CHAPTER 2. HEALTH EFFECTS

2.1 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.

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 by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to copper, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with exposure to copper was also conducted; the results of this review are presented in Appendix C.

Animal and human inhalation studies are presented in Table 2-1 and Figure 2-2, and animal and human oral studies are presented in Table 2-2 and Figure 2-3.

Levels of significant exposure (LSEs) 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. Effects have been classified into "less serious LOAELs" or "serious LOAELs (SLOAELs)." "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 endpoint 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 endpoints. 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.

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

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. The RDA for adult humans is 0.9 mg Cu/day (IOM 2006), and typical diets in the United States contain adequate copper to meet this requirement (NIH 2022). The normal serum copper level in human adults is 10–25 μmol/L (64–160 μg/dL) (IOM 2006). In the human body, copper levels are carefully regulated by transporter proteins that control its absorption, distribution, and excretion (see Chapter 3).

Copper deficiency is relatively rare in humans, but has occurred in infants given formula or cow's milk deficient in copper (IOM 2006). In addition, intake of high levels of zinc or iron may interfere with copper absorption and lead to deficiencies (IOM 2006). Finally, Menke's disease, caused by a mutation in the Menkes P-type ATPase gene, results in impaired copper absorption and copper deficiency (IOM 2006). Copper deficiency is associated with anemia, leukopenia, neutropenia, and osteoporosis (IOM 2006). Several diseases in which copper accumulates in the body have also been identified in humans. These diseases, characterized by severe liver toxicity, are described briefly in Section 2.4, Hepatic.

This toxicological profile is focused on the effects of excess copper exposure from exogenous sources (i.e., not resulting from impaired excretion of copper). Studies of excess copper effects in humans include controlled human studies, epidemiological studies, occupational and community health investigations, and case reports/case series. Controlled human exposure studies are included in the LSE tables for the appropriate exposure routes. Epidemiological studies that met inclusion criteria (see Appendix C, Section C.2.2) are summarized in tables and/or text within each health effect subsection below.

Occupational and community health investigations and case reports/case series are described in text in the corresponding health effect subsection.

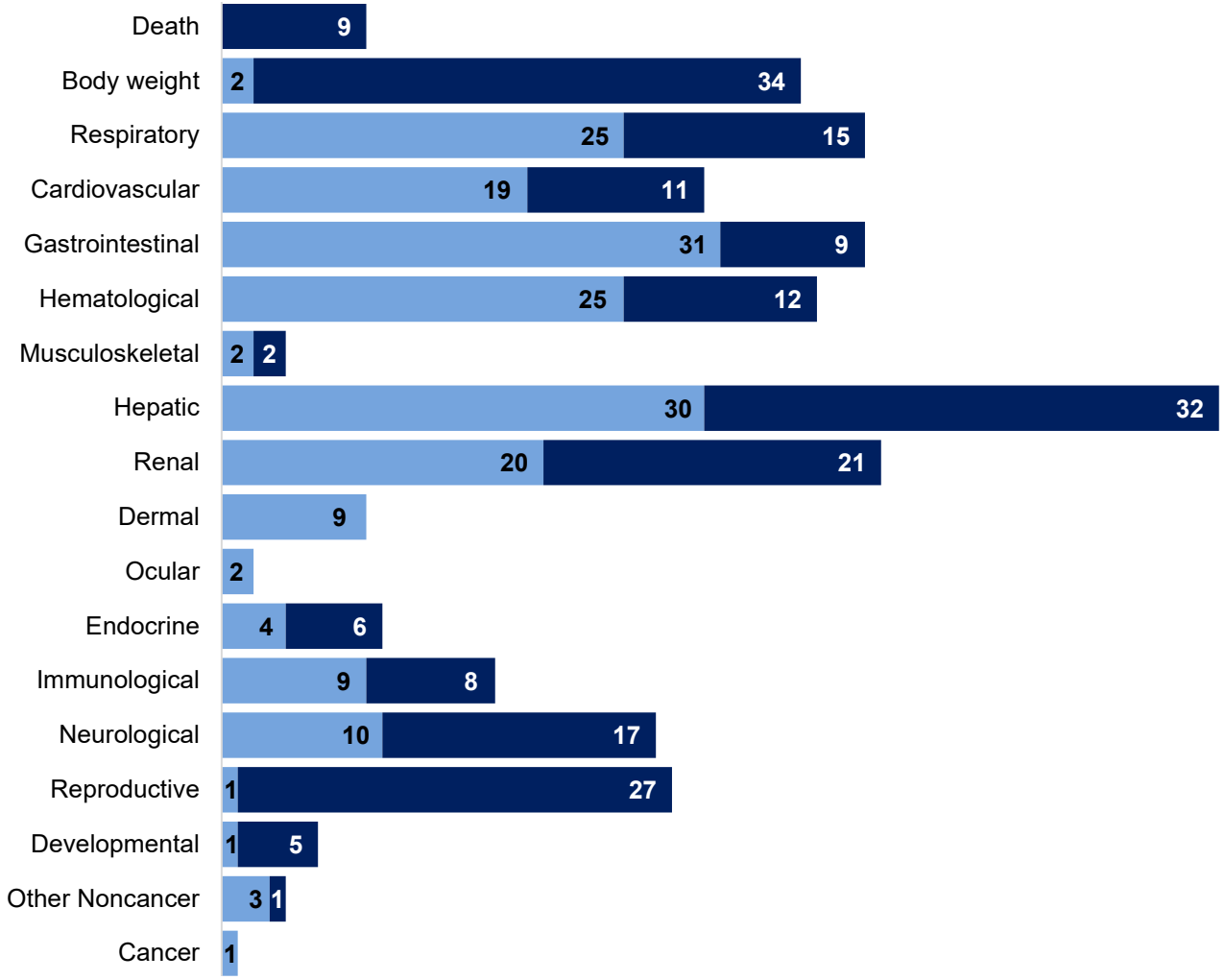
The database of animal studies investigating health effects of copper is large, and the quality of the studies varies widely. Only studies that met inclusion criteria (see Appendix C, Section C.2.2) are discussed in Chapter 2. It is important to note that the majority of animal studies did not report the concentration of copper in the controls' diet or drinking water. As an essential element, copper is typically a constituent of laboratory animal feed, and may also occur in tap water. For the purpose of hazard identification, it is assumed that modern studies provided adequate copper intake in controls to prevent effects of deficiency. Similarly, since copper absorption in the gastrointestinal tract is reduced when zinc intake is high, it is assumed that studies included herein provided adequate, but not excessive, zinc intake for control and exposed animals.

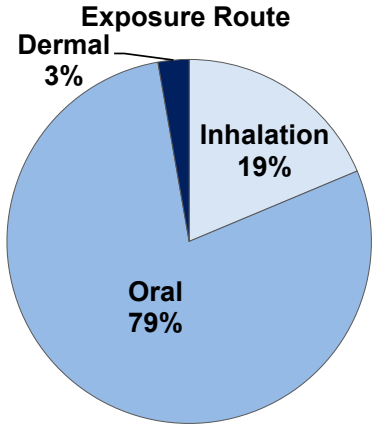
Information in this toxicological profile on health effects of copper comes from 88 human and 94 animal studies that met inclusion criteria. Relevant health effects data for copper are shown in Figure 2-1. As indicated in the figure, the largest numbers of human studies examined gastrointestinal and hepatic effects; the vast majority of these were case reports or case series. Most of the animal studies administered copper orally. The animal studies primarily examined body weight, hepatic, and reproductive effects. Human studies suggest that gastrointestinal effects are a sensitive target of oral exposure to copper, while animal studies suggest that hepatic effects are a sensitive target of oral exposure and respiratory effects are a sensitive target of inhalation exposure.

