximal tubule lumen)		Haywood et al. 1985b NS

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
17	Rat (Fischer- 344)	14 d (W)	Resp	29 M			NTP 1993 copper sulfate
			Cardio	29 M			
			Gastro	29 M			
			Hepatic	29 M			
			Renal		10 M (protein droplets in epithelial cells of proximal tubule)		
			Bd Wt	26 F			

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
18	Rat (Fischer- 344)	14 d (F)	Resp	285 F			NTP 1993 copper sulfate
			Cardio	285 F			
			Gastro	23 F	44 F (hyperplasia of forestomach mucosa)		
			Hemato	93 F	196 F (depletion of hematopoietic cells in bone marrow)		
			Hepatic	92 M	198 M (inflammation)		
			Renal	46 M	92 M (increased protein droplets in cortical tubules)		
			Bd Wt	93 F	196 F (18% decrease in body weight gain)		

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
19	Mouse (B6C3F1)	14 d (W)	Resp	24 M			NTP 1993 copper sulfate
			Cardio	24 M			
			Gastro	24 M			
			Hepatic	24 M			
			Renal	24 M			
			Bd Wt	24 M			

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
20	Mouse (B6C3F1)	14 d (F)	Resp	717 M			NTP 1993 copper sulfate
			Cardio	717 M			
			Gastro	92 M	197 M (hyperplasia of forestomach mucosa)		
			Hepatic	717 M			
			Renal	717 M			
			Bd Wt	717 M			
	INTERMEDIATE EXPOSURE						
	Systemic						
21	Human	daily 2 months (W)	Gastro	0.042[c]	0.091 (gastrointestinal symptoms)		Araya et al. 2003b copper sulfate
			Hepatic	0.17			

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
22	Human	9 months (W)	Gastro	0.319			Olivares et al. 1998 copper sulfate
			Hepatic	0.319			
			Bd Wt	0.319			
23	Human	12 wks (C)	Gastro	0.14			Pratt et al. 1985 copper gluconate
			Hemato	0.14			
			Hepatic	0.14			
24	Rat (Fischer- 344)	3 mo (F)	Hepatic	66 M	89 M (increased number of necroinflammatory foci in the liver)		Aburto et al. 2001b copper sulfate
			Bd Wt	114 M	140 M (15% decreased in terminal body weight)		

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
25	Rat (Sprague-Dawley)	30-58 d (F)	Hepatic	20 F			Cristofori et al. 1992 NS
			Renal	20 F			
26	Rat (Sprague-Dawley)	90 d (W)	Hepatic		8 M (increased aspartate aminotransferase activity)		Epstein et al. 1982 copper acetate
			Bd Wt	8 M			
27	Rat (Fischer- 344)	18 wks (F)	Hepatic		150 M (inflammation and increased serum enzyme activity in adult rats)		Fuentealba et al. 2000 copper sulfate
					120 M (inflammation, necrosis, and increases serum enzyme levels in young rats)		
28	Rat (NS)	3-15 wk (F)	Hepatic		180 M (necrosis)		Haywood 1980 copper sulfate
			Renal		180 M (cytoplasmic droplets and desquamation of epithelial cells in proximal tubules)		

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
29	Rat (Wistar)	2-15 wk (F)	Hepatic		280 M (inflammation, necrosis)	550 M (chronic hepatitis)	Haywood 1985 NS
			Renal		280 M (degeneration of proximal tubule cells)		
			Bd Wt			550 M (weight loss)	
						280 M (50% decrease in body weight gain)	
30	Rat (NS)	3-15 wk (F)	Hepatic		180 M (increased alanine aminotransferase activity)		Haywood and Comerford 1980 copper sulfate
31	Rat (Wistar)	15 wk (F)	Hepatic		320 M (necrosis)	640 M (chronic hepatitis)	Haywood and Loughran 1985 copper sulfate
			Bd Wt			640 M (weight loss)	
						320 M (50% decrease in body weight gain)	

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
32	Rat (Wistar)	4-14 wks (F)	Hepatic		280 M (hepatocellular necrosis)		Haywood et al. 1985a NS
			Renal		280 M (tubular cell necrosis)		
33	Rat (Wistar)	4-15 wk (F)	Renal		200 M (reversible degeneration and necrosis of tubule cells)		Haywood et al. 1985b NS
34	Rat (NS)	30 d (G)	Hemato		100 M (decreased erythrocyte and hemoglobin levels)		Kumar and Sharma 1987 copper sulfate
			Hepatic		100 M (increased glucose, cholesterol, bilirubin, serum enzymes, and decreased total protein levels)		
			Renal		100 M (increased BUN levels)		
35	Rat (Wistar)	15 wks (F)	Cardio		14 M (increased blood pressure)		Liu and Medeiros 1986 copper carbonate

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
36	Rat (Holtzman)	21 wks (F)	Musc/skel	120 M			Llewellyn et al. 1985 copper acetate
			Bd Wt		120 (23% decrease in body weight gain)		

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
37	Rat (Fischer- 344)	13 wk (F)	Resp	134 F			NTP 1993 copper sulfate
			Cardio	134 F			
			Gastro	16 M	33 M (squamous mucosa hyperplasia of forestomach)		
			Hemato	33 M	66 M (decreases in hematocrit, hemoglobin, reticulocytes, mean cell volume, and mean cell hemoglobin levels and increases in platelet levels)		
			Hepatic	8 M	66 M (chronic active inflammation with focal necrosis)		
					16 M (increases serum alanine aminotransferase)		
			Renal	9 F	17 F (increased BUN)	134 F (tubular degeneration)	
			Bd Wt	66 M	140 M (24% decrease in body weight gain)		

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
38	Rat (NS)	20 d (G)	Hemato		100 M (decreases in erythrocyte, hemoglobin, and hematocrit levels)		Rana and Kumar 1980 copper sulfate
			Hepatic		100 M (hepatocellular necrosis)		
			Renal		100 M (tubular cell necrosis)		
39	Mouse (B6C3F1)	13 wk (F)	Resp	814 M			NTP 1993 copper sulfate
			Cardio	814 M			
			Gastro	126 F	267 F (hyperplasia of forestomach mucosa)		
			Hepatic	814 M			
			Renal	814 M			
			Bd Wt	187 M	398 M (12% decrease in body weight gain)		

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
40	Pig (Hampshire)	54 d (F)	Hemato	11	24 (decreased hemoglobin levels)		Kline et al. 1971 copper sulfate
			Bd Wt	11	24 (decreased body weight gain)		
41	Pig (NS)	49 d (F)	Hemato		36 F (decreased hemoglobin levels)		Suttle and Mills 1966a copper carbonate
			Hepatic		36 F (increased asparate aminotransferase activity)		
42	Pig (NS)	6 wks (F)	Hemato		35 F (decreased hemoglobin level)		Suttle and Mills 1966a copper carbonate
			Hepatic		35 F (increased aspartate aminotransferase activity)		
	Immuno/ Lymphoret						
43	Mouse (C57BL/6N)	8 wks (W)			24 (impaired immune function)		Pocino et al. 1990 copper sulfate
44	Mouse (C57BL/6N)	3-5 or 8-10 wks (W)			13 (altered cell-mediated and humoral immunity)		Pocino et al. 1991 copper sulfate

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Neurological						
45	Rat (Sprague-Dawley)	11 mo (W)			36 F (decreased 3,4-dihydroxyphenylacetic acid levels in corpus striatum)		DeVries et al. 1986 copper sulfate
46	Rat (NS)	30 d (F)		23			Murthy et al. 1981 copper sulfate
	Reproductive						
47	Rat (Fischer- 344)	13 wk (F)		66 M 68 F			NTP 1993 copper sulfate
48	Mouse (B6C3F1)	13 wk (F)		398 M 536 F			NTP 1993 copper sulfate
49	Mink (dark mink)	153 or 367 d (F)		12			Aulerich et al. 1982 copper sulfate

Table 3-2 Levels of Significant Exposure to Copper - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Developmental						
50	Rat (Wistar)	60-73 d (W)			130 (delayed growth and development)		Haddad et al. 1991 copper acetate
51	Mouse (C57BL/6N)	1 mo + gd 0-19 (F)		138 F	208 (decreased mean litter size and fetal body weights)		Lecyk 1980 copper sulfate
52	Other (dark mink)	153 or 367 d (F)		13			Aulerich et al. 1982 copper sulfate
	CHRONIC EXPOSURE						
	Death						
53	Mouse (C57BL/6N)	850 d (W)				4.2 (14.7% decrease in lifespan)	Massie and Aiello 1984 copper gluconate
	Systemic						
54	Mouse (C57BL/6N)	850 d (W)	Bd Wt	42 M			Massie and Aiello 1984 copper gluconate

a The number corresponds to entries in Figure 3-2.

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.01 mg Cu/kg/day; the NOAEL was divided by an uncertainty factor of 3 to account for human variability.

c Used to derive an intermediate-duration minimal risk level (MRL) of 0.01 mg Cu/kg/day; the NOAEL divided by an uncertainty factor of 3 to account for human variability.

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = Female; G = gavage; Gastro = gastrointestinal; gd = gestational day; Gn pig = guinea pig; hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; occup = occupational; NS = not specified; Resp = respiratory; (W) = drinking water; wk = week(s)

Figure 3-2. Levels of Significant Exposure to Copper - Oral

Acute (≤14 days)

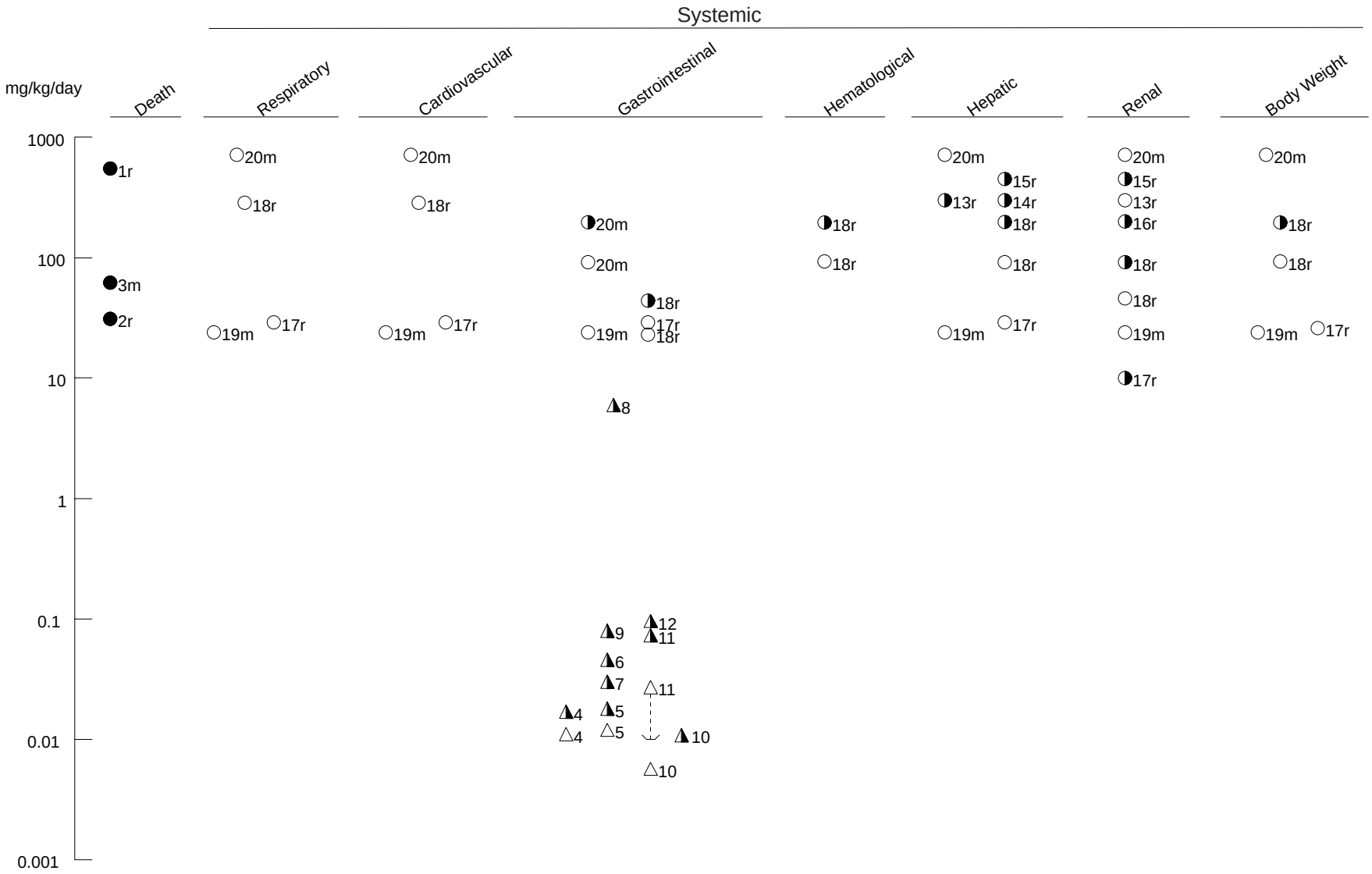


Figure 3-2. Levels of Significant Exposure to Copper - Oral (*Continued*)

Intermediate (15-364 days)

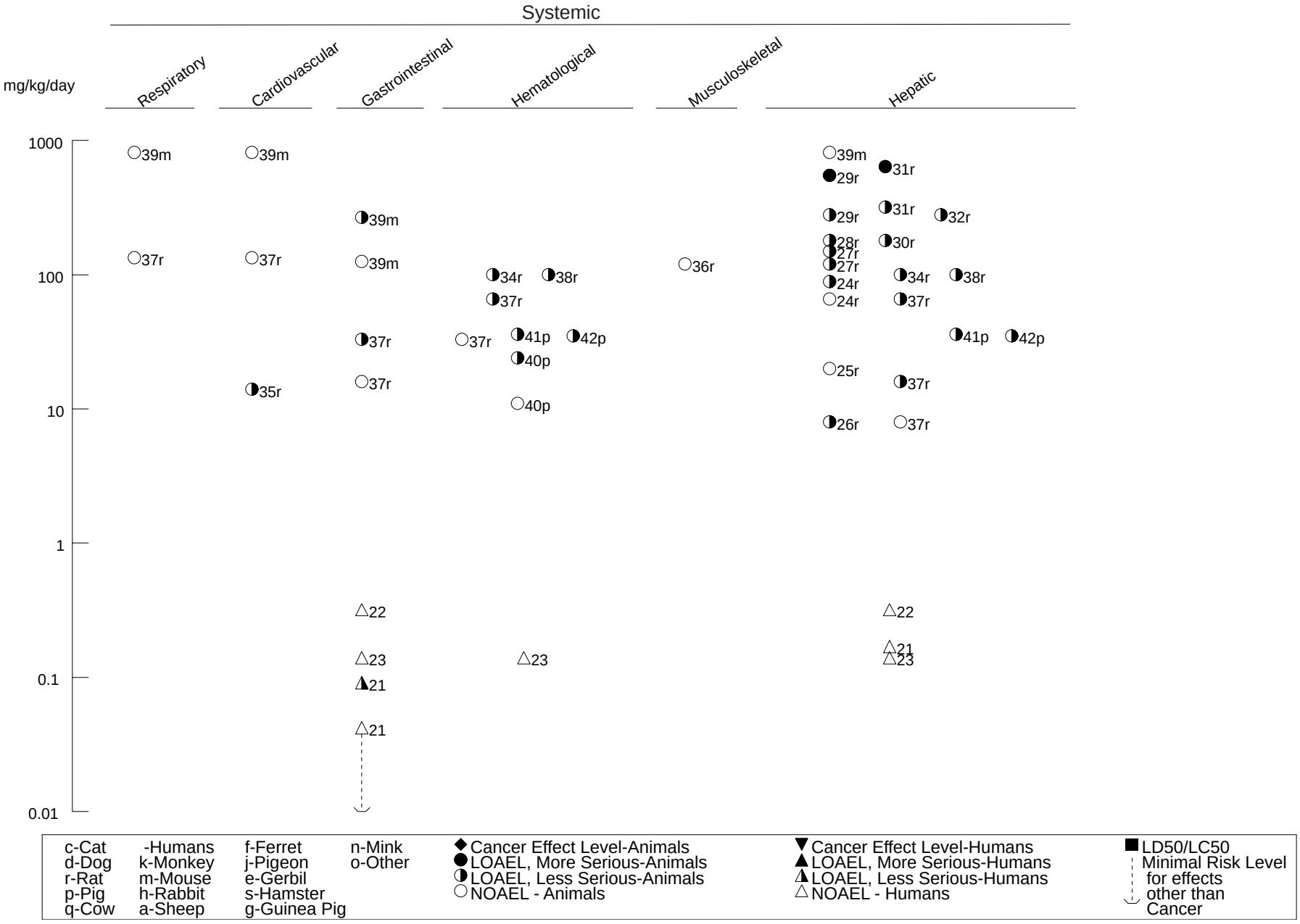


Figure 3-2. Levels of Significant Exposure to Copper - Oral (*Continued*)