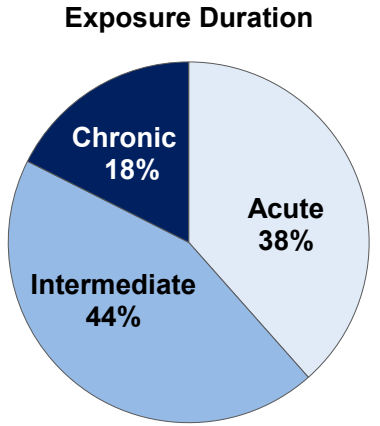
- **Gastrointestinal endpoints:** Gastrointestinal toxicity is a known health effect in humans exposed orally to copper based on a high level of evidence in humans and a high level of evidence in animals.
- **Respiratory endpoints:** Respiratory toxicity is a presumed health effect in humans based on a low level of evidence in humans and a high level of evidence in animals exposed by inhalation.
- **Hepatic endpoints:** Hepatic system toxicity is a presumed health effect in humans based on a high level of evidence in animals.

Figure 2-1. Overview of the Number of Studies Examining Copper Health Effects*

Most studies examined the potential gastrointestinal and hepatic effects of copper.
More studies have evaluated health effects in **animals** than **humans** (counts represent studies examining endpoint).







*Includes studies discussed in Chapter 2. A total of 161 studies (including those finding no effect) have examined toxicity. Studies may have examined more than one endpoint.

Table 2-1. Levels of Significant Exposure to Copper – Inhalation (mg Cu/m^3)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Poland et al. 2022									**Dicopper oxide**
1	Rat (Crl:CD (SD)) 5 M, 5 F	6 hours/day 5 days/week 2 weeks (WB)	0, 0.18, 0.71, 1.78, 8.9	LE, CS, BW, FI, GN, OW, HP	Bd wt	8.9			
					Resp	0.71	1.78		Alveolar histiocytosis in both sexes; increased absolute and relative lung weight in females
					Hepatic	8.9			
					Renal	8.9			
Poland et al. 2022									**Copper sulfate pentahydrate**
2	Rat (Crl:CD (SD)) 5 M, 5 F	6 hours/day 5 days/week 2 weeks (WB)	0, 0.18, 0.71, 1.78, 8.9	LE, CS, BW, FI, GN, OW, HP	Resp	0.18	0.71		Alveolar histiocytosis in both sexes, bronchioloalveolar hyperplasia in males
					Hepatic	8.9			
					Renal	8.9			
INTERMEDIATE EXPOSURE									
Poland et al. 2022									**Dicopper oxide**
3	Rat (Crl:CD (SD)) 10–20 M, 10–20 F	6 hours/day 5 days/week 4 weeks (WB)	0, 0.18, 0.35, 0.7, 1.76	LE, CS, BW, FI, HE, GN, OW, HP	Bd wt	1.76			
					Resp	0.18	0.35		Increased absolute and relative lung weight, neutrophilic inflammation in lungs, alveolar histiocytosis, increased LDH and total protein in BALF
					Hemato	1.76			
					Hepatic	1.76			
					Renal	1.76			
					Neuro	1.76			
Johansson et al. 1983									**Copper chloride**
4	Rabbit (NS) 8 M	1 month 5 days/week 6 hours/day	0, 0.6	IX	Resp	0.6			
					Immuno	0.6			

Table 2-1. Levels of Significant Exposure to Copper – Inhalation (mg Cu/m^3)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Johansson et al. 1984									**Copper chloride**
5	Rabbit (NS) 8 M	4-6 weeks 5 days/week 6 hours/day	0, 0.6	GN, HP	Resp	0.6			

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive sex are presented.

BALF = bronchoalveolar lavage fluid; Bd wt or BW = body weight; CS = clinical signs; F = female(s); FI = food intake; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; IX = immune function; LDH = lactate dehydrogenase; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; Resp = respiratory; WB = whole body

Figure 2-2. Levels of Significant Exposure to Copper – Inhalation
Acute (≤14 days)

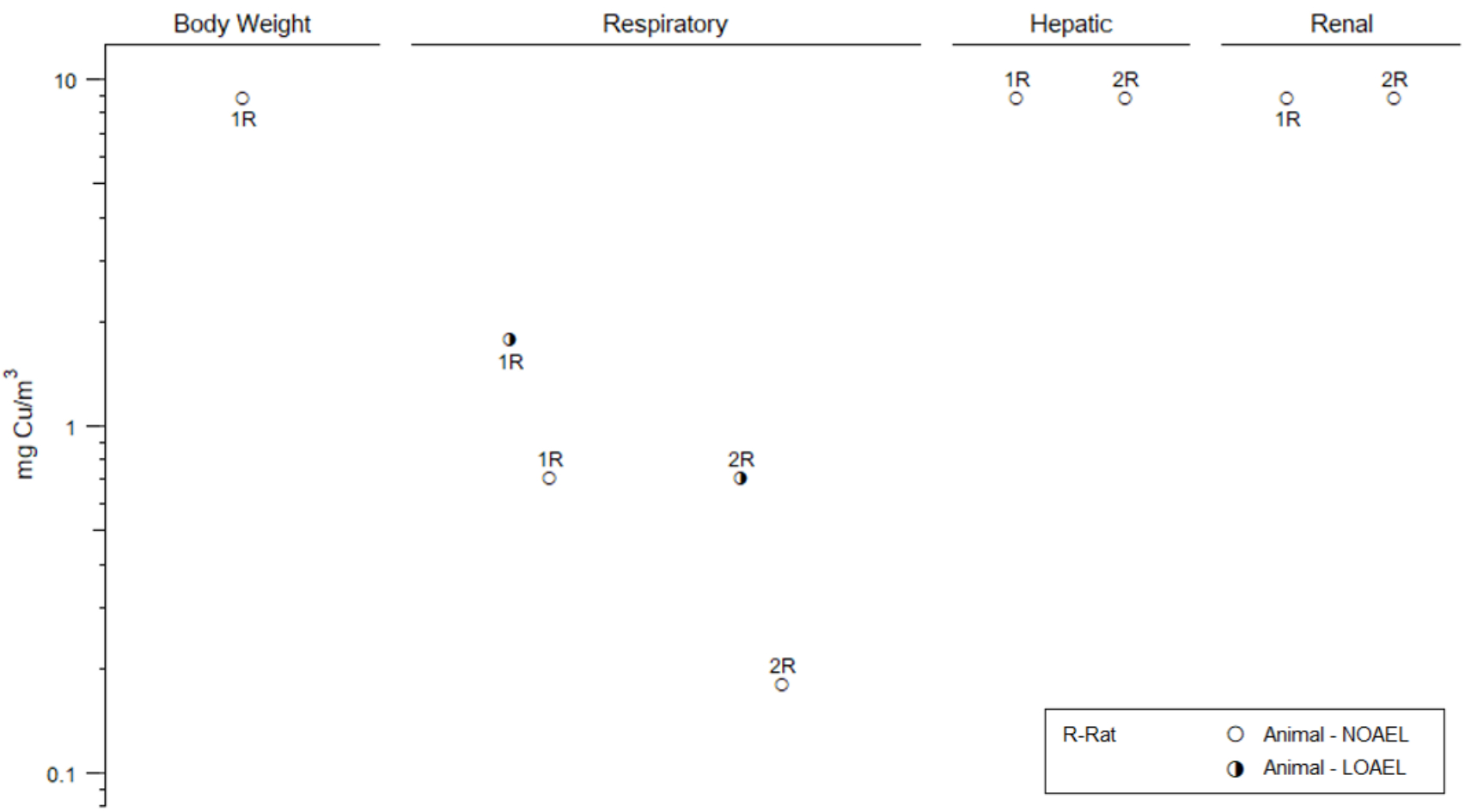


Figure 2-2. Levels of Significant Exposure to Copper – Inhalation
Intermediate (15–364 days)