Intermediate (15-364 days)

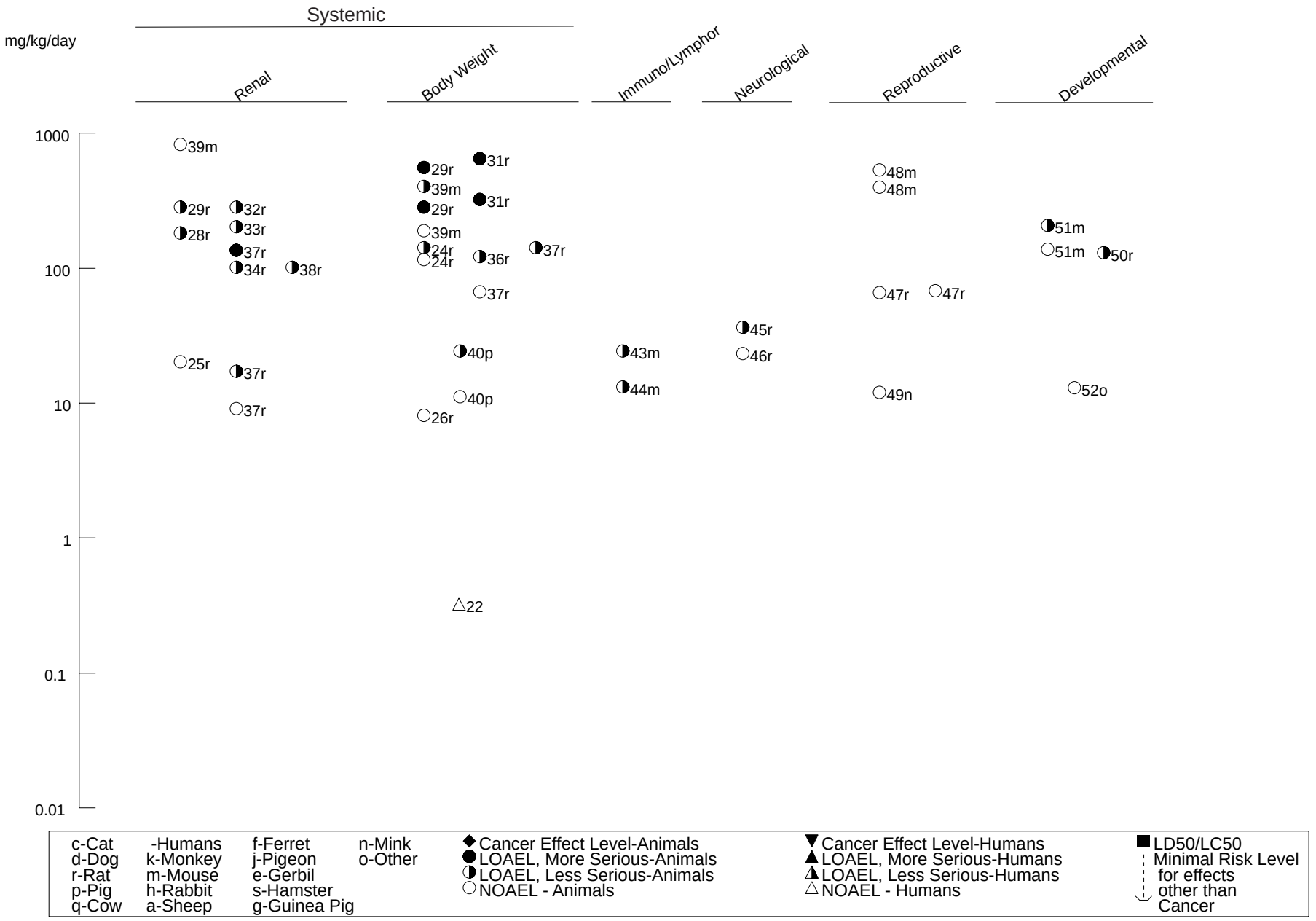
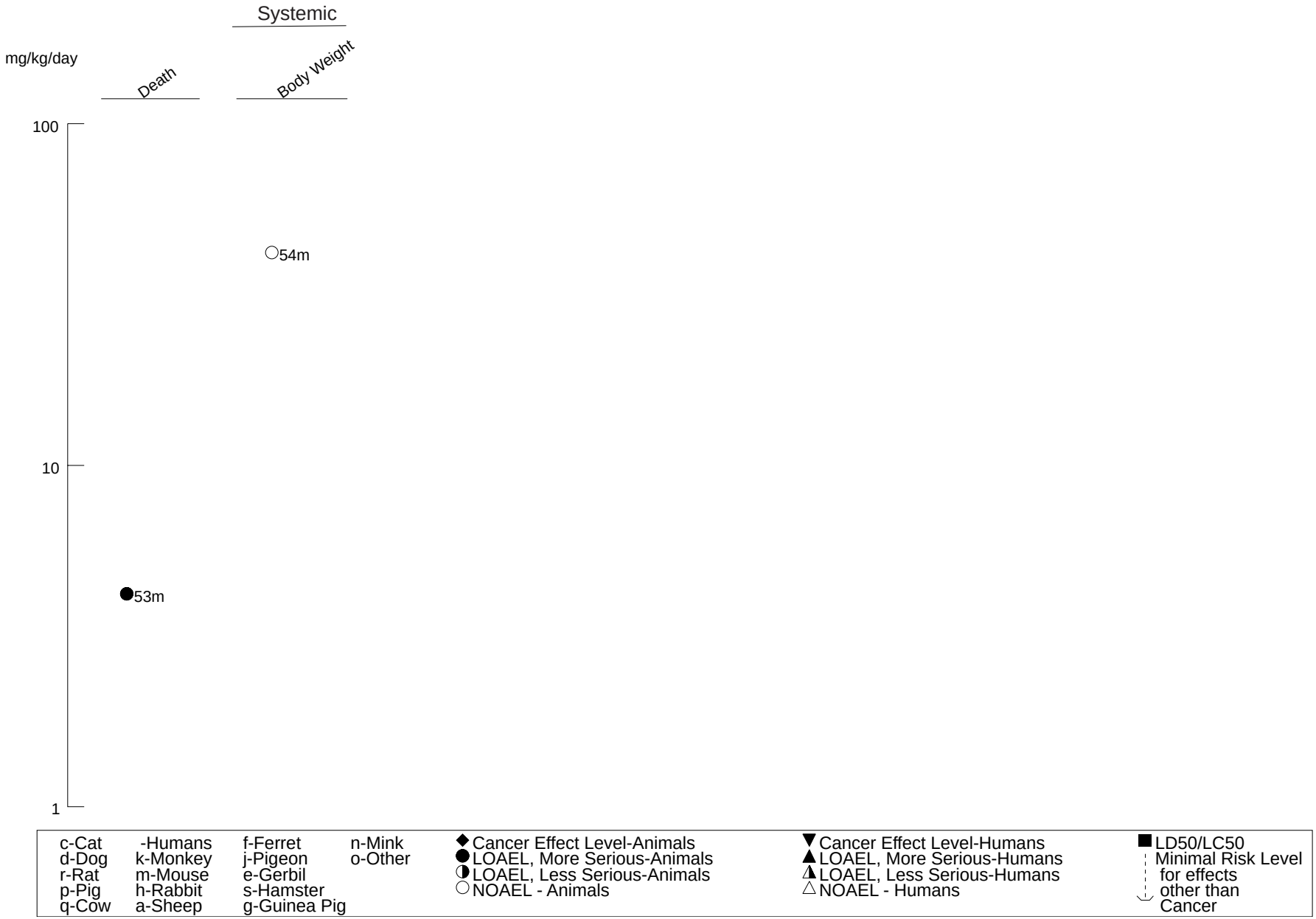


Figure 3-2. Levels of Significant Exposure to Copper - Oral (*Continued*)

Chronic (≥365 days)



Cardiovascular Effects. Several human studies have examined the possible relationship between increased serum copper levels and an increased risk of coronary heart disease. Although a number of studies have found increased risk of coronary heart disease deaths with increasing serum copper levels (Ford 2000), a number of studies have not found a relationship. However, whether copper directly affects atherosclerosis or is a marker of inflammation associated with atherosclerosis remains to be established.

There are limited data on the toxicity of copper to the cardiovascular system. A significant increase in systolic blood pressure was observed in rats exposed to 14 mg Cu/kg/day as copper carbonate in the diet for 15 weeks (Liu and Mederios 1986). No histological alterations were observed in the hearts of rats or mice exposed to 285 or 717 mg Cu/kg/day, respectively, for 14 days or 134 or 814 mg Cu/kg/day for 90 days (NTP 1993).

Gastrointestinal Effects. There are numerous reports of acute gastrointestinal effects in humans after ingestion of large amounts of copper in drinking water or beverages. The most prevalent effects are nausea and vomiting, which typically occur shortly after ingestion and are not persistent (Araya et al. 2001, 2003a, 2003b, 2003c; Chuttani et al. 1965; Eife et al. 1999; Gill and Bhagat 1999; Gotteland et al. 2001; Holleran 1981; Jantsch et al. 1984, 1985; Karlsson and Noren 1965; Knobeloch et al. 1994, 1998; Nicholas and Brist 1968; Olivares et al. 2001; Pizarro et al. 1999, 2001; Semple et al. 1960; Spitalny et al. 1984; Walsh et al. 1977). Abdominal pain and diarrhea have also been reported, but their incidence is typically much lower than nausea and vomiting. Although most of the data on gastrointestinal effects in humans come from case reports of accidental exposure from contaminated beverages with limited information on exposure levels, several recently conducted studies were designed to identify the threshold for gastrointestinal effects. These experiments typically involve adults ingesting a single dose of copper sulfate following an overnight fast (Araya et al. 2001, 2003a, 2003b; Gotteland et al. 2001; Olivares et al. 2001). Olivares et al. (2001) identified the lowest LOAEL for gastrointestinal effects; a significant increase in the incidence of nausea was observed at 4 ppm copper (0.01 mg Cu/kg) and higher. At 6 ppm, a significant increase in the incidence of vomiting was also observed. Administering the copper sulfate in an orange-flavored drink increased the threshold for nausea to 8 ppm (0.022 mg Cu/kg) (Olivares et al. 2001). In two multinational studies conducted by Araya and associates (Araya et al. 2001, 2003a), NOAEL and LOAEL values of 4 and 6 ppm (0.042 and 0.091 mg Cu/kg), respectively, were identified for nausea. Araya et al. (2003a) determined that both the copper concentration and the total copper dose are important variables in predicting a response; as the concentration and dose increase, the probability of eliciting nausea increases.

Similar thresholds for effects were observed in repeated exposure studies (Araya et al. 2003c; Pizarro et al. 1999, 2001). Abdominal pain, nausea, and/or vomiting were observed in women drinking water containing 5 ppm (0.096 mg Cu/kg) copper sulfate or copper oxide for 1 week (Pizarro et al. 2001). The occurrence of gastrointestinal effects (excluding diarrhea) was not significantly different in subjects ingesting copper sulfate and those ingesting copper oxide. In a 2-week exposure study by Pizarro et al. (1999), significant increases in the incidence of gastrointestinal symptoms were observed in subjects exposed to 3 or 5 ppm (0.0731 and 0.124 mg Cu/kg/day), but not to 1 ppm (0.0272 mg Cu/kg/day). The incidences of nausea, vomiting, and/or abdominal pain were 5, 2, 17, and 15% in the control, 1, 3, and 5 ppm groups, respectively. In a similarly designed study, Araya et al. (2003b) examined the occurrence of gastrointestinal symptoms in adults exposed to copper sulfate for 2 months. The incidences of gastrointestinal symptoms were 11.7, 15.3, 18.3, and 19.7% in the control, 2, 4, and 6 ppm groups, respectively. As analyzed using the chi-square test with Bonferroni correction, the incidence was significantly elevated in the 6 ppm (0.17 mg Cu/kg/day) group; if the Bonferroni correction was not used, the incidence was significantly elevated in the 4 ppm (0.091 mg Cu/kg/day) group. A case report by Spitalny et al. (1984) also examined the effects of repeated exposure to copper. Recurrent, acute symptoms, including nausea, vomiting, and abdominal pain, were reported by three of four family members shortly after drinking juice, coffee, or water in the morning. The effects disappeared when the family switched to bottled water. An early morning water sample contained 7.8 ppm copper. A study by Buchanan et al. (1991) also examined individuals with elevated levels of copper in household water. The occurrence of vomiting and nausea with abdominal pain was not significantly different among residents with a first-draw water sample of 3 ppm or higher, as compared to controls with less than 1.3 ppm copper in first-draw sample. The investigators noted that in a case-control study of this population, all of the cases reported that none of the subjects obtained their water immediately from the tap, but most (70%) only let it run for less than 1 minute. The study found that copper content in the tap water used for drinking averaged 14% of first draw samples.

Most of the available human studies examined the relationship between copper exposure and the manifestation of symptoms of gastrointestinal irritation; Gotteland et al. (2001) and Araya et al. (2003c) also looked at physiological alterations. Gotteland et al. (2001) found significant increases in gastric permeability to sucrose following the bolus ingestion of 10 ppm copper as copper sulfate (0.03 mg Cu/kg); no alterations in intestinal permeability to lactulose/mannitol were found. The increased gastric permeability was independent of gastrointestinal symptoms. A significant delay in decreasing the stomach’s antral area was found during the first hour after bolus ingestion of 10 ppm copper as copper

sulfate (0.046 mg Cu/kg) (Arayaet et al. 2003c). This change in antral area is suggestive of a delay in gastric emptying. As with gastric permeability, this effect was independent of gastrointestinal symptoms.

Gastrointestinal effects have also been reported in animal studies. Hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach was observed in rats and mice exposed to 44 and 197 mg Cu/kg/day, respectively, as copper sulfate in the diet for 14 days or 33 and 267 mg Cu/kg/day, respectively, as copper sulfate in the diet for 13 weeks (NTP 1990a). No gastrointestinal effects were observed in rats and mice exposed to 23 or 92 mg Cu/kg/day for 14 days or in rats and mice exposed to 16 or 126 mg Cu/kg/day 13 weeks. Additionally, no gastrointestinal effects were observed in rats and mice exposed to 29 or 24 mg Cu/kg/day as copper sulfate in drinking water (NTP 1990a).

Hematological Effects. There are limited data on the effect of copper on the human hematological system. Acute hemolytic anemia was observed in an 18-month-old child 2 days after he drank a solution containing approximately 3 g of copper sulfate (Walsh et al. 1977). Acute intravascular hemolysis was also reported in 5 of 125 individuals intentionally ingesting a large dose of copper sulfate (Ahasan et al. 1994). No alterations in hematocrit level or mean corpuscular volume were observed in individuals ingesting 0.14 mg Cu/kg/day as copper gluconate in a capsule for 12 weeks (Pratt et al. 1985).

Information on the hematological effects in animals associated with exposure to high levels of copper is also limited to several studies that measured hemoglobin and hematocrit values. Decreased hemoglobin and hematocrit values were observed in rats exposed to ≥66 mg Cu/kg/day (Kumar and Sharma 1987; NTP 1993; Rana and Kumar 1980) for 20–90 days and in pigs exposed to ≥24 mg Cu/kg/day for 48–54 days (Kline et al. 1971; Suttle and Mills 1966a, 1966b). Depletion of hematopoietic cells in the bone marrow was observed in rats exposed to 196 mg Cu/kg/day as copper sulfate in the diet for 14 days (NTP 1993). Contrary to these findings, Liu and Medeiros (1986) observed an increase in hemoglobin levels and no change in hematocrit levels in rats fed a diet containing 14 mg Cu/kg/day as copper carbonate for 20 weeks.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans following oral exposure to copper.