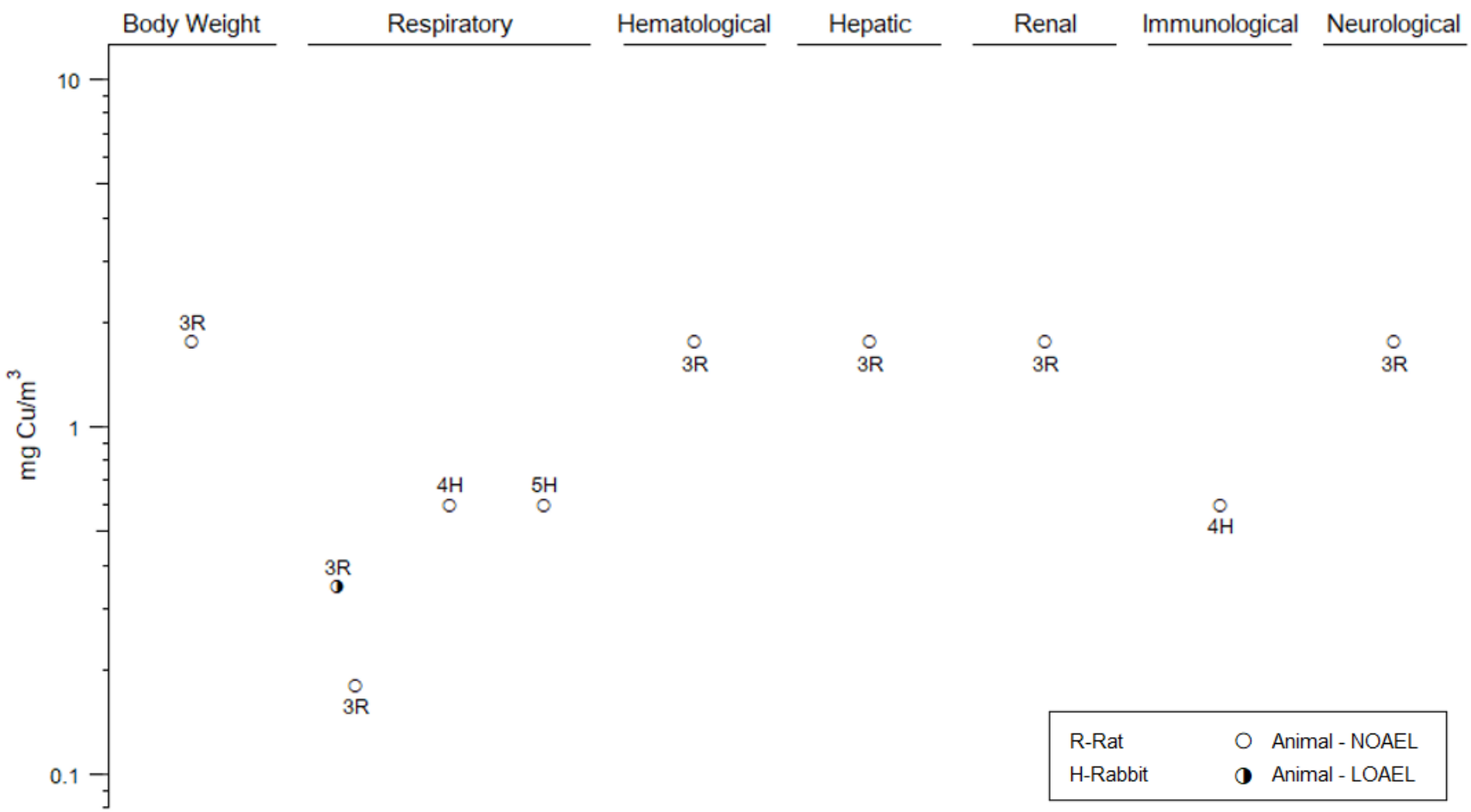


Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Araya et al. 2001									
1	Human 179 B	Once (W)	0, 0.006, 0.012, 0.018, 0.025	CS	Gastro	0.012	0.018		Significantly increased frequency of nausea, 17/179 subjects
Araya et al. 2003a									
2	Human 15 M, 15 F	Once (W)	0, 0.046	OF	Gastro		0.046		Nausea in 9/30 subjects and delayed gastric emptying
Araya et al. 2003c									
3	Human 269 F	Once (W)	0, 0.006, 0.012, 0.018, 0.025	CS, WI	Gastro	0.012	0.018		Nausea in 50/269 subjects.
Gotteland et al. 2001									
4	Human 15 M, 16 F	Once (W)	0, 0.03	CS, OF	Gastro		0.03		Nausea (6/31 subjects) and vomiting (2/31 subjects)
Olivares et al. 2001									
5	Human 30 M, 31 F	Once (W)	0, 0.006, 0.012, 0.018, 0.025, 0.031, 0.037	CS	Gastro	0.006	0.012		Nausea in 5/53 participants
Pizarro et al. 1999									
6	Human 60 F	2 weeks, daily (W)	0.0006, 0.03, 0.07, 0.1	CS, BW, BI	Bd wt	0.1			
					Gastro	0.03	0.07[b]		Abdominal pain, nausea, and/or vomiting
					Hemato	0.1			
					Hepatic	0.1			
Pizarro et al. 2001									
7	Human 45 F	1 week, daily (W)	0, 0.1	CS, BI	Gastro		0.1		Nausea, vomiting, and/or abdominal pain
					Hepatic	0.1			

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Abdel-Baky 2019									
8	Rat (Wistar Albino) 6 M	2 weeks (G)	0, 25.5, 50.9	BC	Renal		25.5		Increased serum urea, uric acid, and creatinine
					Repro		25.5		Decreased serum total testosterone, FSH, LH, and prolactin
Alharbi et al. 2019									
9	Rat (albino) 10 F	7 days, daily (NS)	0, 119	BC, BI, HP	Renal			119	Severely damaged glomeruli corpuscles, hyperplasia of the epithelial cells lining the partial layer of Bowman's capsule, and severely damaged epithelial lining of the proximal and distal convoluted tubules; increased serum urea, creatinine and uric acid levels
Alhusaini et al. 2018a									
10	Rat (Albino) 6 M	7 days, daily (NS)	0, 119	BC, BI, HP	Hepatic		119		Increased hepatic ALT activity
Alhusaini et al. 2018b									
11	Rat (Albino) 8 M	7 days, daily (NS)	0, 39.8	BI, OW, HP	Hepatic			39.8	Marked cellular degeneration and hepatocyte necrosis; increased serum AST, ALT, and LDH activities
Haywood 1980									
12	Rat (NS) 2–4 M	1–2 weeks (F)	0, 300	GN, HP	Hepatic		300		Parenchymal cell hypertrophy
					Renal	300			
Haywood and Comerford 1980									
13	Rat (NS) 4 M	1–2 weeks (F)	0, 300	BC	Hepatic		300		Increased serum ALT activity