Equivocal results on the effects of copper on the musculoskeletal system have been found. Depressed skeletal growth has been observed in rats administered 100 mg Cu/kg/day via gavage; tail length was used

to assess skeletal growth (Rana and Kumar 1980). Using radiographic data, no qualitative or quantitative differences were observed in bones of rats exposed to 120 mg Cu/kg/day as copper acetate in the diet for 21 weeks (Llewellyn et al. 1985). The different outcomes may reflect the different methods used to assess skeletal growth.

Hepatic Effects. With the exception of several defined syndromes—Wilson's disease, Indian childhood cirrhosis, and idiopathic copper toxicosis—liver effects are rarely reported in humans, although this has not been extensively investigated. In a compilation of case reports of individuals intentionally ingesting copper sulfate, jaundice was reported in 11 of 53 individuals (Chuttani et al. 1965). Centrilobular necrosis, biliary stasis, elevated serum bilirubin level and aspartate aminotransferase activity, and elevated bile salts in the urine were found in five of the individuals with jaundice. Jaundice (Akintonwa et al. 1989), centrilobular congestion (Lamont and Duflou 1988), and acute hepatotoxicity (Ahasan et al. 1994) have also been reported in case reports of lethal ingestion of copper sulfate. O'Donohue et al. (1993) reported a case of an adult with jaundice and hepatomegaly following a 3-year exposure to copper supplements. For 2 years, the individual ingested 30 mg/day followed by 1 year of 60 mg/day. In a study of seven adults receiving capsules containing 0.14 mg Cu/kg/day as copper gluconate, no significant alterations in serum aspartate aminotransferase, alkaline phosphatase, serum gamma glutamyl transferase, or lactate dehydrogenase activities were found (Pratt et al. 1985). No alterations in biomarkers of liver damage (serum aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase) were observed in adults exposed to 0.17 mg Cu/kg/day as copper sulfate in drinking water for 2 months (Araya et al. 2003b).

Several studies have examined liver function in infants exposed to elevated levels of copper in drinking water. A no adverse effect level for liver effects was identified in a study of infants (3 months of age at study initiation) exposed to 0.315 mg Cu/kg/day as copper sulfate in drinking water for 9 months (Olivares et al. 1998). No alterations in total bilirubin levels or serum alanine aminotranferase, aspartate aminotransferase, or gamma-glutamyl transferase activities were found. A higher percentage of copper-exposed infants (30.4%) were withdrawn from the study, as compared to the control group (11.1%). The reasons for being withdrawn from the study were blood sampling refusal (eight infants in the copper group and two infants in the control group), protocol transgression (four infants in the copper group and no infants in the control group), and change of address (five infants in the copper group and one infant in the control group). Two recent surveys of infants exposed to 0.8 mg Cu/L in household water did not find significant alterations in serum parameters of liver function or alterations in liver ultrasound imaging tests (Zietz et al. 2003a, 2003b).

There is strong evidence to suggest that Wilson's disease, Indian childhood cirrhosis, and possible idiopathic copper toxicosis are caused by an increased genetic susceptibility to copper toxicity.

Wilson's Disease. Wilson's disease is an autosomal recessive genetic disorder with a worldwide occurrence of 1 in 30,000 to 1 in 100,000 depending on the population (Llanos and Mercer 2002; Scheinberg and Sternlieb 1996). It is characterized by high levels of copper in the liver and low levels of serum ceruloplasmin. The accumulation of copper in the liver is due to a genetic defect in one of the Cu-ATPases (ATP7B), resulting in impaired biliary excretion of copper. One of the early manifestations of the disease, typically at 8–12 years of age, is liver damage. Three types of liver damage are seen—cirrhosis, chronic active hepatitis, and fulminant hepatic failure. It is unlikely that the manifestation of Wilson's disease is related to exposure to high levels of copper; high levels of hepatic copper have been observed in affected individuals consuming average copper intakes (Scheinberg and Sternlieb 1996).

Indian Childhood Cirrhosis (ICC). ICC is a type of cirrhosis typically seen in infants and young children (6 months to 5 years of age with a mean of 18 months) living in rural areas of the Indian subcontinent. Other features include high rates of parental consanguinity and up to 22% of siblings affected (Pandit and Bhave 1996; Tanner 1998). Two of the most discriminatory features of ICC are coarse, dark brown orcein staining (representing copper) and intralobular pericellular fibrosis (Pandit and Bhave 1996). Liver copper levels ranging from 790 to 6,654 µg/g dry weight (mean of 939 µg/g) were found in 53 children diagnosed with ICC, as compared to levels of 8–118 µg/g (mean 42–45 µg/g) in 12 controls aged 6 months to >1 year (Bhave et al. 1982); interpretation of these study results is limited by the small number of controls and the lack of detail on the control group.

In a study of 100 children with ICC and 100 age-, sex-, and caste-matched controls, it was determined that ICC was attributable to the early introduction of cow or buffalo milk feeds contaminated with copper from brass vessels, which were used to store and heat the milk (Bhave et al. 1987). Although a cause and effect relationship between high copper intake and ICC has not been firmly established, there is strong evidence to support an association. In another study in which the parents of 100 children with ICC were advised to use aluminum or stainless steel vessels for preparing infant milk feeds, only 1 of 86 younger siblings of the children with ICC developed ICC (this child was known to have received copper-contaminated milk) as compared to 30 of 125 older siblings (Tanner 1998).

Idiopathic Copper Toxicosis (ICT). Although there are limited data on ICT, it is also believed to be caused by an autosomal-recessive inherited defect in copper metabolism and excess dietary copper

(Müller et al. 1998; Wijmenga 2002). In the literature, ICT is also referred to as Indian childhood cirrhosis-like liver disease, copper-associated liver disease in childhood, and Tyrollean infantile cirrhosis. In the last 25 years, there have been <200 cases of ICT reported in a number of countries including Australia, Austria, Germany, Ireland, Italy, Kuwait, Mexico, United Kingdom, and United States. With the exception of a study of ICT in 138 children living in Tyrol Austria (Müller et al. 1996), most papers describe the clinical course of 1–4 children. Compiling the data from these studies, Müller et al. (1998) found a number of patterns: (1) the age of onset of clinical symptoms occurs before the age of 2 years (infantile onset) or before the age of 5 years (late onset), although onset as late as 10 years has also been observed; (2) rapid progression and death within 2 weeks to 11 months; (3) very high copper levels in the liver, 190–3,360 µg/g dry weight (normal is <50 µg/g); (4) abnormal biochemical markers of liver damage such as aminotransferases, alkaline phosphatase, bilirubin, albumin, and prothrombin time; and (5) marked panlobular and pericellular fibrosis associated with an usually mild inflammatory infiltrate, ballooning degeneration of hepatocytes, and an abundance of Mallory bodies. The high levels of copper in the liver, the identification of environmental copper exposure, and the similarity of the clinical presentation and histopathology with ICC suggest that copper is the causative agent. As with ICC, an increased genetic susceptibility to copper toxicity has been suggested. A genealogic investigation conducted by Müller et al. (1996) provided suggestive evidence that the disease is transmitted in an autosomal recessive mode.

The hepatotoxicity of copper in animals has been described and investigated in a number of acute- and intermediate-duration oral exposure studies. The majority of these studies used rats; a small number of studies used pigs and mice. In addition to these studies, there are a number of studies in animals with similar genetic defects as Wilson's disease, including Long Evans Cinnamon (LEC) rats and Bennington terrier dogs. The results of these studies were not considered relevant to healthy humans and will not be discussed. The earliest symptoms of hepatotoxicity in rats orally exposed to copper are increases in serum chemistry enzymes, particularly alanine aminotransferase and asparate aminotransferase (Epstein et al. 1982; Fuentealba et al. 2000; Haywood 1980; Haywood and Comerford 1980; Kumar and Sharma 1987; NTP 1993; Sugawara et al. 1995). Continued exposure or exposure to higher concentrations can result in inflammation, parenchymal cell hypertrophy, and hepatocellular necrosis (Aburto et al. 2001b; Fuentealba et al. 2000; Haywood 1980, 1985; Haywood and Loughran 1985; Haywood et al. 1985a; NTP 1993). At very high doses, chronic hepatitis (Haywood 1985; Haywood and Loughran 1985) has also been observed.

Studies in rats provide information on the dose-response relationships as a function of exposure duration. The highest NOAEL and lowest LOAEL for liver effects in acutely exposed rats are 92 and 198 mg Cu/kg/day, respectively, administered as copper sulfate in the diet for 14 days (NTP 1993). Chronic inflammation was observed at the LOAEL; a LOAEL for serum chemistry changes was not identified in the available acute exposure studies because the only study testing low doses (NTP 1993) did not assess this parameter. The threshold for hepatoxicity in rats following intermediate-duration exposure appears to be between 8 - 16 mg Cu/kg/day. NTP (1993) found a significant increase in serum alanine aminotransferase activity in Fischer 344 rats exposed to 16 mg Cu/kg/day as copper sulfate in the diet for 13 weeks; no effects were observed at 8 mg Cu/kg/day. However, Epstein et al. (1982) found a significant increase in asparate aminotransferase in Sprague-Dawley rats exposed to 8 mg Cu/kg/day as copper sulfate in drinking water for 90 days; differences in the exposure route and rat strain may have contributed to these differences. Histological damage (chronic active inflammation and focal hepatocellular necrosis) has been observed at 66 mg Cu/kg/day (administered as copper sulfate in the diet for 90 days) and higher (NTP 1993). Severe hepatic damage (chronic hepatitis) has been observed in rats exposed to >550 mg Cu/kg/day as copper sulfate in the diet for 15 weeks (Haywood 1985; Haywood and Loughran 1985).

The available rat hepatoxocity data, along with toxicokinetic data, suggest that there are three phases of copper toxicity in the rat. In the first phase, copper levels increase in the liver, with minimal to no damage to hepatic tissues. As the hepatic copper levels increase, inflammation and necrosis occur. Thereafter, the copper levels in the liver begin to decrease and the parenchymal tissue begins to regenerate. At this point, the animal develops a tolerance to copper. Haywood et al. (1985a) speculated that the tolerance resulted from a shift in the liver from copper storage and biliary excretion to copper transport and renal clearance. Tolerance appears to protect the animals from subsequent liver toxicity. For example, no adverse liver effects were observed in rats exposed to 640 mg Cu/kg/day as copper sulfate in the diet when this exposure was preceded by a 15-week exposure to 320 mg Cu/kg/day as copper sulfate in the diet. This is in contrast to the severe hepatocellular necrosis that was observed in animals exposed to a control diet for 15 weeks followed by a 3-week exposure to 640 mg Cu/kg/day (Haywood and Loughran 1985). The time course of each phase of liver toxicity appears to be dose-related. At higher doses, the onset of the necrosis and regeneration occurred earlier as compared to lower doses. Additionally, there appears to be an upper limit of copper intake, which would induce copper tolerance; doses that exceed this level would result in permanent damage to the liver. Dietary exposure of rats to ≥550 mg Cu/kg/day as copper sulfate for 15 weeks resulted in chronic hepatitis with no evidence of regeneration of parenchymal tissue (Haywood and Loughran 1985).

There are limited experimental data on the hepatoxocity of copper in other animal species. Pigs fed a diet providing 35–36 mg Cu/kg/day for 7 weeks had a significant increase in aspartate aminotransferase activities (Suttle and Mills 1966a, 1966b). It appears that rats and pigs are equally sensitive to high levels of copper in the diet or drinking water. In contrast, mice do not appear to be as sensitive to the hepatic toxicity of copper as rats. No hepatic effects were observed in mice exposed to 814 mg Cu/kg/day for 13 weeks as compared to rats, which exhibited an increase in alanine aminotransferase activity at 16 mg Cu/kg/day and chronic active inflammation at 66 mg Cu/kg/day (NTP 1993).

Renal Effects. There is limited information on the renal toxicity of copper in humans. Congestion of the glomeruli and denudation of tubular cells were observed in four individuals consuming a single lethal dose of copper sulfate (Chuttani et al. 1965). Acute renal failure was reported in 5 of 125 individuals intentionally ingesting large doses of copper sulfate (Ahasan et al. 1994). Hematuria, glycosuria, cylindruria, and proteinuria, indicative of renal tubular damage, were observed in a child who drank a solution containing approximately 3 g of copper sulfate (Walsh et al. 1977).

A number of animal studies confirm that the kidney is a target of copper toxicity. Renal toxicity as a result of copper loading follows a specific time course (Haywood 1980, 1985; Haywood et al. 1985a, 1985b). No treatment-related effects were observed in rats exposed to 300 mg Cu/kg/day as copper sulfate in the diet for 1–2 weeks (Haywood 1980). However, eosinophilic droplets were observed in the epithelial cell cytoplasm of the proximal convoluted tubules in rats exposed to 450 mg Cu/kg/day for 2 weeks (Haywood et al. 1985a). The number of eosinophilic droplets increased with increasing duration (Haywood 1980, 1985). Exposure to 100–280 mg Cu/kg/day for 3–5 weeks resulted in necrosis and degeneration of proximal tubule cells (Haywood 1985; Haywood et al. 1985a, 1985b; Rana and Kumar 1980). After 9 weeks, extensive desquamation of the epithelial cells of the proximal convoluted tubules was evident in rats exposed to 180 mg Cu/kg/day (Haywood 1980). Complete regeneration of the proximal tubules was observed after 15 weeks of copper treatment in rats exposed to 180–280 mg Cu/kg/day (Haywood 1980, 1985; Haywood et al. 1985a, 1985b). In contrast to the Haywood and associates studies, a 13-week study by NTP (1993) did not find evidence of regeneration of renal tissue. An increase in protein droplets in epithelial cell cytoplasm and the lumen of the proximal convoluted tubules was observed in rats exposed to 10 or 92 mg Cu/kg/day as copper sulfate in drinking water or diet, respectively, for 2 weeks or to 33 mg Cu/kg/day as copper sulfate in the diet for 13 weeks. At 134 mg Cu/kg/day, karyomegaly and tubule cell degeneration were also observed. Additional renal effects observed in the intermediate-duration study included an increase in serum urea nitrogen levels in females exposed to ≥17 mg Cu/kg/day, increased urinary glucose output in males exposed to ≥66 mg

Cu/kg/day, and increased urinary aspartate aminotransferase and N-acetyl-β-glucosaminidase activities in male and female rats exposed to 140 or 134 mg Cu/kg/day, respectively. The NTP (1993) study identified a NOAEL of 9 mg Cu/kg/day. No effects were observed in mice fed a diet for 13 weeks which provided 814 mg Cu kg/day as copper sulfate (NTP 1993).

Body Weight Effects. No studies were located regarding body weight effects in humans following oral exposure to copper.

Dietary exposure studies have reported 12–24% decreases in body weight gain in rats following exposure to 120–140 mg Cu/kg/day for 2–15 weeks (Llewellyn 1985; NTP 1993), in mice following exposure to 398 mg Cu/kg/day for 13 weeks (NTP 1993), or in pigs (magnitude of decreased weight gain not reported) following exposure to 24 mg Cu/kg/day for 54 days (Kline et al. 1971). No effect levels of 66 (NTP 1993), 187 (NTP 1993), and 11 mg Cu/kg/day (Kline et al. 1971) have been reported in rats, mice, and pigs, respectively; Epstein et al. (1982) also reported no adverse effects on body weight gain in rats exposed to 8 mg Cu/kg/day in drinking water. More severe decreases in body weight gain and weight loss have also been reported (Haywood 1985; Haywood and Loughran 1985); the weight loss was reported at lethal concentrations. Only one study examined the effect of copper on body weight gain following chronic-duration exposure (lifetime exposure beginning at 58 days of age); this study found no biologically significant effect in mice exposed to 42 mg Cu/kg/day as copper gluconate in drinking water (Massie and Aiello 1984).

3.2.2.3 Immunological and Lymphoreticular Effects

Information on the immunotoxicity of copper following oral exposure is limited to two drinking water studies in which mice were exposed to several concentrations of copper sulfate for 8 weeks (Pocino et al. 1990) or copper chloride for 3–5 or 8–10 weeks (Pocino et al. 1991). In these studies, groups of mice underwent several tests to assess immune function: *in vitro* lymphoproliferative responses to *Escherichia coli* lipopolysaccharide (LPS), and concanavalin A (Con A), induction and evaluation of antibody response to sheep red blood cells, evaluation of autoantibody production, and induction and elicitation of delayed-type hypersensitivity response (only tested in the Pocino et al. 1991 study). At the lowest dose tested (13 mg Cu/kg/day as copper chloride), impaired cellular (proliferative response to LPS) and humoral (autoantibody production) immunity were observed. Impaired performance on the remaining immune function tests were observed at ≥26 mg Cu/kg/day as copper chloride (Pocino et al. 1991) or

≥24 mg Cu/kg/day as copper sulfate (Pocino et al. 1990). The LOAEL values from these studies are presented in Table 3-2 and Figure 3-2.

3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans following oral exposure to copper.

No effects on spontaneous motor activity (assessed using an actophometer), learning ability (assessed using a pole climbing chamber), or relearning capacity and memory (assessed using a Y-maze) were observed in rats fed a diet containing 23 mg Cu/kg/day as copper sulfate (Murthy et al. 1981). This study found no alterations in brain dopamine or norepinephrine levels. De Vries et al. (1986) also did not find significant alterations in corpus striatial dopamine levels in rats exposed to 36 mg Cu/kg/day as copper sulfate in drinking water for 11 months. However, a 25% decrease in a dopamine metabolite, 3,4-di-hydroxyphenylacetic acid, was found in the corpus striatum.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following oral exposure to copper.

Reproductive performance, as assessed by the length of gestation, number of kits whelped, and average kit weight, was not adversely affected in minks fed a diet containing 12 mg Cu/kg/day as copper sulfate (Aulerich et al. 1982). No other oral exposure studies examined reproductive function. The intermediate-duration study by NTP (1993) did not find any histological alterations or alterations in sperm morphology or vaginal cytology in male and female rats exposed to 66 and 68 mg Cu/kg/day, respectively, or in male and female mice exposed to 398 and 536 mg Cu/kg/day, respectively. The NOAEL values for reproductive effects are reported in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects of humans following oral exposure to copper.

There are limited data on the developmental toxicity of copper in experimental animals. Delayed growth and development were observed in the offspring of rats exposed to 130 mg Cu/kg/day as copper sulfate in

the diet for 7 weeks prior to mating and during gestation (Haddad et al. 1991). In 11.5-day-old embryos, significant decreases in mean somite number, crown-rump length, and yolk sac diameter were observed. In 21.5-day-old fetuses and newborns, delayed ossification was observed in the cervical and cauda vertebrae, sternum, metacarpals, forelimb phalanges, metatarsals, and hindlimb phalanges. Exposure of mouse dams to a higher dose, 208 mg Cu/kg/day as copper sulfate in the diet, resulted in decreased mean litter size and decreased fetal body weights; the statistical significance of these effects is not known (Lecyk 1980). No statistically significant alterations in newborn mortality or body weight were observed in the offspring of mink exposed to 13 mg Cu/kg/day as copper sulfate in the diet (Aulerich et al. 1982). There was a trend toward increased kit mortality between birth and 4 weeks of age in the offspring of mink exposed to 6 or 13 mg Cu/kg/day. The incidences were 12, 9, 19, 38, and 32% in the 1, 6, 3, 6, and 13 mg Cu/kg/day groups, respectively; the statistical significance of this effect was not reported. The NOAEL values and all reliable LOAEL values for developmental effects in each species are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located regarding carcinogenic effects in humans following oral exposure to copper.

Several oral studies have examined the carcinogenicity of copper compounds in animals. These studies did not find increases in the occurrence of tumors in mice exposed to 86 mg Cu/kg/day as the pesticide, copper 8-hydroxyquinoline (BRL 1968), liver tumors in rats exposed to 130 mg Cu/kg/day as copper acetate (Kamamoto et al. 1973), or large intestine tumors in rats exposed to 9 mg Cu/kg/day as an unspecified copper compound (Greene et al. 1987). These studies are limited in scope and it can not be determined whether the maximum threshold dose (MTD) was achieved. An increased occurrence of hepatocellular carcinomas has been reported in Long-Evans Cinnamon rats (Sawaki et al. 1994), an animal model for Wilson's disease. However, liver cancer has not been reported in individuals with Wilson's disease; thus the significance of this finding in not known.

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding death in humans and animals following dermal exposure to copper.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, dermal, or body weight effects in humans or animals following dermal exposure to copper.

Hematological Effects. Hemolytic anemia was observed in a severely burned and debilitated child in whom copper sulfate crystals were being applied to granulation tissue. Increased serum and urine copper levels were observed (Holtzman et al. 1966). Because the skin was severely damaged, this study cannot be used to predict the dermal toxicity of copper following exposure to intact skin. No studies were located regarding hematological effects in animals following dermal exposure to copper.

Ocular Effects. Eye irritation has been reported by factory workers exposed to copper dust (Askergren and Mellgren 1975). No studies were located regarding ocular effects in animals following exposure to copper.

3.2.3.3 Immunological and Lymphoreticular Effects

In some individuals, exposure to copper metal produces pruritic dermatitis. Saltzer and Wilson (1968) reported a case of a woman who had recurrent pruritus on her ring finger and wrist caused by copper metal in her ring and wristwatch. Allergic contact dermatitis has been observed in individuals following a patch test using a copper penny and/or a copper sulfate solution (Barranco 1972; Saltzer and Wilson 1968).

No studies were located regarding the following health effects in humans and/or animals after dermal exposure to copper:

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.2.4 Other Routes of Exposure

Cardiovascular Effects. A dramatic decrease in pulse pressure and heart rate was observed in New Zealand white rabbits infused with 2.5 mg Cu/kg as copper sulfate with an infusion pump in the femoral vein for 10–15 minutes (Rhee and Dunlap 1990). Systolic and diastolic pressure initially increased, then rapidly decreased.

Reproductive Effects. Intraperitoneal exposure to 0.95 or 1.4 mg Cu/kg/day for 26 days resulted in significant decreases in testes, seminal vesicle, and ventral prostate weights and in plasma testosterone levels in Wistar rats (Chattopadhyay et al. 1999); decreases in testicular Δ5-3β-hydroxysteroid dehydrogenases and 17β-hydroxysteroid dehydrogenase activities were also observed at 1.4 mg Cu/kg/day. An in vitro study (Holland and White 1988) demonstrated that cupric ions and cuprous ions decrease human spermatozoa motility.

Cancer. Several studies have examined the carcinogenicity of copper compounds following parenteral administration. No significant alterations in tumor incidence were observed in male Wistar rats receiving subcutaneous injections of 2 mg Cu/kg/day as copper acetate (Yamane et al. 1984), in male and female F344 rats receiving intramuscular injections of 0.25 or 0.41 mg Cu/kg/day as finely ground copper (Furst 1971), or in Wistar rats receiving 150 mg Cu/kg as copper oxide, 150 mg Cu/kg as copper sulfide, or 70 mg Cu/kg as copper sulfate (Gilman 1962). An increase in the occurrence of renal cell carcinoma was observed in male Wistar rats receiving 3–5 mg Cu/kg as cupric nitrilotriacetate 5 days/week for 12 weeks (Toyokuni et al. 1996); cupric nitrilotriacetate is a chelated compound of copper that is water soluble. A study by BRL (1968) found a slight, but statistically significant, increase in the incidence of reticulum cell sarcomas in mice 18 months after receiving a single subcutaneous injection of copper 8-hydroxyquinoline; the significance of this finding is not known.

3.3 GENOTOXICITY

No studies were located regarding genotoxicity in humans after inhalation, oral, or dermal exposure to copper or its compounds. Several studies have assessed the genotoxicity of copper sulfate following oral or parenteral exposure; the results of these *in vivo* genotoxicity studies are summarized in Table 3-3. Significant increases in the occurrence of micronuclei and chromosomal aberrations have been observed in chick bone marrow cells and erythrocytes (Bhunya and Jena 1996) and mouse bone marrow cells (Agarwal et al. 1990; Bhunya and Pati 1987). A study by Tinswell and Ashby (1990) did not find

Table 3-3. Genotoxicity of Copper *In Vivo*

Species (test system)	End point	Results	Reference	Compound
Drosophila melanogaster (injection into larvae)	Recessive lethals	+	Law 1938	Copper sulfate
White Leghorn chick bone marrow cells (intraperitoneal injection and oral exposure)	Chromosomal aberrations	+	Bhunya and Jena 1996	Copper sulfate
White Leghorn chick bone marrow cells (intraperitoneal injection and oral exposure)	Micronuclei	+	Bhunya and Jena 1996	Copper sulfate
White Leghorn chick erythrocytes (intraperitoneal injection and oral exposure)	Micronuclei	+	Bhunya and Jena 1996	Copper sulfate
Inbred Swiss mice bone marrow cells (intraperitoneal and/or subcutaneous injection)	Chromosomal aberrations	+	Bhunya and Pati 1987	Copper sulfate
Inbred Swiss mice bone marrow cells (intraperitoneal and/or subcutaneous injection)	Micronuclei	+	Bhunya and Pati 1987	Copper sulfate
Inbred Swiss mice (intraperitoneal injection)	Sperm abnormalities	+	Bhunya and Pati 1987	Copper sulfate
CBA mice bone marrow cells (intraperitoneal injection)	Micronuclei	–	Tinwell and Ashby 1990	Copper sulfate
Swiss mice (intraperitoneal injection)	Chromosomal aberrations	+	Agarwal et al. 1990	Copper sulfate

+ = positive results; – = negative results

increases in the number of micronuclei in mouse bone marrow cells. Increases in the occurrence of recessive lethals (Law 1938) and sperm abnormalities (Bhunya and Pati 1987) have also been observed in Drosophila and mice, respectively.

Several studies copper sulfate and copper chloride genotoxicity did not find significant increases in the occurrence of reverse mutations in *Salmonella typhimurium* (Marzin and Phi 1985; Tso and Fung 1981; Wong 1988) or *Saccharomyces cerevisiae* (Singh 1983). In contrast, Demerec et al. (1951) found an increased occurrence of reverse mutations in *Escherichia coli*. Positive results have been found in studies testing for DNA damage. Errors in DNA synthesis by viral DNA polymerase (Sirover and Loeb 1976), a reduction in DNA synthesis (Garrett and Lewtas 1983; Sirover and Loeb 1976), and an increase in the occurrence of DNA strand breaks (Sideris et al. 1988; Sina et al. 1983) have been observed. The increase in sister chromatid exchange in Chinese hamster cells (Sideris et al. 1988) is consistent with the clastogenic effects observed in *in vivo* assays. The results of these studies are summarized and are presented in Table 3-4.

3.4 TOXICOKINETICS

Physiologically normal levels of copper in the body are held constant by alterations in the rate and amount of copper absorption, compartmental distribution, and excretion.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

No studies were located regarding the rate and extent of absorption following inhalation exposure of humans to copper. There are limited data on copper absorption in animals. Copper oxide was observed in alveolar capillaries 3 hours after albino rats were exposed to a welding dust aerosol generated from pure copper wires (no additional exposure information was provided) (Batsura 1969). The half-time of copper sulfate in the lungs was estimated to be 7.5 hours after intratracheal instillation of 20 µg copper per Wistar rat (Hirano et al. 1990).

Table 3-4. Genotoxicity of Copper *In Vitro*

Species (test system)	End point	Results		Reference	Compound
		With activation	Without activation		
Prokaryotic organisms:					
Salmonella typhimurium TA102	Reverse mutation	NT	–	Marzin and Phi 1985	Copper sulfate
S. typhimurium TA98, TA102, TA1535, TA1537	Reverse mutation	–	–	Wong 1988	Copper chloride
S. typhimurium TA100	Reverse mutation	NT	–	Tso and Fung 1981	Copper chloride
Escherichia coli	Reverse mutation	NT	+	Demerec et al. 1951	Copper sulfate
Avian myeloblastosis virus, DNA polymerase	Errors in DNA synthesis	NT	+	Sirover and Loeb 1976	Copper chloride
Bacillus subtilis	rec assay	NT	–	Nishioka 1975	Copper chloride
Eukaryotic organisms:					
Fungi:					
Saccharomyces cerevisiae	Reverse mutation	NT	–	Singh 1983	Copper sulfate
S. cerevisiae	Recombination	NT	–	Sora et al. 1986	Copper sulfate
Mammalian cells:					
Chinese hamster ovary cells	DNA synthesis	NT	+	Garrett and Lewtas 1983	Copper chloride
Rat hepatocytes	DNA strand breaks	NT	+	Sina et al. 1983	Copper sulfate
Chinese hamster V79 cells	DNA strand breaks	NT	+	Sideris et al. 1988	Copper nitrate
Chinese hamster V79 cells	Sister chromatid exchange	NT	+	Sideris et al. 1988	Copper nitrate

+ = positive results; – = negative results; DNA = deoxyribonucleic acid; NT = not tested

3.4.1.2 Oral Exposure

Copper is absorbed from the stomach and small intestine; there appear to be species differences in the site of maximal absorption. The site of maximal copper absorption is not known for humans, but it is assumed to be the stomach and duodenum because of the rapid appearance of ^{64}Cu in the plasma after oral administration (Bearn and Kunkel 1955). In rats, copper is primarily absorbed from the duodenum, and to a lesser extent from the stomach (Van Campen and Mitchell 1965); in Golden hamsters, copper is primarily absorbed from the lower small intestine (25–35 cm from the pylorus) (Crampton et al. 1965).

Copper is absorbed from the gastrointestinal tract as ionic copper or bound to amino acids. Absorption of the latter apparently involves at least two kinetically distinguishable processes. The first mechanism transports copper from the mucosal side of the intestine to the serosal side. Only a small fraction of the ingested copper is transported via this mechanism (Crampton et al. 1965; Gitlan et al. 1960). The second mechanism of copper absorption involves the delivery of copper to the absorptive surface, mucosal uptake and binding to metallothionein or another intestinal binding protein (Evans and LeBlanc 1976). The copper bound to metallothionein can be slowly released to the blood (Marceau et al. 1970) or is excreted when the mucosal cell is sloughed off.

A number of human studies have examined the oral absorption of ^{64}Cu; the average absorption efficiencies ranged from 24 to 60% in presumably healthy adults (Jacob et al. 1987; Johnson et al. 1988b; Strickland et al. 1972; Turnlund et al. 1982, 1983, 1985, 1988a; 1988b; 1989; Weber et al. 1969).

Numerous factors may affect copper absorption. These factors include: the amount of copper in the diet (Farrer and Mistilis 1967; Strickland et al. 1972; Turnland et al. 1989), competition with other metals, including zinc, iron, and cadmium (Davies and Campbell 1977; Hall et al. 1979; Haschke et al. 1986; Hoogenraad et al. 1979; Prasad et al. 1978; Turnland et al. 1988a) and age (Varada et al. 1993). The absorption of copper appears to be inversely related to the amount of copper in the gastrointestinal tract (Strickland et al. 1972; Turnland et al. 1989). In a study of 11 young men administered various copper doses in food over a period of 42–98 days, absorption efficiencies of 55–56, 36, and 12% were found at doses of 0.785, 1.68, and 7.53 mg/day, respectively (Turnland et al. 1989). In humans, the amount of stored copper does not appear to influence copper absorption (Strickland et al. 1972). In rats, the absorption of copper appears to be inversely related to the amount of cadmium in the diet (Davies and Campbell 1977). A significant decrease in copper absorption was observed when the copper:cadmium

ratio was 1:4. The amount of copper retained in the intestinal mucosal cells was also inversely related to cadmium dietary concentration. In addition, increased levels of zinc in the diet result in decreased in copper absorption in humans and rats (Hall et al. 1979; Hoogenraad et al. 1979; Prasad et al. 1978). Turnland et al. (1988a) found that diets low in zinc (below the dietary requirement) decreased copper absorption in humans; 48.1% of radiolabeled copper was absorbed when the diet contained 1.3 mg copper and 16.5 mg zinc (dietary requirement is 15 mg zinc), and 37.2–38.5% of radiolabelled copper was absorbed when the diet contained 1.3 mg copper and 5.5 mg zinc. A decrease in copper absorption has been observed in infants with high intakes of iron (Haschke et al. 1986). Apparently conflicting results have been reported on the effect of ascorbic acid on copper absorption in humans. Based on a decrease in serum ceruloplasmin levels, Finley and Cerklewski (1983) concluded that a diet high in ascorbic acid resulted in a decrease in copper status. In a study by Jacob et al. (1987), copper absorption was not affected by a high ascorbic acid intake. A decrease in serum ceruloplasmin activity was also found; however, the amount of ceruloplasmin protein was not affected.