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Husain et al. 2021									
14	Rat (Wistar) 6 M	Once (W)	0, 2.4, 7.1, 14, 19	LE, HP	Gastro	7.1	14		Histopathological changes in the duodenum (loss of regular arrangement of enterocytes and their brush borders, necrotic debris, and increased lymphocytes and plasma cells)
Husain et al. 2023									
15	Rat (Wistar) 6 M	Once (GW)	0, 2, 7.1, 14, 19	LE, BC, HP	Renal		2		Mild interstitial bleeding in kidneys; increased BUN and serum creatinine
Sarawi et al. 2022									
16	Rat (Wistar) 8 M	7 days (G)	0, 39.8	BC, BI, HP, RX	Repro			39.8	Absence of mature spermatozoa, degeneration of seminiferous tubules, and loss of spermatogenic series; decreased serum FSH, LH, and testosterone
Al-Musawi et al. 2022									
17	Mouse (BALB/c) 6 M	2 weeks (G)	0, 6.4, 8.9	BW, OW, HP, RX	Bd wt			6.4	28% decrease in body weight
					Repro			6.4	Infertility
Babaei et al. 2012									
18	Mouse (NMRI) 6 F	14 days, daily (G)	0, 39.8, 79.6	BC, HP	Repro			39.8	Decreased number of antral follicles and ovarian cell damage
Kadammattil et al. 2018									
19	Mouse (Swiss albino) F NS	7 days, daily (days 7–12 of pregnancy) (NS)	0, 4.0	DX, RX	Repro	4			
					Develop	4			

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kadammattil et al. 2018									
20	Mouse (Swiss albino) 2 NS	Once (NS)	39.8	LE	Death			39.8	LD_{50} (up and down method)
Kadammattil et al. 2018									
21	Mouse (Swiss albino) M NS	Once	0, 4.0	RX	Repro	4			
Yamamoto et al. 2004									
22	Shrew (*Suncus murinus*) 4 F	Once (G)	0, 2.5, 31	CS, FI	Gastro	2.5	31		15 episodes of emesis in 4/4 animals
INTERMEDIATE EXPOSURE									
Araya et al. 2003b, 2004									
23	Human 327–355 B	2 months, daily (W)	0, 0.001, 0.055, 0.11, 0.17	CS, WI, BC, BI	Gastro	0.055	0.11		Significant increase in gastrointestinal symptoms (65/355 subjects)
					Hepatic	0.17			
Harvey et al. 2003									
24	Human 12 M	6 weeks, daily (F)	0.009 (control), 0.02, and 0.08	HE, BC, BI	Hemato	0.02			
O'Connor et al. 2003									
25	Human 11 M, 11 F	6 weeks, daily (F)	M: 0.018, 0.058; F: 0.017, 0.067	BW, BC, BI	Hepatic	0.067 F 0.058 M			
Olivares et al. 1998									
26	Human 48–80 B	9 months, daily (W)	0.0378–0.174, 0.0522–0.319	CS, BW, BC	Bd wt	0.319			
					Gastro	0.319			
					Hepatic	0.319			

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Pratt et al. 1985									
27	Human 3 M, 4 F	12 weeks (C)	0, 0.15	BC	Gastro	0.15			
					Hemato	0.15			
					Hepatic	0.15			
Rojas-Sobarzo et al. 2013									
28	Human 30 M	6 months	0, 0.1	BC, BI	Hepatic	0.1			
Abe et al. 2008									
29	Rat (Fischer-344) 6–8 M	6 weeks daily (F)	0, 62	BW, HP	Bd wt	62			
					Hepatic	62			
Adele et al. 2023									
30	Rat (Wistar) 5 F	5 weeks (NS)	0, 39.8	BW, HE, OW	Bd wt	39.8			
					Hemato		39.8		Decreased erythrocyte count, hemoglobin, and hematocrit
					Hepatic	39.8			
					Immuno		39.8		Decreased WBC count
Ali et al. 2023									
31	Rat (Sprague-Dawley) 4 F	4 months (W)	0, 11.3	HP	Cardio			11.3	Increased cardiac injury score (myocyte damage and necrosis), mast cell infiltration and collagen deposition in the heart
Ali et al. 2023									
32	Rat (Sprague-Dawley) 6 F	4 months (W)	0, 11.3	BW, OW, HP	Bd wt	11.3			
					Cardio			11.3	Fibrosis and collagen deposition in the heart; myocardial damage; increased absolute and relative heart weight

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Arafa et al. 2019									
33	Rat (Wistar) 10 M	90 days, daily (G)	0, 50.9	CS, BI, HP	Cardio		50.9		Increase in systolic blood pressure
					Repro			50.9	Reductions in relative testicular weight, serum testosterone, and serum LH
Arowoogun et al. 2021									
34	Rat (Wistar) 5 M	7 weeks 3 times/week (GO)	0, 79.6	BW, HP	Neuro			79.6	Focal areas of necrosis and degenerated neurons in the cerebellum (not reported quantitatively); ~35% decrease in brain AChE activity
Babaei and Abshenas 2013									
35	Rat (Sprague-Dawley) 12 M	56 days, daily (G)	0, 79.6	OW, HP, RX	Repro			79.6	Reduced testicular weight; decreased sperm count, percentage of live spermatozoa, and sperm motility
Chen et al. 2023									
36	Rat (Wistar) 10 F	35 days (G)	0, 6, 12, 25	OW, HP	Bd wt	6	12		10% decrease in body weight
					Repro		6		Decreased percentage preantral ovarian follicles; increased percentages of antral and atretic follicles
Chung et al. 2009									
37	Rat (Sprague-Dawley) 12 M, 12 F	M: 30 days F: 38 days (GW)	0, 0.83, 3, 13, 51	LE, CS, BW, FI, HE, BC, UR, GN, OW, HP, RX, DX	Death			51 F	3/12 died
					Bd wt	51			
					Resp	51			
					Cardio	51			
					Gastro	0.83 F	3 F		Increased incidences of squamous cell hyperplasia in the stomach
						3 M	13 M		Increased incidences of squamous cell hyperplasia in the stomach

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hemato	51 F			
						13 M	51 M		Decreased erythrocyte count, hemoglobin, hematocrit, MCV, and MCH; increased platelets, WBCs, and neutrophils
					Hepatic	51			
					Renal	51			
					Endocr	51			
					Immuno	51			
					Neuro	51			
					Repro	51			
					Develop	13	51		Increased percentage of runt pups (weighing 1/3 less than control mean weight) and pups with icterus
De Vries et al. 1986									
38	Rat (Sprague-Dawley) 8 F	11 months, daily (W)	0, 46	BI	Neuro		46		Decreased 3,4-dihydroxyphenylacetic acid levels in corpus striatum
Draper et al. 2023									
39	Rat (Sprague-Dawley) 6 M	28 days (GW)	0, 161.5	HP	Resp		161.5		Slight histological changes in the lungs (thickened interalveolar septa, stratified epithelia, smooth muscle disruption, epithelial desquamation)
Epstein et al. 1982									
40	Rat (Sprague-Dawley) 8 M	90 days, daily (W)	0, 8.6	BW, WI, BC	Bd wt	8.6			
					Hepatic		8.6		Increased serum AST activity