Studies in humans and animals provide suggestive evidence of age-related changes in copper absorption. Varada et al. (1993) found that copper absorption was linear and nonsaturable in suckling (16 days of age) and weanling (21–22 days of age) rats. In contrast, copper absorption was saturable in adolescent rats (6 weeks of age). The levels of copper retained in the intestine were greater in the suckling rats than in the weanling or adolescent rats. However, the increased retention was not related to increased metallothionen levels; the levels of metallothionen (after zinc induction) were higher in the adolescent rats compared to the younger rats. A linear relationship between copper intake and retention was also found in a balance study of infants (aged 2–16 weeks) (Dörner et al. 1989). Olivares et al. (2002) did not find significant differences in copper absorption between 1-month-old and 3-month-old infants. The relatively small range of doses used in this study does not allow for a determination of whether copper absorption is saturable in infants. Several studies of adults did not find differences in copper absorption between male and female adults aged 20–83 years (Johnson et al. 1992) or between elderly men (65–74 years) and young men (22–30 years) (Turnland et al. 1982, 1988b).

Human studies did not find that increased levels of fiber (α-cellulose or phytate) (Turnland et al. 1985) or ascorbic acid (Turnland et al. 1987) significantly altered copper absorption. However, a study in rats found an increase in fecal excretion of copper (and a decrease in apparent absorption) in rats fed a high fiber (potato fiber or sugar beet pulp) diet (Gralak et al. 1996). The administration of copper in infant formula or in a solution high in fulvic acid did not appear to influence copper uptake from the intestinal lumen into the intestinal mucosa of suckling rats, as compared to copper in drinking water (Lind and

Glynn 1999). However, the absorption rate of copper into the circulatory system was decreased when administered in the infant formula or fulvic acid solutions. Gender does not appear to influence copper absorption. Johnson et al. (1992) found that women aged 20–59 years absorbed more copper (66.1–74.1%) than similarly aged men (62.0–69.2%); however, when net copper absorption was normalized by body weight, no sex-related differences in absorption were found. No sex-related differences in net copper absorption were found in older (60–83 years) men and women.

3.4.1.3 Dermal Exposure

The available *in vivo* data do not provide information on the rate and extent of absorption through intact skin following dermal exposure of humans or animals to copper. Following a copper azide explosion that yielded metallic copper and nitrogen fumes, a small increase in serum copper levels was found in the affected worker (Bentur et al. 1988). Similarly, animal studies demonstrate that copper can pass through dermal barriers when applied with an appropriate vehicle, (e.g., salicylic acid or phenylbutazone) (Beveridge et al. 1984; Walker et al. 1977). *In vitro* studies suggest that copper is poorly absorbed through intact skin. Less than 6% of copper deposited on *ex vivo* human skin samples was absorbed (Pirot et al. 1996a, 1996b); copper chloride was absorbed to a higher extent than copper sulfate (Pirot et al. 1996a).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No studies were located regarding the rate and extent of distribution of copper following inhalation exposure of humans or animals.

3.4.2.2 Oral Exposure

Following ingestion of copper, copper levels in the blood rapidly rise. The copper is predominantly bound to albumin. There is some evidence that albumin plays a passive role in copper transport, carrying a large portion of the exchangeable copper in the circulation and releasing this to other carriers for actual cell-specific uptake. There is also evidence that transcuprein is another plasma protein carrier (Weiss and Linder 1985). Thus, dietary copper is transported to, and enters, the liver and kidney. Copper then reemerges into the plasma bound to the ceruloplasmin. Ceruloplasmin, which tightly binds six or seven

copper atoms (Musci et al. 1993; Saenko et al. 1994), is the most abundant copper protein in the plasma; 60–95% of the plasma copper is bound to ceruloplasmin (Harris 1993). Copper is transported from the liver to other tissues via ceruloplasmin. Ceruloplasmin does not enter the cell (Percival and Harris 1990). Copper, probably as Cu(I) rather than Cu(II) (Dameron and Harris 1989; Percival and Harris 1989), enters the cell via a carrier-mediated process. The membrane-bound copper transporting adenosine triphosphatase (Cu-ATPase), which selectively binds copper ions, transports copper ions into and out of cells (Harris et al. 1998). In most organs and tissues, copper turnover is biphasic (Levenson and Janghorbani 1994). In the plasma, the half-lives of the first and second components were 2.5 and 69 days, respectively. It is likely that the first order component is ceruloplasmin associated copper. The respective calculated copper half-lives for other tissues are 3.9 and 21 days for the liver, 5.4 and 35 days for the kidney, and 23 and 662 days for the heart; copper turnover in the brain appears to be monophasic with a half-life of 457 days.

3.4.2.3 Dermal Exposure

No studies were located regarding the rate and extent of distribution of copper following dermal exposure of humans or animals to copper.

3.4.3 Metabolism

The metabolism of copper consists mainly of its transfer to and from various organic ligands, most notably sulfhydryl and imidazole groups on amino acids and proteins. Several specific binding proteins for copper have been identified that are important in the uptake, storage, and release of copper from tissues.

In the liver and other tissues, copper is stored bound to metallothionein and amino acids and in association with copper-dependent enzymes. Several studies have shown that copper exposure induces metallothionein synthesis (Mercer et al. 1981; Wake and Mercer 1985). Increased levels of metallothionein may be associated with resistance to copper toxicity in pigs (Mehra and Bremner 1984). Ceruloplasmin is synthesized in the liver. Copper is incorporated into the molecule, and it is released from the liver. Copper exposure has also been shown to induce ceruloplasmin biosynthesis (Evans et al. 1970b; Haywood and Comerford 1980).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No studies were located regarding the rate and extent of excretion of copper following inhalation exposure of humans or animals.

3.4.4.2 Oral Exposure

Bile is the major pathway for the excretion of copper. After the oral administration of radioactive copper as copper acetate in healthy humans, 72% was excreted in the feces (Bush et al. 1955). A considerable fraction of fecal copper is of endogenous biliary origin. The remainder of the fecal copper is derived from unabsorbed copper and copper from desquamated mucosal cells. Copper in bile is associated with low molecular weight copper binding components as well as macromolecular binding species (Gollan and Dellar 1973). Reabsorption of biliary copper is negligible (Farrer and Mistilis 1967).

Normally, 0.5–3.0% of daily copper intake is excreted into the urine (Cartwright and Wintrobe 1964).

3.4.4.3 Dermal Exposure

No studies were located regarding the rate and extent of excretion of copper following dermal exposure of humans or animals to copper.

3.4.4.4 Other Routes of Exposure

Biliary excretion of copper following intravenous administration does not increase proportionally with dosage, suggesting that the hepatobiliary transport of copper is saturable (Gregus and Klaassen 1986). Thus, at high copper intakes, urinary copper excretion increases (Gitlan et al. 1960).

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological

processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-3 shows a conceptualized representation of a PBPK model.

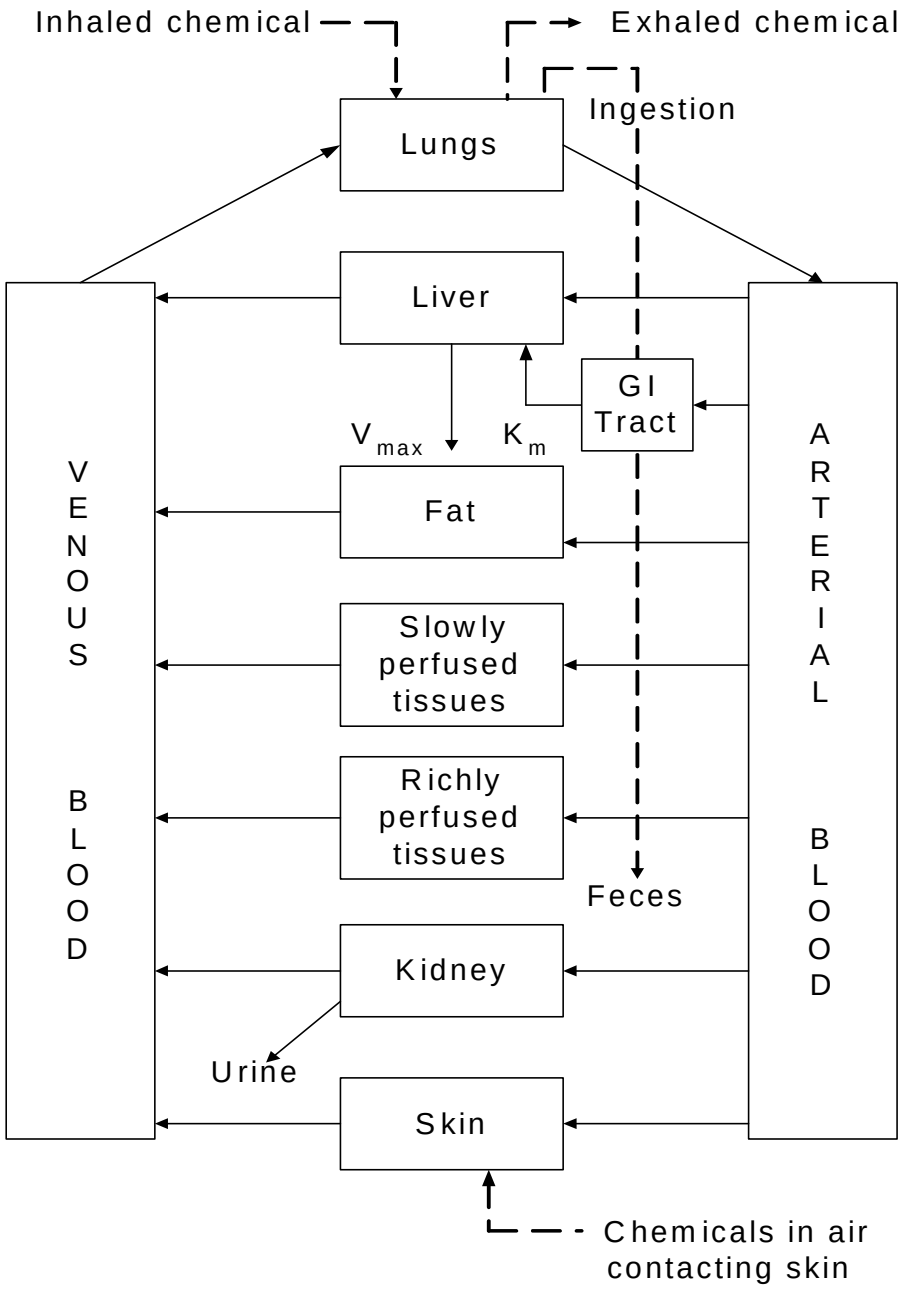
A PBPK model for copper has not been identified.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Copper is an essential element required for the normal functioning of more than 30 enzymes. The ability of copper to cycle between an oxidized state, Cu(II), and reduced state, Cu(I), is used by cuproenzymes involved in redox reactions. However, it is this property of copper that is also potentially toxic because the transitions between Cu(II) and Cu(I) can result in the generation of superoxide radicals and hydroxyl radicals (Camakaris et al. 1999). Under most circumstances, a number of homeostatic mechanisms maintain a physiologically essential concentration of copper. Copper homeostasis involves regulation of absorption, cellular uptake, intracellular transport, sequestration/storage, cellular efflux, and excretion from the body. Turnland et al. (1989) demonstrated that copper absorption from the gastrointestinal tract is inversely proportional to dietary intake; as dietary copper increases, absorption efficiency decreases. At dietary concentrations of 0.785, 1.68, and 7.53 mg/day (the recommended dietary allowance [RDA] for copper is 0.900 mg/day), 56, 36, and 12%, respectively, of the radiolabelled copper was absorbed. How the absorption of copper is regulated is not fully understood. *In vitro* studies provide evidence that copper uptake into intestinal cells appears to be saturable (Arredondo et al. 2000). This study also provides suggestive evidence that copper uptake into the intestinal cell and efflux are influenced by intracellular copper concentrations. There is evidence that copper diffuses across the intestinal cell membrane; however, it is unlikely that this is the only absorption mechanism. It is possible that recently identified copper transporters (hCtr1 and hCtr2) play a role in the regulation of copper uptake. The Menkes protein (MNK), a copper-translocating P-type ATPase, may be involved in the transport of copper across the basolateral membrane of intestinal cells (Pena et al. 1999). MNK protein is involved the delivery of copper to copper-dependent enzymes and the efflux of copper from the cell. The export of

Figure 3-3. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan et al. 1994

copper via the MNK protein appears to be regulated by intracellular copper concentration. Exposure to copper produces a conformational change in the MNK protein resulting in the formation of a copper cluster, which allows access to the phosphorylation site that upon phosphorylation, initiates copper translocation (Dameron and Harrison 1998). Once copper is released from the intestinal cells, it is transported bound to albumin and histidine to the liver via the portal circulation. Once in the hepatic cells, copper complexes with small cytoplasmic proteins known as copper chaperones. These copper chaperones are involved in intracellular distribution of copper ions. In the liver, another P-type ATPase, Wilson protein (WND), delivers copper to ceruloplasmin, which is then released to the blood for distribution to other tissues and organs. Under conditions of elevated copper, WND is involved in the release of copper at the canalicular membrane with ensuing biliary excretion of copper. The liver plays a critical role in copper homeostasis as both the storage site for this metal and as part of the physiologic route for excretion through the biliary system. The molecular mechanisms determining biliary copper excretion are becoming clearer due to the better understanding of genetic defects, such as Wilson's disease. Specifically, the Wilson protein localized to the trans-Golgi network of hepatocytes not only delivers copper to ceruloplasmin, but also is essential for biliary copper excretion. Recently, other proteins have also been identified that interact with Wilson protein and appear to be equally important in the process of biliary copper excretion (Tao et al. 2003).

3.5.2 Mechanisms of Toxicity

Although a number of studies have investigated the mechanisms of copper hepatotoxicity in rats, it is not known whether rats would be a good model for human liver toxicity unrelated to a genetic defect in copper metabolism. Lysosomes serve an important role in hepatic copper metabolism. Excess copper is sequestered within hepatocyte lysosomes where it is complexed with metallothionein. However, this protective mechanism is saturable and liver lesions can develop above the saturation limit. In copper loaded rats, lysosomes become enlarged and more fragile with decreased membrane fluidity (Myers et al. 1993). The results of the Haywood et al. (1985a) study do not suggest that liver damage is due to rupturing of lysosomes because lysosomal instability precedes and is not synchronous with liver damage. It is speculated that saturation of the lysosomes results in an accumulation of copper in the nucleus and subsequent nuclear damage (Fuentealba and Haywood 1988; Fuentealba et al. 1989; Haywood et al. 1985a). The mechanism by which copper accumulates in the nucleus and the mechanisms by which it provokes injury are not clear. It has been suggested that excess copper results in oxidative damage, including lipid peroxidation. Increases in the level of thiobarbituric acid reactive substance (TBARS), a measure of lipid peroxidation, have been found in copper-loaded rats (Myers et al. 1993; Sokol et al.

1993). However, a study by Aburto et al. (2001b) did not find significant alterations in the levels of malaondialdehyde, a lipid peroxidation by product, prompting the study authors to postulate that lipid peroxidation does not play a major role in copper toxicity although it may occur as a terminal event as a consequence of cell injury. Sokol et al. (1990, 1993) suggested that oxidant injury to hepatocyte mitochondria may be one of the initiating factors in hepatocellular damage. Numerous studies have shown that rats can develop tolerance to high levels of copper. After 3–5 weeks of copper loading resulting in tissue damage, the copper levels in the liver begin to decline and the tissue begins to regenerate (Haywood and Loughran 1985). It is believed that the mechanism involved in tolerance development is the increased synthesis of metallothionein (Evering et al. 1991a, 1991b; Freedman and Peisach 1989).