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Fuentealba et al. 2000									
41	Rat (Fischer-344) 4–5 M, 4–11 F	12–18 weeks (F)	Young rats, M: 0, 150; F: 0, 170 Adult rats, M: 0, 120; F: 0, 130	BC, HP, DX	Death			150 F	2/8 young female rats died during experiment
					Hepatic			120 M	Multifocal hepatitis, widespread single cell necrosis, and increased serum ALT and SDH activities in adult rats after 18 weeks
Gupta et al. 2021									
42	Rat (Wistar) 5 M	24 weeks (GW)	0, 8.0	BW, FI, WI, BC, BI, OW, HP, RX	Bd wt	8			
					Repro			8	Shrunken seminiferous tubules; decreases in the following: absolute testis weight, sperm count, percent motile sperm, and percent viable sperm; and an increase in morphological abnormalities in sperm
Haywood 1980									
43	Rat (NS) 2–4 M	3–15 weeks (F)	0, 180	GN, HP	Hepatic			180	Massive necrosis, inflammatory cell infiltration, bile duct hyperplasia, progressing to fine diffuse fibrosis by 15 weeks
					Renal		180		Cytoplasmic droplets and desquamation of epithelial cells in proximal tubules
Haywood and Comerford 1980									
44	Rat (NS) 4 M	3–15 weeks (F)	0, 180	BC	Hepatic		180		Increased ALT activity
Haywood and Loughran 1985									
45	Rat (Wistar) 24 M	5–15 weeks (F)	0, 320, 420, 530, 640	BW, HP	Bd wt			320	~50% decrease in terminal body weight
					Hepatic			320	Diffuse and extensive necrosis by week 5

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kalita et al. 2020									
46	Rat (Wistar) 6 M	1 month, daily (G)	0, 25.5	BW, BI, HP, NX	Bd wt	25.5			
					Neuro			25.5	Reduced locomotor activity (reduced distance traveled and time moving); reduced grip strength; and reduced latency to fall time on the rotarod test and increased time resting
Kumar and Sharma 1987									
47	Rat (Albino) 15 M	30 days, daily (G)	0, 39.8	BW, BC, BI	Hemato		39.8		Decreased erythrocyte count and hemoglobin
					Hepatic		39.8		Increased serum ALT with increased cholesterol and bilirubin and decreased total protein levels
					Renal		39.8		Increased urea levels
Kumar et al. 2015, 2016a, 2016b									
48	Rat (Wistar) 18 M	30, 60, or 90 days, daily (G)	0, 25.5, 50.9	BW, BC, HE, HP, NX	Bd wt			25.5	26% decrease in body weight at 90 days
					Hemato		25.5		Decreased hemoglobin at 60 and 90 days
					Hepatic			25.5	Hepatocellular degeneration and hemorrhage, massive fatty change and centrilobular necrosis, occasional hepatic cell necrosis; increased ALT, AST, and bilirubin at 90 days
					Renal			25.5	Hemorrhage, inflammatory and cellular damage in kidneys, and degeneration of renal intertubular space and Bowmen's capsule; increased BUN and BUN/creatinine ratio after 90 days

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro			25.5	Impaired motor coordination and cognitive function (grip strength, latency to fall time, and attention scores); gliosis; pyknotic nuclei, and glial nodule formation in brain after 90 days
Kumar et al. 2019									
49	Rat (Sprague-Dawley) 5 M	16 weeks, daily (G)	0, 2.6, 5.1	CS, BW, BC, NX	Bd wt	5.1			
					Neuro			2.6	Decreased locomotor activity and neuromuscular coordination, decreased passive avoidance response, less exploration time
Liu and Medeiros 1986									
50	Rat (Wistar) 10 M	15 weeks (F)	0, 14	CS, BW, FI, WI, BC, UR, OW	Cardio		14		Increased blood pressure
Liu et al. 2016									
51	Rat (Wistar) 10 M	30 days, daily (G)	0, 39.8, 79.6, 159	OW, HP, RX	Repro		39.8	79.6	LOAEL: Decreased sperm count and serum LH and FSH SLOAEL: Marked reduction in sperm count and increase in sperm malformation rate; significant reductions in serum testosterone, FSH, and LH
Llewellyn et al. 1985									
52	Rat (Holtzman) 10 M	21 weeks (F)	0, 120	BW, FI, WI, OW	Bd wt		120		Decreased body weight gain (23%)
					Musc/skel	120			
Murthy et al. 1981									
53	Rat (NS) 48 M	Daily, 30 days (F)	0, 23	CS, BW	Neuro		23		Increase in dopamine and norepinephrine with 21% casein diet, and decrease in 5-hydroxy-tryptamine with 10% casein diet

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 1993									
54	Rat (Fischer-344) 5 M, 5 F	6–15 days, daily (W)	M: 0, 10, 29, 36, 45, 96; F: 0, 10, 26, 31, 71	CS, BW, WI, GN, HP	Death			31 F	5/5 died
								36 M	5/5 died
					Bd wt	26 F		31 F	46% decrease in body weight
						29 M		36 M	48% decrease in body weight
					Resp	26 F			
						29 M			
					Cardio	26 F			
						29 M			
					Gastro	26 F			
						29 M			
					Hepatic	26 F			
						29 M			
					Renal	26 F			
							10 M		Protein droplets in epithelial cells of proximal tubule
					Endocr	26 F			
						29 M			
					Immuno	26 F			
						29 M			
					Neuro	26 F			
						29 M			
					Repro	26 F			
						29 M			

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 1993									
55	Rat (Fischer-344) 5 M, 5 F	15 days, daily (F)	M: 0, 23, 46, 92, 198, 324; F: 0, 23, 44, 93, 196, 285	CS, BW, FI, WI, GN, OW, HP	Bd wt	196 F	285 F		13% decrease in body weight
						92 M	198 M		18% decrease in body weight
					Resp	285 F			
						324 M			
					Cardio	285 F			
						324 M			
					Gastro	23 F	44 F		Hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach
						23 M	46 M		Hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach
					Hemato	93 F		196 F	Depletion of hematopoietic cells in bone marrow
						92 M		198 M	Depletion of hematopoietic cells in bone marrow
					Hepatic	196 F	285 F		Minimal to mild mononuclear inflammatory cell infiltrate in three of five females
						92 M	198 M		Minimal to mild mononuclear inflammatory cell infiltrate in 4/5 males
					Renal	44 F	93 F		Increased protein droplets in cortical tubules
						46 M	92 M		Increased protein droplets in cortical tubules

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Endocr	285 F			
						324 M			
					Immuno	285 F			
						324 M			
					Neuro	285 F			
						324 M			
					Repro	285 F			
						324 M			
NTP 1993									
56	Rat (Fischer-344) 10 M, 10 F	13 weeks, daily (F)	M: 0, 8, 16, 33, 66, 140 F: 0, 9, 17, 34, 68, 134	CS, BC, BI, UR, GN, OW, HP	Bd wt	134 F			
						66 M		140 M	24% decrease in body weight by end of experiment
					Resp	134 F			
						140 M			
					Cardio	134 F			
						140 M			
					Gastro	17 F	34 F		In 7/10 females, hyperplasia of limiting ridge that forms the junction of the forestomach squamous mucosa with the glandular gastric mucosa
						16 M	33 M		In 10/10 males, hyperplasia of limiting ridge that forms the junction of the forestomach squamous mucosa with the glandular gastric mucosa
					Hemato	134 F			
						33 M	66 M		Decreased hematocrit, hemoglobin, mean cell volume and mean cell hemoglobin levels; and increased reticulocytes and platelets