Studies in monkeys, dogs, and ferrets provide strong evidence that copper-induced emesis results from stimulation of the vagus nerve. Abdominal vagotomy resulted in a dramatic decrease in the occurrence of emesis in dogs (Fukui et al. 1994) and ferrets (Makale and King 1992) orally exposed to copper sulfate and in monkeys receiving oral or intravenous injections of copper sulfate (Fukui et al. 1993). In monkeys, administration of compounds that block 5-HT_3 receptors also resulted in a decrease in emesis following oral or intravenous administration of copper sulfate (Fukui et al. 1993). In contrast, 5-HT_3 blockers did not affect the occurrence of emesis in dogs (Fukui et al. 1994) or ferrets (Bhandari and Andrew 1991) receiving an oral dose of copper sulfate, but compounds that block 5-HT_4 receptors did inhibit copper-induced vomiting. Fukui et al. (1994) suggested that copper sulfate caused gastrointestinal irritation that resulted in the release of 5-HT and evoked emesis by activation of abdominal visceral afferents through 5-HT_4 receptors.

3.5.3 Animal-to-Human Extrapolations

The toxicity of copper has been assessed in a number of experimental animal species, and sensitivity to copper toxicity is highly species dependent. Ruminants are more susceptible than nonruminant species. NTP (1993) demonstrated that rats are much more sensitive than mice to the hepatotoxicity of copper. In rats, dietary exposure to 16 mg Cu/kg/day for 13 weeks resulted in an increase in alanine aminotransferase activity; chronic active liver inflammation was observed at 66 mg Cu/kg/day. In contrast, no evidence of liver damage was observed in mice exposed to 814 mg Cu/kg/day for 13 weeks.

Most of the experimental data on the toxicity of copper come from studies in which rats were used; however, the relevance of this species to human toxicity has not been fully evaluated. The dietary

requirement for copper in rats is 5 mg Cu/kg diet (NRC 1995); a commonly used diet for rats (AIN76, AIN 93G, AIN93M) has a cupric carbonate concentration of 300 mg/kg diet (160 mg Cu/kg diet). An intermediate-duration exposure to approximately 250 mg Cu/kg diet resulted in mild liver effects (increased serum alanine aminotransferase) (NTP 1993). It is unlikely that humans would tolerate prolonged exposure to a copper dose that is 50 times higher than the dietary requirement (0.65 mg Cu/kg/day); gastrointestinal disturbances were observed in women ingesting 0.0731 mg Cu/kg/day in drinking water (Pizarro et al. 1999). Thus, the applicability of these animal data to humans is not known.

The Long-Evans Cinnamon rat is often used as a model for Wilson's disease. This rat strain shares many characteristics with Wilson's disease: accumulation of liver copper, decreased serum copper and ceruloplasmin levels, and impaired biliary excretion of copper (Sugawara et al. 1991, 1992, 1994; Suzuki et al. 1995).

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others believe that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics include the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial,

scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992). However, in the case of ambient human exposures, the validity of these possibilities has yet to be established conclusively.

There is no evidence that copper interferes with the normal function of the neuroendocrine axis.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects to humans from exposures during the period from conception to maturity at 18 years of age, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any direct or indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Potentially relevant animal and *in vitro* models are also discussed.

Children should not be considered small adults. They may differ from adults in their exposures and in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6 Exposures of Children.

Children may differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life at which particular structures and/or functions will be most sensitive to perturbation. Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Also, the distribution of xenobiotics may be different; for example, infants

have a larger proportion of their bodies as extracellular water, their brains and livers are proportionately larger, and the composition and quality of their lipid depots differ from those of adults (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form and/or in detoxification. There may also be differences in excretion, particularly in newborns who have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). In addition, children and adults may differ in their capacity to repair damage from chemical insults. In general, children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to the development of cancer.

Certain characteristics of the developing human may increase exposure or susceptibility to certain toxicants, whereas others may decrease susceptibility to the same toxicant. For example, although infants breathe more air per kilogram of body weight than adults, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Copper is an essential element required for normal growth and development and for a variety of metabolic functions including iron metabolism, cross-linking of connective tissue, and lipid metabolism. Signs of copper deficiency in infants and children include anemia that is unresponsive to iron supplementation, neutropenia, bone abnormalities, and hypopigmentation of the hair (Cordano 1998; Danks 1988).

Exposure to excess levels of copper has been associated with adverse health effects in infants and children. There is an extensive body of literature on two syndromes that have been associated with exposure to high levels of copper, Indian childhood cirrhosis and idiopathic copper toxicosis. Both are characterized by severe liver damage in infants and children (<5 years of age). In the case of Indian childhood cirrhosis, excessive copper exposure has been traced to the use of brass or copper containers for storage and heating of milk. Doses as high as 0.930 mg/kg/day have been estimated; this dose is

approximately 30 times higher than the dietary requirement for copper (Tanner 1998). Idiopathic copper toxicosis (also referred to as non-Indian childhood cirrhosis) has also been linked to exposure to high levels of copper in drinking water and/or the use of copper utensils (Wijemenga 2002). A common finding in both syndromes is the early dietary introduction of non-mother's milk and/or formula. Genealogical investigations provide suggestive evidence that both syndromes are transmitted in an autosomal recessive mode. However, the mechanism of action has not been identified. It is possible that the genetic defect results in reduced copper efflux from the liver. Very high levels of copper have been detected in the livers of affected infants; copper levels ranging from 790 to 6,654 µg/g dry weight (mean of 939 µg/g) have been reported in infants diagnosed with Indian childhood cirrhosis (levels in control infants ranged from 8 to 118 µg/g (Bhave et al. 1982). Support for the genetic component comes from the finding that decreasing copper exposure levels dramatically decreases the occurrence of Indian childhood cirrhosis (Tanner 1998). Additionally, no alterations in serum biomarkers of liver damage (alanine aminotransferase activity, aspartate aminotransferase activity, gamma glutamyl transferase activity, and total bilirubin levels) were observed in infants ingesting water containing 2 mg/L copper (0.319 mg/kg/day) (Olivares et al. 1998) or infants living in households with tap water copper levels of 0.8 mg/L (Zietz et al. 2003a, 2003b). Together, these data suggest that exposure to copper levels exceeding the copper metabolic capacity of certain individuals with a genetic defect is the causative agent for severe liver damage.

Another adverse health effect that has been reported in infants and children is gastrointestinal upset. This effect, which is one of the most commonly reported adverse health effect in adults, is manifested in nausea, vomiting, abdominal pain, and/or diarrhea. Symptoms usually occur shortly after ingesting a copper-contaminated beverage or drinking water containing a high level of copper. In most of the reports of gastrointestinal upset in children (Gill and Bhagat 1999; Karlsson and Noren 1965; Knobeloch et al. 1994; Spitalny et al. 1984; Walsh et al. 1977), no reliable information on copper concentration or dose was reported. In one report where school-age children ingested a beverage stored in an old urn, the concentration of copper in the beverage was estimated to be 300 mg/L (Gill and Bhagat 1999). Another study reported vomiting in infants ingesting a single dose of 7.5 mg/L copper sulfate (Karlsson and Noren 1965). Knobeloch et al. (1994) noted that children appear to be more sensitive to the gastrointestinal effects of copper than adults. This statement was based on two surveys of residents with elevated copper levels in the drinking water. In the first survey, it appears that children who were described as "unusually irritable" or had recurrent headaches were categorized as having gastrointestinal upset. In the second survey, mothers were asked to recall the frequency of gastrointestinal effects for all family members. A significantly higher percentage of children, as compared to adults, were reported to have gastrointestinal

effects. It is difficult to determine what role recall bias had in the results and how well the mothers knew of symptoms of gastrointestinal upset in the adult members of the household. The available data are inconclusive to assess accurately whether there is an age-related difference in the gastrointestinal toxicity of copper.

The potential age-related differences in the toxicity of copper has been assessed in rats exposed to 120 mg Cu/kg/day as copper sulfate in the diet for 12 weeks (Fuentealba et al. 2000). The observed liver effects were more severe in young rats (exposed *in utero*, during lactation, and for 12 weeks post weaning) as compared to the effects observed in adult rats. The copper levels in the liver were also higher in the young rats (1,553–1,635 versus 472–534 µg/g). The doses used in this study are very high, 1,000 times higher than the rat dietary requirement of 0.15–0.30 mg/kg/day (AIN 1977). It is not known if increased liver sensitivity would also occur at lower copper doses. Although these data are suggestive that children may be more sensitive to the hepatotoxicity of high doses of copper, uncertainty in the use of rats as a model for human toxicity limits the extrapolation of these study results to humans.

Several studies have investigated the potential developmental toxicity of dietary copper sulfate; the results suggest that *in utero* exposure to copper can result in delays in growth and development in the offspring of rats exposed to 130 mg Cu/kg/day (Haddad et al. 1991) and mice exposed to 208 mg Cu/kg/day (Lecyk 1980). No developmental effects were observed in the offspring of mink exposed to 13 mg Cu/kg/day (Aulerich et al. 1982).

There is concern that toxicokinetic differences between infants and adults may result in increased sensitivity in infants. During the second half of pregnancy, particularly in the third trimester, the fetus accumulates copper at a rate of 50 g/kg/day (Widdowson et al. 1974). Approximately half of the copper in the fetus is stored in the liver, mostly bound to metallothionen. Additionally, the rate of transfer of copper from the liver to the bile or blood is decreased due to the immaturity of the liver. The magnitude of the amount of copper in the fetal liver is similar to levels observed in Wilson’s disease; however, the fetal/neonatal liver tolerates these high concentrations (Olivares et al. 2000). After birth, the copper levels in the liver steadily decrease from about 51 µg/g at birth to 5.7 µg/g at 6–14 months of age (Klein et al. 1991; Olivares et al. 2000).

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to copper are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by copper are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that may result in an increase in absorbed dose, a decrease in the dose-level required for biological effectiveness, or a target tissue response. Biomarkers of susceptibility are discussed in Section 3.10 “Populations that are Unusually Susceptible.”

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Copper

Copper levels can readily be measured in tissues, body fluids, and excreta. Depending on the dose and exposure duration, inhalation and/or oral exposure to copper can result in increased levels of copper in serum, urine, hair, and liver. Increased whole blood and serum copper levels have been reported in humans following intentional ingestion of a single dose of 1–30 g of copper as copper sulfate (Chuttani et al. 1965). The serum and whole blood levels of copper ranged from 239 to 346 and from 383 to 684 µg/100 mL, respectively; the serum and whole blood levels in non-exposed individuals were 151.6 and 217 µg/100 mL, respectively. Following chronic inhalation exposure to 111–464 mg Cu/m^3 copper dust, plasma serum levels of >200 µg/100 mL were observed in 16% of factory workers exposed to copper dust (Suciu et al. 1981). However, increased serum copper levels may only be reflective of recent exposure. Chuttani et al. (1965) observed that serum ionic copper rapidly diminished to normal levels following an acute bolus dose.

A relationship between blood copper levels and the severity of symptoms has not been established. Among individuals intentionally ingesting a single dose of copper sulfate (1–30 g), Chuttani et al. (1965) noted that there did not appear to be any difference between serum copper levels in individuals only exhibiting gastrointestinal effects and those with more severe symptoms (jaundice, renal manifestations, or shock). In contrast, whole blood copper levels were much higher in the individuals with severe symptoms (798 µg/100 mL) compared to those with mild symptoms (287 µg/100 mL).

Copper levels in hair and nails can also be used to assess copper exposure. In a study of preschool children, the levels of copper in hair and toenail samples were log-normally distributed (Wilhelm et al. 1991). The geometric mean concentrations of copper in hair and toenails were 10.6 µg/g (range of 5.4–20.7 µg/g) and 7.5 µg/g (range of 3.0–18.6 µg/g), respectively. Based on a hair growth rate of 10 mm per month, the copper levels in the first 2 cm proximal to the scalp would represent copper intake over 2 months (Hopps 1977). In contrast, toenail samples would represent copper intake over 12–18 months, based on a toenail growth rate of 1 mm/month (Fleckman 1985). Increased hair copper levels have been reported in workers exposed to 0.64–1.05 mg/m^3 of an unspecified copper compound; the concentration of copper in the hair was 705.7 µg/g, as compared to a 8.9 µg/g concentration in non-exposed workers (Finelli et al. 1981), and increased hair and fingernail copper levels were observed in children with Indian childhood cirrhosis (Sharda and Bhandari 1984).

3.8.2 Biomarkers Used to Characterize Effects Caused by Copper

The harmful health effects of copper occur over a wide range of copper intakes from too little copper in the diet to excessive copper exposure.

Low Intakes of Copper. The nutritional requirements of copper and the health effects associated with copper deficiency have been reviewed by numerous authors (Gallagher 1979; Mason 1979; O'Dell 1984). Copper deficiency is rarely observed in humans; the existence of covert copper deficiency among segments of the population is unknown. The limited data available on human health effects of inadequate copper intakes are derived mostly from case reports of severely malnourished children, patients maintained by total parenteral nutrition without copper, and children with Menkes' disease (a genetic disorder resulting in impaired copper absorption). Copper deficiency is characterized by hypochromic anemia, abnormalities of connective tissues, and central nervous system disorders. Sudden death associated with spontaneous rupture of a major blood vessel or the heart itself has been observed in some animal species.

The manifestations of copper deficiency are related to a decrease in several of the copper-containing metalloenzymes. The most severe biochemical alteration is decreased cytochrome oxidase activity; this is manifested as poor growth, anemia, and central nervous system effects. The decreased oxidative metabolism associated with decreased cytochrome oxidase results in poor growth in infants, weight loss, and emaciation. The hypochromatic anemia observed during copper deficiency is not distinguishable from iron deficiency anemia; however, it is not responsive to iron administration. A decrease in protoheme synthesis, a result of decreased cytochrome oxidase, has also been observed. As with anemia, the central nervous system effects, primarily the result of hypomyelination, are associated with low activity levels of cytochrome oxidase; the decreased synthesis of phospholipids observed in copper deficiency may also contribute to the development of central nervous system effects. In addition to the decrease in cytochrome oxidase, a decrease in lysyl oxidase is also observed. Lysyl oxidase is involved in the formation of cross-links in collagen and elastin. Depending on the species, this impairment results in bone disorders, a defective cardiovascular system, or abnormal lung structure.

Exposure to Excess Levels of Copper. No copper-specific biomarkers of effects have yet been identified. The most notable sign of toxicity in humans ingesting a beverage or water containing copper is gastrointestinal distress. Symptoms (typically nausea, vomiting, and abdominal pain) usually occur shortly after ingesting the contaminated beverage. The liver is another sensitive target of copper toxicity. Alterations in a number of serum enzymes have been observed in humans and animals with copper-

induced liver damage (Chuttani et al. 1965; Epstein et al. 1982; Haywood 1980; Haywood and Comerford 1980; Müller et al. 1998; NTP 1993; Sugawara et al. 1995). The affected serum enzymes include serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase. Increases in serum bilirubin levels have also been observed in humans. Animal studies demonstrate that the rise in serum enzyme activities are the first evidence of liver damage. However, alterations in serum enzyme levels are not unique to copper-induced liver damage.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Numerous studies have demonstrated the interaction between copper and several other metals. Dietary zinc strongly affects copper absorption. A diet high in zinc can result in copper deficiency. Reductions in erythrocyte superoxide dismutase, indicative of marginal copper deficiency, have been found in studies of women ingesting zinc supplements (50 mg zinc/day) for 10 weeks (Yadrick et al. 1989) and men ingesting 50 mg zinc/day for 6 weeks (Fisher et al. 1984). The exact mechanism of the zinc-copper interaction is not known. However, increased dietary zinc results in induction of metallothionein synthesis in the intestine and metallothionein has a greater binding capacity for copper than for zinc. Thus, the dietary copper is sequestered in the intestinal mucosal cell and eventually is excreted in the feces when the mucosal cell is sloughed off (Hall et al. 1979; Whanger and Weswig 1971). Because exposure to excess dietary zinc results in decreased copper absorption, it is often used as a treatment for Wilson's disease (Brewer et al. 1993). An oral/intraperitoneal study in mice provides some evidence that zinc and copper may interact at sites other than the intestine. In this study on the influence of zinc on mitigating the immunotoxicity of copper, mice were exposed to copper sulfate in the drinking water for 8 weeks and received an intraperitoneal injection of zinc sulfate once a week (Pocino et al. 1990). Decreases in the magnitude of the proliferative response to con A or LPS and the antibody response to sheep red blood cells were observed in the copper-exposed mice, but not the mice receiving copper and zinc. However, zinc did not modify the increased production of auto-antibodies reactive with bromelain-treated mouse red blood cells.