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	34 F		68 F	Chronic active inflammation with focal necrosis in 1/10 males; increased serum ALT
						16 M	33 M		Chronic active inflammation with focal necrosis in 1/10 males; 112% increase in serum ALT
					Renal	9 F	17 F		Increased BUN and cytoplasmic alteration in kidneys of 1/10 females
						16 M	33 M		Cytoplasmic alteration in kidneys of 3/10 males
					Endocr	134 F			
						140 M			
					Neuro	68 F		134 F	Gliosis in brain in 10/10 rats
						140 M			
					Repro	68 F 140 M		134 F	Chronic active inflammation of clitoral gland in 10/10 rats
Parlak Ak et al. 2021									
57	Rat (Sprague-Dawley) 6 M	21 days (G)	0, 127	OW, HP, RX	Repro			127	Histopathological changes in the testes (shrinkage of seminiferous tubules, vacuoles, loss of germ cells, interstitial edema); decreased sperm concentration and motility; and increased percentage of abnormal sperm
Patwa and Flora 2020									
58	Rat (Sprague-Dawley) 9 M	16 weeks (GW)	0, 8.0	BW, BC, BI, OW, HP, IX	Bd wt	8			
					Hepatic			8	Marked necrosis of hepatocytes, distorted sinusoidal space, and central vein distortion in liver

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Patwa et al. 2022									
59	Rat (Sprague-Dawley) 6 M	16 weeks (NS)	0, 8	NX	Neuro			8	Decreased spontaneous locomotor activity in open field test, impaired memory function in passive avoidance and novel object exploration tests, increased anxiety in elevated plus maze test
Rana and Kumar 1980									
60	Rat (Albino) 10 M	20 days, daily (G)	0, 39.8	CS, BW, BC, GN, HP	Bd wt			39.8	>28% decrease in body weight
					Hemato		39.8		Decreased erythrocyte count, hemoglobin, and hematocrit
					Musc/skel		39.8		Depressed skeletal growth assessed by tail length
					Hepatic			39.8	Centrilobular necrosis and perilobular sclerosis with nuclear edema in liver
					Renal		39.8		Engorgement of uriniferous tubules, necrosis of the tubules, nuclear pyknosis and cell proliferation in medullary region
Sakhaee et al. 2012									
61	Rat (Wistar) 20 M	8 weeks, daily (G)	0, 39.8, 79.6	BC, BI, HP, RX	Hepatic			39.8	Multifocal hepatitis, cell swelling in hepatocytes, centrilobular hepatocellular necrosis, and mild bile retention
					Renal		39.8		Mild tubular necrosis and hyaline cast formation in renal tubules
					Repro			39.8	Decreases in sperm concentration, motility, and viability
Seven et al. 2018									
62	Rat (Sprague-Dawley) 6 M	21 days, daily (G)	0, 199	BW, BI, HP	Bd wt	199			
					Other noncancer		199		Decreased food consumption

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Seven et al. 2020									
63	Rat (Sprague-Dawley) 6 M	21 days (GW)	0, 128	OW, HP, NX	Repro			128	Histopathological changes in the testes (loss, disorganization and vacuolation of germinal epithelium; interstitial edema); decreased sperm concentration and percent motile sperm; increased percentage of abnormal sperm
Sugawara et al. 1995									
64	Rat (Fischer-344) 6 F	60 days (F)	0.124, 17, 34, 68	BW, BC	Hepatic	17	34		Increased serum ALT and AST activities
Temiz et al. 2021									
65	Rat (Wistar Albino) 8 M	2 times/week 28 days (G)	0, 3.9	BC, BI, HP	Hepatic		3.9		Increased serum AST, ALT, and LDH; centrilobular and vacuolar degeneration, dilatation of sinusoid, focal necrosis, and inflammatory cell infiltration in all or most animals
Yu et al. 2021a									
66	Rat (Sprague-Dawley) 24 M	24 weeks (F)	15, 30, 60, 120	BW, BC, BI, OW, HP	Hepatic	30	60		Decreased hepatocyte count and percentage of hepatocyte area; hepatic cords were damaged, disordered or even absent, and increased number of cells with hyperchromatic nuclei and concentrated cytoplasm

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Yu et al. 2023									
67	Rat (Sprague-Dawley) 10 M	12 weeks (G)	0, 20, 40, 80, 160	BW, FI, HP, NX	Bd wt		160		11% decrease in terminal body weight
					Neuro	40		80	Impaired spatial learning and memory (assessed in Morris water maze test); histopathological changes in the brain (pyknosis, hyperemia, neuronal edema, vacuolation)
Adeleke et al. 2023									
68	Mouse (Swiss) 10 M	28 days (G)	0, 10, 20, 39.8	HP, NX	Neuro			10	Decreased density of viable neurons in the brain; increased immobility in the tail suspension and forced swim tests
Babaei et al. 2012									
69	Mouse (NMRI) 6 F	35 days, daily (G)	0, 39.8, 79.6	BC, HP	Repro			39.8	Significant decrease in number of ovarian follicles and corpus lutea, and ovarian cell damage
Chen et al. 2020									
70	Mouse (CD-1) 15 M	8 weeks (G)	0, 10, 39.8, 59.7	BC, HP, RX	Repro	10	39.8		Decreased sperm count and sperm motility
Dab et al. 2023									
71	Mouse (Swiss (albino)) 8 M	20 days (G)	0, 16	BW, BC	Bd wt		16		Decreased body weight gain (78%)
					Hepatic		16		Increased serum ALT
Dai et al. 2020									
72	Mouse (C57BL/6) 10 M	28 days (G)	0, 12.8, 25.5, 50.9	BW, BC, BI, OW, HP	Bd wt	50.9			
					Renal	12.9	25.5		Increased serum BUN and creatinine; tubular degeneration, necrosis, tubular dilation, cast formation, and glomerular degeneration

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Dai et al. 2023									
73	Mouse (C57BL/6) 10 M	28 days (NS)	0, 39.8	LE, BW, OW, HP	Bd wt		39.8		~10% decrease in body weight
					Renal			39.8	Severe tubular dilation, degeneration, and necrosis; increased BUN and serum creatinine
Guo and Wang 2021									
74	Mouse (ICR) 60 M	42 days (G)	0, 3.9, 7.8, 15.6	OW, HP, RX	Repro		3.9		Increased sperm malformations and decreased sperm motility and concentration
Isibor et al. 2022									
75	Mouse (Swiss) 10 M	28 days (GW)	0, 39.8	BI, NX	Neuro		39.8		Impaired spatial memory function (Y-maze test); 60% increase in brain AChE activity
Kheirandish et al. 2014									
76	Mouse (NMRI) 15 M	56 days, daily (G)	0, 79.6	GN, HP	Repro			79.6	Shrinkage of seminiferous tubules and moderate to severe degeneration of germinal layers, significantly decreased seminiferous tubule diameter, Sertoli cell nuclei diameter and epithelial height; and significantly lower meiotic index and spermatogenesis
Kvietkauskaite et al. 2004									
77	Mouse (BALB/c) 10 M	19 weeks (W)	0, 22, 42	BW, HE, BC, BI, OW, HP	Bd wt	22	42		10.3% decrease in body weight
					Hemato	42			
					Immuno		22		Decreased percent of natural killer and suppressor cells and altered immunoregulatory index

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Liu et al. 2020a, 2020b, 2021a, 2021b									
78	Mouse (ICR) 4 M, 4 F	21 or 42 days (GW)	0, 4, 8 or 16	BW, BC, OW, HP	Bd wt		4	8	LOAEL: 15% decrease in terminal body weight SLOAEL: >20% decrease in terminal body weight
					Hepatic	4	8		Disorganized hepatic cords, hepatocyte degeneration (granular and vacuolar)
NTP 1993									
79	Mouse (B6C3F1) 5 M, 5 F	8–15 days, daily (W)	M: 0, 10, 24, 57, 133, 367; F: 0, 15, 36, 62, 174, 330	BW, WI, GN, HP	Death			62 F	3/5 died
								57 M	1/5 died
					Bd wt	36 F		62 F	34% weight loss in survivors
						24 M		57 M	22% weight loss
					Resp	36 F			
						24 M			
					Cardio	36 F			
						24 M			
					Gastro	36 F			
						24 M			
					Hepatic	36 F			
						24 M			
					Renal	36 F			
						24 M			
					Endocr	36 F			
						24 M			
					Neuro	36 F			
						24 M			
					Repro	36 F			
						24 M			

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 1993									
80	Mouse (B6C3F1) 5 M, 5 F	15 days, daily (F)	M: 0, 43, 92, 197, 294, 717; F: 0, 53, 104, 216, 398, 780	CS, BW, FI, WI, GN, OW, HP	Bd wt	780 F			
						717 M			
					Resp	780 F			
						717 M			
					Cardio	780 F			
						717 M			
					Gastro	104 F	216 F		Two of five females had minimal hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge of the forestomach at its junction with the glandular gastric mucosa
						92 M	197 M		Three of five males had minimal hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge of the forestomach at its junction with the glandular gastric mucosa
					Renal	780 F			
						717 M			
NTP 1993									
81	Mouse (B6C3F1) 10 M, 10 F	13 weeks, daily (F)	M: 0, 44, 97, 187, 398, 815; F: 0, 52, 126, 267, 536, 1,058	CS, BW, FI, GN, OW, HP	Bd wt	267 F	536 F	1,058 F	LOAEL: 12% decrease in body weight SLOAEL: 24% decrease in body weight
						97 M	187 M		10% decrease in body weight
					Resp	1,058 F			
						815 M			
					Cardio	1,058 F			
						815 M			

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Gastro	126 F	267 F		In 5/10 females, hyperplasia of forestomach mucosa
						97 M	187 M		In 2/10 males, hyperplasia of forestomach mucosa
					Hepatic	1,058 F			
						815 M			
					Renal	1,058 F			
						815 M			
					Endocr	1,058 F			
						815 M			
					Neuro	267 F			
						187 M			
					Repro	536 F	1058 F		Cyst in clitoral gland in 8/10
						815 M			
Peng et al. 2020									
82	Mouse (C57BL/6) 10 M	4 weeks (GW)	0, 80.0	BC, HP	Renal			80	Increased BUN and serum creatinine; marked tubular degeneration, dilation, and necrosis in kidneys
Sakhaee et al. 2014									
83	Mouse (NMRI) 12 M	42 days, daily (G)	0, 79.6	BC, BI, GN, HP	Hepatic		79.6		Increased serum AST and ALT
					Repro			79.6	Degenerative changes in seminiferous tubules; significantly decreased sperm concentration, motility, and viability
Sakhaee et al. 2016a									
84	Mouse NMRI 6 M	28 days, once every 2 days (GW)	0, 39.8	HP, RX	Repro			39.8	Depletion and vacuolation of seminiferous epithelium; significant decreases in sperm count, motility, and viability

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Sakhaee et al. 2016a									
85	Mouse NMRI 6 M	42 days, once every 2 days (GW)	0, 39.8	HP, RX	Repro			39.8	Degeneration of the seminiferous tubules; significant decreases in sperm count, motility, and viability
Sakhaee et al. 2016b									
86	Mouse (NMRI) 6 M	42 days (GW)	0, 39.8	HP, RX	Repro			39.8	Disorganization and vacuolation of seminiferous epithelium; significant decreases in sperm count, motility, and viability
Seffner et al. 1997									
87	Guinea pig (albino) 5–8 NS	6 months, daily (W)	<1.04, 18.4	DX	Develop	18.4			
Li et al. 2021									
88	Rabbit (Rex) 20 M, 20 F	5 weeks (F)	0.60, 2.72, 4.83	BW, FI, BC, OW	Bd wt	4.83			
Aulerich et al. 1982									
89	Mink (dark mink) 12 M, 12 F	153 or 367 days (F)	M: 0, 1.5, 3, 6, 12; F: 0 1.6, 3, 6, 13	DX	Repro Develop	12 13			
Kline et al. 1971									
90	Pig (Hampshire-Yorkshire) 12 NS	88 days (F)	0.1, 1.7, 2.3, 2.7	BW, FI, HE, BC	Bd wt Hemato	1.7 2.7	2.3		Decreased body weight gain (17%)
Suttle and Mills 1966									
91	Pig (NS) 6 F	46 days, daily (F)	0, 16.5	BW, BC, BI	Bd wt Hemato Hepatic		16.5 16.5 16.5		Decreased body weight gain (22%) Decreased hemoglobin Jaundice in 2/6 animals

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Suttle and Mills 1966									
92	Pig (NS) 6 F	49 days, daily (F)	0, 18.7	BW, BC, BI	Bd wt		18.7		Decreased body weight gain (27%)
					Hemato		18.7		Decreased hemoglobin at 6 weeks, and increased erythrocyte count
					Hepatic		18.7		Severe transient jaundice 5/6 animals between weeks 3 and 6; increased AST activity
Zhang et al. 2020									
93	Pig (NS) 6 M, 6 F	6 weeks (F)	0, 0.35, 1.80, 3.62	BW, FI, BC, OW, HP	Bd wt	3.62			
					Hepatic	3.62			
CHRONIC EXPOSURE									
Araya et al. 2012									
94	Monkey (Tufted Capuchin) 2 M, 2 F	3 years daily (F)	0, 5 increased to 7.5 over first 2 months	CS, BW, FI, BI, HP, OF	Bd wt	7.5			
					Hemato		7.5		Decreased hemoglobin
					Hepatic	7.5			
Araya et al. 2012									
95	Monkey (Tufted Capuchin) 2 M, 2 F	3 years daily (milk)	0, 3.5 increased to 5.5 over first 2 months	CS, BW, FI, BI, HP, OF	Bd wt	5.5			
					Hemato	5.5			
					Hepatic	5.5			

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg Cu/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Massie and Aiello 1984									
96	Mouse (C57BL/6N) 8 M	850 days (W)	0, 4.2, 8.5, 42	CS, BW	Death			42	14.4% decrease in mean survival time and 12.8% decrease in maximum lifespan
					Bd wt	42			

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.02 mg Cu/kg/day based on benchmark dose modeling of gastrointestinal symptoms in volunteers. The $BMDL_{10}$ of 0.055 mg Cu/kg/day was divided by an uncertainty factor of 3 for human variability to derive the MRL. This MRL was also considered protective for intermediate-duration exposure and adopted for the intermediate-duration oral MRL. See Appendix A for more detailed information regarding the MRL.