Several other divalent cations compete with copper for intestinal absorption. Exposure to dietary cadmium (Evans et al. 1970a), ferrous iron (Wapnir et al. 1993; Yu et al. 1994), and stannous tin (Pekelharing et al. 1994; Wapnir et al. 1993) can result in decreased copper absorption. In the case of cadmium, the decrease is related cadmium induction of metallothionein and the binding of copper to it. Tetrathiomolybdate is used for the treatment of Wilson's disease (Brewer 1995) and excessive dietary molybdenum can also result in decreased uptakes and, therefore, copper utilization and toxicity. Two

mechanisms of action of tetrathiomolybdate have been proposed: it reacts with copper-metallothionein to form a soluble complex which is excreted (Ogra et al. 1996) and it can complex with nonceruloplasmin plasma copper, preventing its cellular absorption (Brewer 1995).

Because selenide is a strong reducing agent (Frost 1972), it has been postulated that selenium may play a role in detoxifying copper. Aburto et al. (2001a, 2001b) examined the possible interaction between copper and selenium. Selenium did not influence the hepatotoxicity of copper in rats fed diets with excess levels of copper and inadequate, adequate, or excess levels of dietary selenium. Hepatic copper levels and histological alterations were not significantly different in rats receiving a high copper/high selenium diet as compared to rats receiving a high copper/adequate selenium diet.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to copper than will most persons exposed to the same level of copper in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). Whatever the basis of the increased susceptibility may be, the result is reduced detoxification or excretion of copper or compromised functioning of organs affected by copper. Populations at greater risk due to unusually high exposure to copper are discussed in Section 6.7, Populations with Potentially High Exposures.

A number of populations of individuals unusually susceptible to copper toxicity have been identified. The increased susceptibility to copper toxicity is associated with genetic defects that impair copper homeostatic mechanisms. Wilson's disease, also referred to as hepatolenticular degeneration, is an autosomal recessive disorder with a worldwide incidence of 1 in 30,000 (Scheinberg and Sternlieb 1996). The primary genetic defect in Wilson's disease is in ATP7B, which encodes a P-type ATPase (Wilson protein), which delivers copper to ceruloplasmin. The genetic defect results in impaired biliary excretion of copper and an accumulation of copper in the liver. As described by Brewer and Yuzbasiyan-Gurkan (1992), the progression of the disease begins with an accumulation of copper in the liver, damage to the liver, and subclinical liver cirrhosis. Over time, the individual will develop hepatic, neurological, and psychiatric symptoms. The hepatic effects are characterized by jaundice, hypoalbuminemia, ascites, coagulation defects, hyperammonemia, hepatic encephalopathy, and/or liver failure; in the cases of massive liver failure, large amounts of copper are released from the liver resulting in hemolytic anemia. Neurological symptoms include tremors and other movement disorders and speech abnormalities. Psychiatric and behavioral symptoms are often found in individuals also manifesting neurological other

symptoms. The psychiatric symptoms include reduced performance in school or work, inability to cope, depression, very labile moods ranging from mania to depression, sexual exhibitionism, and frank psychosis. Individuals with Wilson's disease have low serum ceruloplasmin levels, elevated urinary copper levels, and elevated liver copper levels; Kayser-Fleischer rings, which result from corneal copper deposits, are also detected in individuals with Wilson's disease. Individuals who are heterozygotes for Wilson's disease may also be unusually susceptible to the toxicity of copper. Increases in urinary copper and hepatic concentrations and decreased copper incorporation into ceruloplasmin have been observed in heterozygotes. These findings may suggest that long-term exposure to elevated levels of copper may result in copper overload. Although the incidence of heterozygotes is not known, NAS (2000) estimates that 1 in 40,000 individuals (approximately 1% of the U.S. population) may be heterozygotes for Wilson's disease.

Indian childhood cirrhosis (ICC) and idiopathic copper toxicosis (ICT) are two syndromes that result in severe, often fatal, liver cirrhosis in infants and young children. Although the basis of the defect has not been firmly established, it is believed to be due to an inherited autosomal recessive defect in copper metabolism aggravated by high copper intake (Bhave et al. 1982, 1987; Müller et al. 1996, 1998). ICC occurs in infants and children living in rural areas of the Indian subcontinent who are introduced early to cow or buffalo milk that is stored or heated in brass or copper vessels. Copper is believed to be the causative agent because the milk has very high copper levels, very high copper levels are found in the liver, and replacing the brass or copper vessels with aluminum or stainless steel vessels eliminates the occurrence of ICC in siblings of ICC affected children (Bhave et al. 1982; Tanner 1998). A high degree of parental consanguinity, the occurrence of ICC in children, but not the parents, and the fact that 22% of siblings affected suggest an autosomal recessive component to the disease (Pandit and Bhave 1996; Tanner 1998). For ICT, which includes Tyrolean infantile cirrhosis, sources of high copper exposure have been identified. For the 138 cases of ICT in children living in the Tyrolean region of Austria, the source of the copper was the use of a water/unpasteurized cow's milk mixture that was heated in a copper pot (Müller et al. 1996). For the other cases of ICT that have been identified in a number of countries, the source of the excess copper intake was drinking water (Müller et al. 1998). The similarity of ICT to ICC has prompted investigators to suggest that ICT may also be due to an autosomal recessive genetic defect in copper metabolism and excessive copper intake at a very young age. A genealogical investigation by Müller et al. (1996) provides supportive evidence for a genetic basis of the disease.

It has been postulated that individuals with a deficiency of the enzyme glucose-6-phosphate dehydrogenase would be susceptible to the toxic effects of oxidative stressors such as copper (Calabrese

and Moore 1979; Chugh and Sakhuja 1979). This has not been supported by epidemiological or experimental data. In the blood, most of the copper is bound to ceruloplasmin. With the exception of ingestion of a very large dose of copper salts, the levels of nonceruloplasmin bound copper remain low following copper exposure. Thus, it is unlikely that this relatively small change in free copper would alter the survival of glucose-6-phosphate dehydrogenase deficient red cells.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to copper. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to copper. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to copper:

Ellenhorn MJ, Schonwald S, Ordog G, et al., eds. 1997. Medical toxicology: Diagnosis and treatment of human poisoning. Second edition. Baltimore, MD: Williams & Wilkins, 1554-1556.

Goldfrank LR, Flomenbaum FE, Lewin NA, et al., eds. 1998. Goldfrank's toxicologic emergencies. Sixth edition. Stamford, CT: Appleton & Lange, 1339-1340.

Haddad LM, Shannon MW, Winchester JF, eds. 1998. Clinical management of poisoning and drug overdose. Third edition. Philadelphia, PA: WB Saunders, 165.

3.11.1 Reducing Peak Absorption Following Exposure

Following ingestion of copper or copper compounds, milk or water should be given immediately after ingestion and/or prior to vomiting. Because of the strong emetic properties of copper and copper compounds, vomiting usually occurs shortly after ingestion. Induction of vomiting and gastric lavage are contraindicated following ingestion of caustic copper salts, such as copper sulfate. Gastric lavage may be indicated after ingestion of noncorrosive copper compounds (HSDB 2002).

For individuals with Wilson's disease, the administration of a diet high in zinc is used as a maintenance treatment (Brewer et al. 1989). The zinc interferes with copper absorption by inducing intestinal metallothionein resulting in increased copper sequestration (Brewer et al. 1992).

3.11.2 Reducing Body Burden

A number of methods have been employed to reduce copper body burden. These methods range from the use of chelating agents to increases in dietary levels of zinc and molybdenum. Gao et al. (1989) tested the *in vitro* effectiveness of five chelating agents using human serum albumin. The agents (in order of decreasing effectiveness) were ethylenediaminotetraacetate (EDTA), diethylene triaminopentaacetate, ethylene glycol-*bis*-(aminoethylether)-tetraacetate, nitrilotriacetate, and iminodiacetate. The *in vivo* effectiveness of these agents has not been established. D-penicillamine is often used to decrease the elevated levels of hepatic copper in individuals with Wilson's disease (Walshe 1996; Walshe and Yealland 1993) and idiopathic childhood cirrhosis (Rodeck et al. 1999). However, a number of potential side effects have been associated with penicillamine treatment (Brewer and Yuzbasiyan-Gurkan 1992). A variety of other chelating agents have been tested in copper loaded rats. Tetraethylenepentamine pentahydrochloride (TETREN) was more effective in increasing urinary excretion of copper than 1,4,7,11-tetraazaundecane tetrahydrochloride (TAUD) or penicillamine, which were equally effective (Domingo et al. 2000). TETREN did not result in a decrease in copper levels in the liver, although a significant decrease in kidney copper levels was observed. In contrast, TAUD and penicillamine reduced the levels of copper in the liver. None of the three chelating agents affected the amount of copper excreted into the feces.

The known interaction between copper and molybdenum have been used to treat individuals with Wilson's disease. The administration of tetrathiomolybdate to individuals with neurological or psychiatric symptoms associated with Wilson's disease has resulted in an improvement or reversal of symptoms (Brewer 1995). In blood plasma, tetrathiomolybdate complexes with nonceruloplasmin plasma copper, preventing its cellular absorption. Studies in Long-Evans Cinnamon rats, a model for Wilson's disease, and sheep have found that administration of tetrathiomolybdate results in a dramatic decrease in the levels of copper in the liver (Humphries et al. 1988; Kumaratilake and Howell 1989; Ogra et al. 1996) and decreased liver damage (Humphries et al. 1988). Tetrathiomolybdate also reacts with copper bound to metallothionein resulting in a soluble copper-tetrathiomolybdate complex (Ogra et al. 1996). The addition of molybdenum to a high sulfur, low copper diet can result in a decrease in liver and plasma copper levels in copper loaded sheep (van Ryssen 1994).

Although zinc is used in the treatment of Wilson's disease to decrease the absorption of copper, zinc does not appear to be effective in reducing the copper body burden. No alterations in hepatic copper levels were observed in sheep administered a low copper, high zinc diet (van Ryssen 1994). A reduction in hepatic copper levels has been observed in dogs administered a high zinc diet (Brewer et al. 1992);

however, it is believed that the reduction was secondary to the induction of copper deficiency and the mobilization of copper from the liver (van Ryssen 1994).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

There are limited data on methods for interfering with the mechanisms of action of copper. An *in vitro* study suggested that lazaroids (21-aminosteroids) may have a protective effect against copper-induced erythrocyte lipid peroxidation (Fernandes et al. 1992). Oxidative damage to the erythrocyte membrane may be the cause of the hemolysis observed following exposure to very high doses of copper.

3.12 ADEQUACY OF THE DATABASE

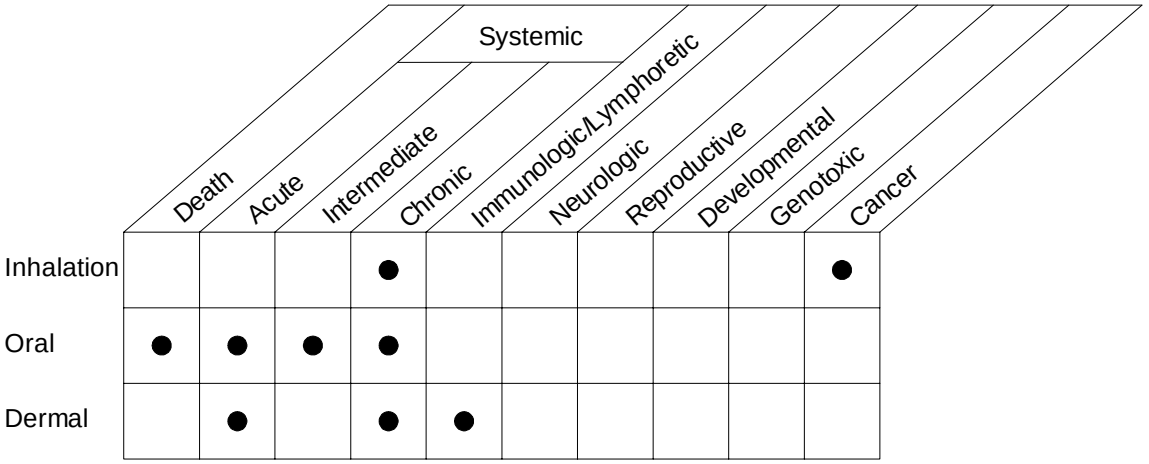
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of copper is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of copper.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

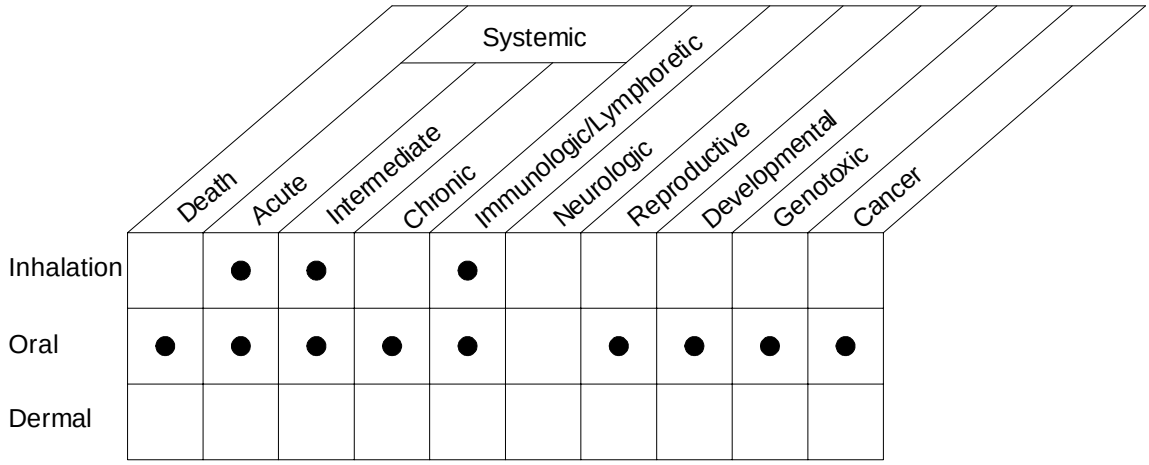
3.12.1 Existing Information on Health Effects of Copper

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to copper are summarized in Figure 3-4. The purpose of this figure is to illustrate the existing information concerning the health effects of copper. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a “data

Figure 3-4. Existing Information on Health Effects of Copper



Human



Animal

● Existing Studies

need." A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments.

Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

The toxicity of inhaled copper has been investigated in a couple of occupational exposure studies. These studies examined a limited number of systemic end points, and exposure is poorly characterized. There are numerous reports and studies on the toxicity of ingested copper in humans. Most of the reports and studies focused on the gastrointestinal effects following acute exposure to copper in drinking water or other beverages. Data on other health effects in humans comes from individuals with Wilson's disease, Indian childhood cirrhosis, and idiopathic copper toxicosis. These diseases/syndromes are the result of genetic defect(s) resulting in impaired copper kinetics; the latter two syndromes are also associated with exposure to high levels of copper in drinking water or milk (due to storage of milk in brass vessels). These studies provide information on potential targets of toxicity, primarily the liver.

Information on the dermal toxicity of copper is limited to reports of contact dermatitis in individuals and eye irritation in workers exposed to copper dust.

As with the human database, there are limited data on the toxicity of inhaled copper in animals. The available studies have primarily focused on potential respiratory effects. There is a more extensive database on the toxicity of ingested copper in animals. These studies have found a number of systemic effects, including gastrointestinal, hepatic, and renal effects following acute, intermediate, and chronic exposure. Immunological and developmental effects have also been reported in animal studies. Several studies have also examined potential neurological and reproductive targets, but have not found effects. Carcinogenic effects were not found in several animal studies; however, the studies are limited in scope and tested low doses. No animal studies examining the dermal toxicity of copper were identified.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. No data were located regarding health effects after acute inhalation exposure to copper in humans. Animal data are limited to information from studies in mice and hamsters conducted by Drummond et al. (1986). Respiratory tract irritation and impaired immune function were

observed. This study was not selected as the basis for an acute-duration inhalation MRL because it only examined a limited number of end points, and the liver and kidney, which are targets following oral exposure, were not examined; in addition, the animals were only exposed for 3 hours/day. Additional inhalation studies are needed to identify the critical targets of toxicity and to establish concentration-response relationships for copper. The most commonly reported effect in humans acutely exposed to copper is gastrointestinal upset. The reported symptoms include nausea, vomiting, abdominal pain, and diarrhea (Chutanni et al. 1965; Gill and Bhagat 1999; Gotteland et al. 2001; Nicholas and Brist 1968; Olivares et al. 2001; Pizarro et al. 1999, 2001; Semple et al. 1960; Walsh et al. 1977). Hepatic and renal effects have also been seen in individuals ingesting lethal doses of copper sulfate (Chuttani et al. 1965). Animal studies support the identification of the gastrointestinal tract, liver, and kidneys as sensitive targets of copper toxicity. Hyperplasia of the forestomach has been observed in rats and mice exposed to copper sulfate in the diet for 14 days (NTP 1993). Hepatic effects ranging from increases in alanine aminotransferase activity to hepatocellular necrosis and renal effects (protein droplets in proximal tubules) have been observed in rats exposed to fairly high doses of copper sulfate in the diet (Haywood 1980; Haywood and Comerford 1980; Haywood et al. 1985b; NTP 1993). Decreases in body weight gain have also been observed in rats (NTP 1993). The acute-duration oral database was considered adequate for derivation of an MRL. The MRL was based on gastrointestinal upset in women ingesting drinking water containing copper sulfate for 2 weeks (Pizarro et al. 1999). There are limited data on the dermal toxicity of copper. Pruritic dermatitis and allergic contact dermatitis have been reported in humans exposed to copper. No animal studies were identified. These data provide suggestive evidence that copper may be irritative to the skin; additional dermal studies are needed to determine whether copper exposure will also result in systemic effects.

Intermediate-Duration Exposure. No studies were located regarding health effects in humans after intermediate-duration inhalation. Only one animal inhalation exposure study was located. This study did not find any adverse histological alterations in the lungs or functional alterations in alveolar macrophages of rabbits exposed to copper chloride (Johansson et al. 1983, 1984). Because the lungs were the only tissues examined, these studies were not considered suitable for derivation of an intermediate-duration inhalation MRL for copper. Additional studies are needed to identify the critical targets of toxicity and establish concentration-response relationships for inhaled copper. Three experimental human studies and two community-based studies have examined the oral toxicity of copper in healthy humans. The primary focus of these studies was examination of the potential of low doses of copper to induce hepatic effects in adults (Araya et al. 2003b; Pratt et al. 1985) or infants (Olivares et al. 1998; Zietz et al. 2003a, 2003b); no adverse effects were found. The Araya et al. (2003b) study also assessed the potential for gastrointestinal

effects in adults and found significant increases in the incidence of effects as a function of dose/duration. A number of animal studies have reported adverse liver and kidney effects following intermediate-duration oral exposure to copper compounds (Epstein et al. 1982; Fuentealba et al. 2000; Haywood 1980, 1985; Haywood and Comerford 1980; Haywood and Loughran 1985; Haywood et al. 1985a, 1985b; Kumar and Sharma 1987; NTP 1993). The observed liver and kidney effects demonstrated dose- and duration-response relationships. The studies by Haywood and associates demonstrate that rats can develop a tolerance to copper following repeated oral exposure. Studies in other animal species are needed to determine if this phenomenon is unique to rats or is observed in other species as well. Other systemic effects that have been reported in animals include hyperplasia of the forestomach mucosa (NTP 1993), decreased erythrocyte and hemoglobin levels (Kumar and Sharma 1987; Rana and Kumar 1980; Suttle and Mills 1966a), and decreased body weight gain or weight loss (Haywood 1985; Haywood and Loughran 1985; Kline et al. 1971; Llewellyn 1985; NTP 1993). For the most part, these studies involved dietary exposure of rats to copper sulfate; additional studies in other species would be useful for identifying a model for human toxicity. The Araya et al. (2003b) human study was used as the basis of an intermediate-duration oral MRL for copper. No data on the dermal toxicity of copper following intermediate-duration exposure were identified. Studies are needed to identify the critical targets of copper toxicity following dermal exposure.

Chronic-Duration Exposure and Cancer. Systemic effects such as nausea (Suciu et al. 1981), hepatomegaly (Suciu et al. 1981), decreased hemoglobin and erythrocyte levels (Finelli et al. 1981), and respiratory irritation (Askergren and Mellgren 1975; Suciu et al. 1981) have been observed in workers exposed to copper dust. The mild gastrointestinal effects observed in some workers were attributed to swallowing airborne copper dust (Suciu et al. 1981). The poor characterization and/or the lack of controls preclude deriving a chronic-duration inhalation MRL based on the occupational exposure studies. Additional studies are needed to identify the critical targets of toxicity of inhaled copper. There are numerous reports of severe health effects in infants and children ingesting copper-contaminated milk or water containing high levels of copper (Müller et al. 1996, 1998; Pandit and Bhave 1996; Tanner 1998). Indian childhood cirrhosis and idiopathic copper toxicosis are characterized by severe liver cirrhosis occurring before the age of 5 years. There is suggestive evidence that both of these syndromes are related to increased dietary intake of copper in conjunction with increased genetic susceptibility. Nausea, vomiting, and abdominal pain were reported by members of a family with very high levels of copper in the drinking water (Spitalny et al. 1984). The animal database on the oral toxicity of copper following chronic-duration exposure is limited to one study (Massie and Aiello 1984) that found a decrease in lifespan and no effect on body weight gain in mice exposed to copper gluconate for 850 days. No other

end points of toxicity were examined in this study. The database was considered inadequate for derivation of a chronic-duration oral MRL. Additionally, studies that examine a variety of end points are needed to identify the critical targets of toxicity and establish dose-response relationships. Information on the dermal toxicity of copper is limited to a report of ocular irritation in workers exposed to copper dust (Askergren and Mellgren 1975). Additional dermal toxicity studies are needed to identify the critical targets of toxicity following dermal exposure.

Data on the carcinogenicity of copper in humans are limited to a study of copper miners (Chen et al. 1993) and a follow-up to this study (Chen et al. 1995). Increased risk of cancer, stomach cancer, and lung cancer were observed. Because the workers were also exposed to radon and radon daughters, silica, iron, titanium, sulfur, and arsenic, a causal relationship between copper and increased cancer risk can not be established. No studies examining the association between copper ingestion and cancer risk in humans were identified. Several animal studies have examined the carcinogenic potential of ingested copper (BRL 1968; Greene et al. 1987; Kamamoto et al. 1973). These studies are limited in scope, the studies by Green et al. (1987) and Kamamoto et al. (1973) only examined one potential target, and tested fairly low doses of copper. No dermal carcinogenicity studies in humans or animals were identified. Additional studies by the inhalation, oral, and dermal routes are needed to assess the carcinogenic potential of copper in humans.

Genotoxicity. No data on the genotoxicity of copper in humans were located; studies of workers or individuals accidentally exposed to high levels of copper would provide value information on its genotoxic potential in humans. The available genotoxicity data suggest that copper is a clastogenic agent (Agarwal et al. 1990; Bhunya and Jena 1996; Bhunya and Pati 1987; Sideris et al. 1988). However, mixed results have been found in point mutation assays (Demerec et al. 1951; Marzin and Phi 1985; Singh 1983; Tso and Fung 1981; Wong 1988). Additional studies are needed to assess copper's potential to induce point mutations. Several studies have also shown that exposure to copper can result in DNA damage (Garrett and Lewtas 1983; Sideris et al. 1988; Sina et al. 1983).

Reproductive Toxicity. There are no human studies and two animal studies that examined the potential of copper to induce reproductive effects. These studies did not find any adverse alterations in reproductive performance in mink (Aulerich et al. 1982), sperm morphology in rats and mice (NTP 1993), or vaginal cytology in rats or mice (NTP 1993). The NTP (1993) study also did not find histological alterations in reproductive tissues. Multigeneration or continuous breeding studies would provide information on the reproductive effects of copper in animals, which may be used to assess possible reproductive effects in humans exposed to high levels of copper.

Developmental Toxicity. Developmental studies by the oral route in rats (Haddad et al. 1991) and mice (Lecyk 1980) have shown that high copper intakes can result in impaired growth. The developmental toxicity of copper in humans has not been adequately investigated. No data were located regarding developmental effects of copper after inhalation or dermal exposures in humans or animals. Further studies in other animal species would provide valuable information on the potential of copper to adversely affect development. Such information might be relevant to humans.

Immunotoxicity. There are limited data on the immunotoxic potential of copper and its compounds. Reports on humans developing dermatitis after dermal exposure to copper (Barranco 1972; Saltzer and Wilson 1968) suggest that copper is an allergen. This is supported by a report of a woman developing dermatitis after insertion of a copper IUD (Barranco 1972). Immunological effects also have been observed in mice (Drummond et al. 1986) following acute inhalation exposure to copper sulfate. In addition, impaired immune function has been observed in mice exposed to copper chloride (Pocino et al. 1991) or copper sulfate (Pocino et al. 1990) in drinking water. Intermediate-duration studies concentrating on immunologic effects in different species would be useful for establishing dose-response relationships and assessing whether there are species differences. More studies in humans and animals that examine the immune response to copper exposure and the mechanisms involved therein would be useful.

Neurotoxicity. Neurological impairment has been observed in factory workers exposed to copper dust. No effects on neurobehavioral performance were observed in rats exposed to copper in the diet (Murthy et al. 1981). However, this study did find alterations in the levels of a dopamine metabolite, suggesting that copper may adversely affect the nervous system. Additional studies are needed to further investigate the neurotoxic potential of copper; these studies should assess the potential of copper to perturb dopaminergic pathways and related functions.

Epidemiological and Human Dosimetry Studies. Several studies have examined the toxicity of inhaled copper in workers (Askergren and Mellgren 1975; Finelli et al. 1981; Suciu et al. 1981). These studies have primarily focused on the respiratory tract, although health examinations revealed other adverse effects (e.g., hepatomegaly). Chen et al. (1993, 1995) examined the carcinogenic potential of inhaled copper. In general, these studies are limited by poor exposure characterization, co-exposure to several toxic and/or carcinogenic compounds (e.g., arsenic, cadmium, radon, lead), and limited number of end points examined. Occupational exposure studies examining populations of workers exposed to

copper and with minimal exposure to other metals would be useful in assessing the toxicity of inhaled copper. These studies should examine a wide variety of end points, particularly the gastrointestinal tract, liver, and kidneys, which are targets of toxicity following oral exposure.

There are numerous reports of accidental or intentional ingestion of copper. The most commonly reported effect in these studies is gastrointestinal upset. There have also been several experimental studies designed to identify a no effect level for gastrointestinal upset following short-term (2 weeks or less) exposure to copper in drinking water (Olivares et al. 2001; Pizarro et al. 1999, 2001). There are several subpopulations of individuals exposed to higher than normal levels of copper; these groups include communities with higher than normal levels of copper in drinking water and individuals ingesting higher than normal levels of copper in the form of supplements. Studies of these groups that involved examination for a variety of potential effects (including gastrointestinal, hepatic, and renal effects, which have been shown to be sensitive end points in animal studies) could provide useful information on the toxicity of copper in otherwise healthy humans. In addition, if the study group included both children and adults, these data could address the issue of age-related differences in toxicity.

Biomarkers of Exposure and Effect.

Exposure. Copper levels can be measured in tissues, body fluids, and excreta. Whole blood, serum, and urine copper levels have been established in healthy individuals. It has been demonstrated that copper levels in the body increase with increased exposure after acute poisoning. Similarly, increased copper levels were observed in workers after occupational exposure. Serum and urine copper levels, plasma ceruloplasmin levels, and clinical manifestations are specific indicators of copper status. It is doubtful that a single “specific” biomarker of intoxication resulting from exposure to a specific metal will be found. In any case, elevated tissue copper levels should be a sufficient indicator of exposure and the possibility of intoxication.

Effect. There are no specific biomarkers for copper toxicity. Individuals with Wilson's disease are usually diagnosed by examining serum and urine copper levels, plasma ceruloplasmin levels, and clinical manifestations. However, the relationship between serum and urine levels of copper and health effects are not known. Studies examining the possible correlation between blood levels or excreta levels of copper with effects would facilitate medical surveillance leading to early detection and possible treatment.

Absorption, Distribution, Metabolism, and Excretion. The absorption, distribution, metabolism, and excretion of copper administered orally have been studied in animals and, to some

extent, in humans. Furthermore, alterations in copper absorption, distribution, and excretion have been studied in deficiency and toxicity states. Despite the information on copper absorption, there is very little information on differences between absorption rates of the various Cu(II) compounds and differences between the bioavailability of copper from food and water.

Several studies have shown that ingested or implanted metallic copper results in increased serum copper levels and liver toxicity (Keller and Kaminski 1984; Yelin et al. 1987). Studies on the release of copper ions from both ingested and implanted metallic copper would be useful.

There is very limited information on copper absorption following inhalation exposure, and data on the absorption of copper through the skin are limited. Further studies in animals on the rate and extent of copper absorption following exposure from both the inhalation route and the dermal route would more fully characterize copper toxicokinetics in animals and by extrapolation in humans.

There is evidence that animals develop a tolerance to continued high doses of copper; more information on the mechanism(s) involved might be useful to establish if humans also could develop tolerance, as well as to provide insight for the development of more effective and efficient treatment of copper toxicity.

Comparative Toxicokinetics. The metabolism of copper has been studied in rats, pigs, hamsters, and humans. However, there are no comparative studies on the effects of high copper intakes on the distribution of copper in the body or the development of tolerance to continued high intakes of copper. Furthermore, the animal species that might serve as the best model for extrapolating results to humans is not known.

Methods for Reducing Toxic Effects. Methods for reducing the toxic effects of copper have primarily focused on reducing body burdens. Many of these methods have been designed for individuals with Wilson's disease; however, it is likely that these would also be effective in other instances of copper intoxication. D-penicillamine (Rodeck et al. 1999; Walshe 1996; Walshe and Yealland 1993) is the most commonly used palliative agent for Wilson's disease; however, it has a number of potentially deleterious side effects. Studies in animals suggest that TETREN and TAUD may also be effective chelating agents (Domingo et al. 2000). Other treatment methods include administration of tetrathiomolybdate (Humphries et al. 1988; Kumaratilake and Howell 1989; Ogra et al. 1996), diets high in molybdenum and sulfur (van Ryssen 1994), and diets high in zinc (Brewer et al. 1992; van Ryssen 1994). Further studies

are needed to identify treatments that would interfere with copper's mechanism of toxicity and reduce body burden with minimal side effects.

Children's Susceptibility. There are some data on the toxicity of copper in infants and children. Severe liver damage has been reported in infants and children. These effects are typically clustered in geographically regions and have been grouped into two syndromes: Indian childhood cirrhosis and idiopathic copper toxicosis. Both of these syndromes are associated with elevated copper intakes and early dietary introduction of milk and/or formula, and are believed to have a genetic component. Very high levels of copper are found in the livers of affected children, suggesting that the mechanism of action is related to impaired copper efflux. Additional studies are needed to determine the mechanism of toxicity and to ascertain copper's role in the observed effects. Information that would provide a better understanding of copper absorption and excretion in early infancy and homeostatic mechanisms in infants would also provide valuable documentation on these syndromes and their relationship to copper.

Child health data needs relating to exposure are discussed in Section 6.8.1 Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Ongoing studies pertaining to copper have been identified and are shown in Table 3-5 (FEDRIP 2003).

Table 3-5. Ongoing Studies on Copper

Investigator	Affiliation	Research description	Sponsor
Turnland JR	Agricultural Research Service, Davis, California	Influence of high copper intake on copper homeostasis and mineral metabolism	USDA
Kelvay LM	Agricultural Research Service, Grand Forks, North Dakota	Determination of a no effect level for copper	USDA
Reeves PG	Agricultural Research Service, Grand Forks, North Dakota	Correlation between sperm motility and copper status in humans and animals	USDA
Harris ED	Texas A & M University	Copper metabolism and homeostasis in humans and animals	CSREES TEX
Thiele DJ	University of Michigan at Ann Arbor	Copper homeostasis	NIGMS
Culotta VC	John Hopkins University	Intracellular pathways of copper trafficking	NIEHS
Gitlin JD	Washington University	Copper chaperones	NIDDKD

CSREES TEX = Cooperative State Research Education and Extension Service, Texas; NIDDKD = National Institute of Diabetes and Digestive and Kidney Disease; NIEHS = National Institute of Environmental Health and Science; NIGMS = National Institute of General Medical Sciences; USDA = U.S. Department of Agriculture