AChE = acetyl cholinesterase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; B = both males and females; BC = serum (blood) changes; Bd wt or BW = body weight; BI =biochemical indices; BUN = blood urea nitrogen; (C) = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental effects; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FSH = follicle-stimulating hormone; (G) = gavage; Gastro = gastrointestinal; GN = gross necropsy; (GO) = gavage in oil; (GW) = gavage in water; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; IX = immune function; LD_{50} = median lethal dose; LDH = lactate dehydrogenase; LE = lethality; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OF = organ function; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive function; SDH = sorbitol dehydrogenase; SLOAEL = serious lowest-observed-adverse-effect level; UR = urinalysis; (W) = water; WBC = white blood cell; WI = water intake

Figure 2-3. Levels of Significant Exposure to Copper – Oral
Acute (≤14 days)

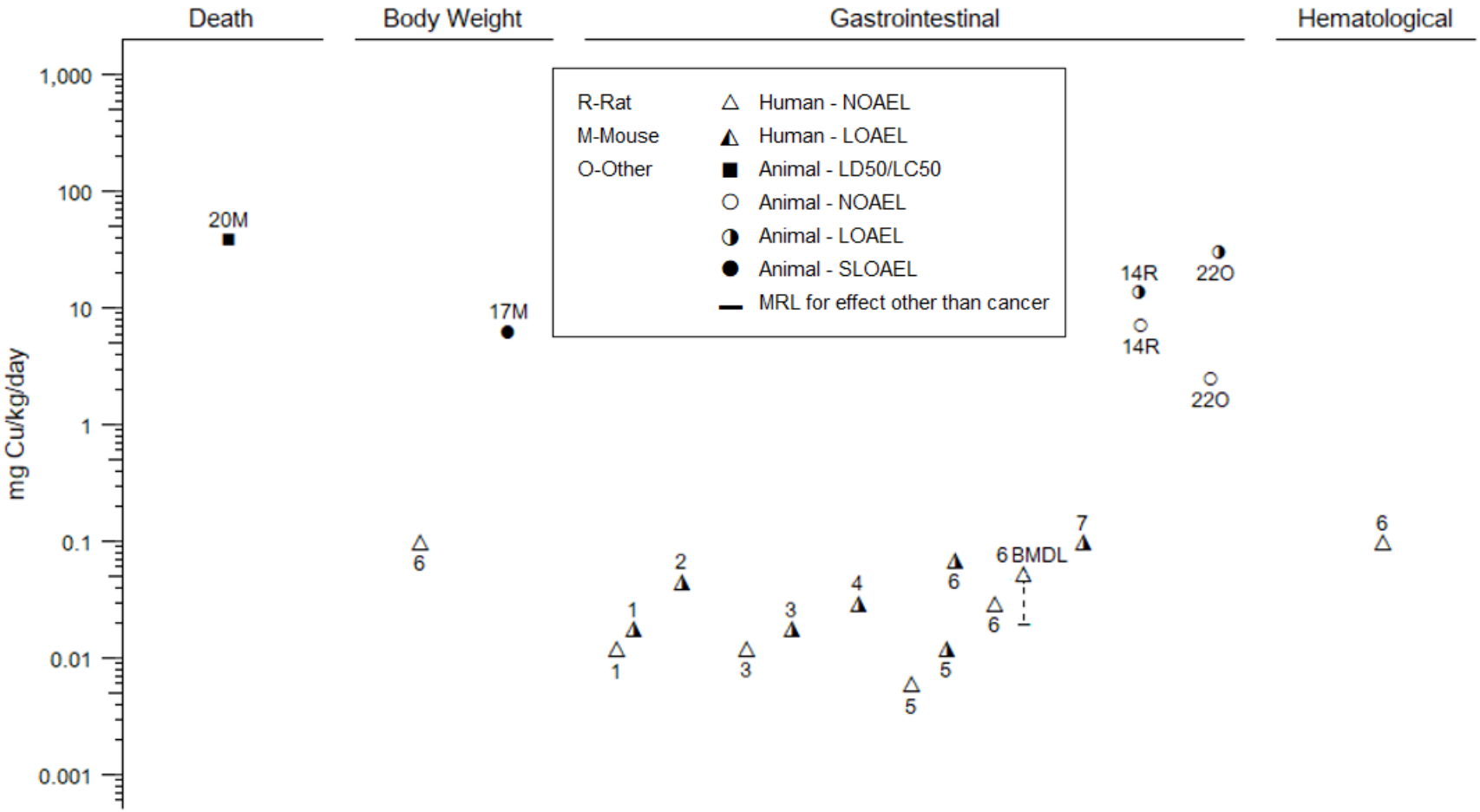


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Acute (≤14 days)

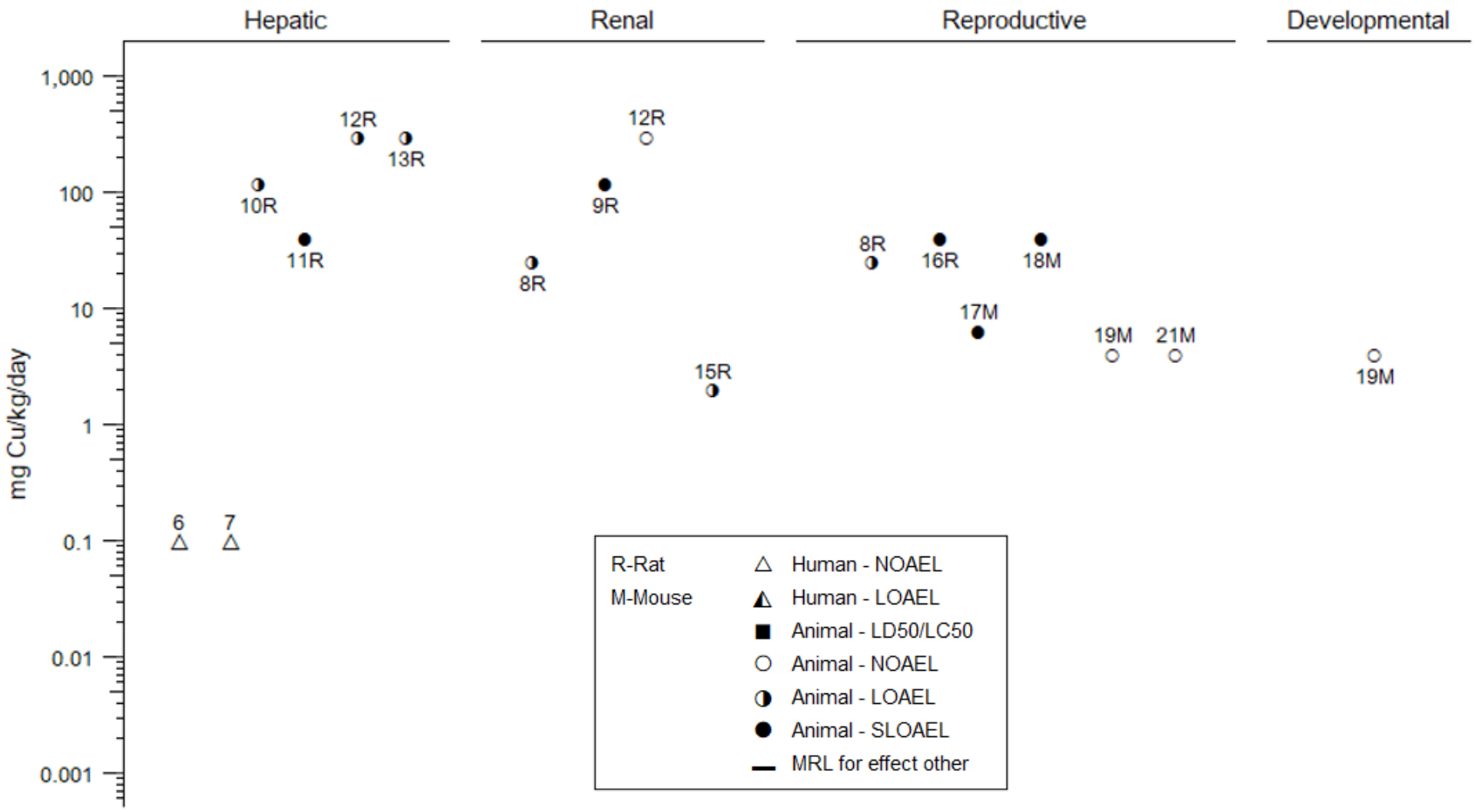


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

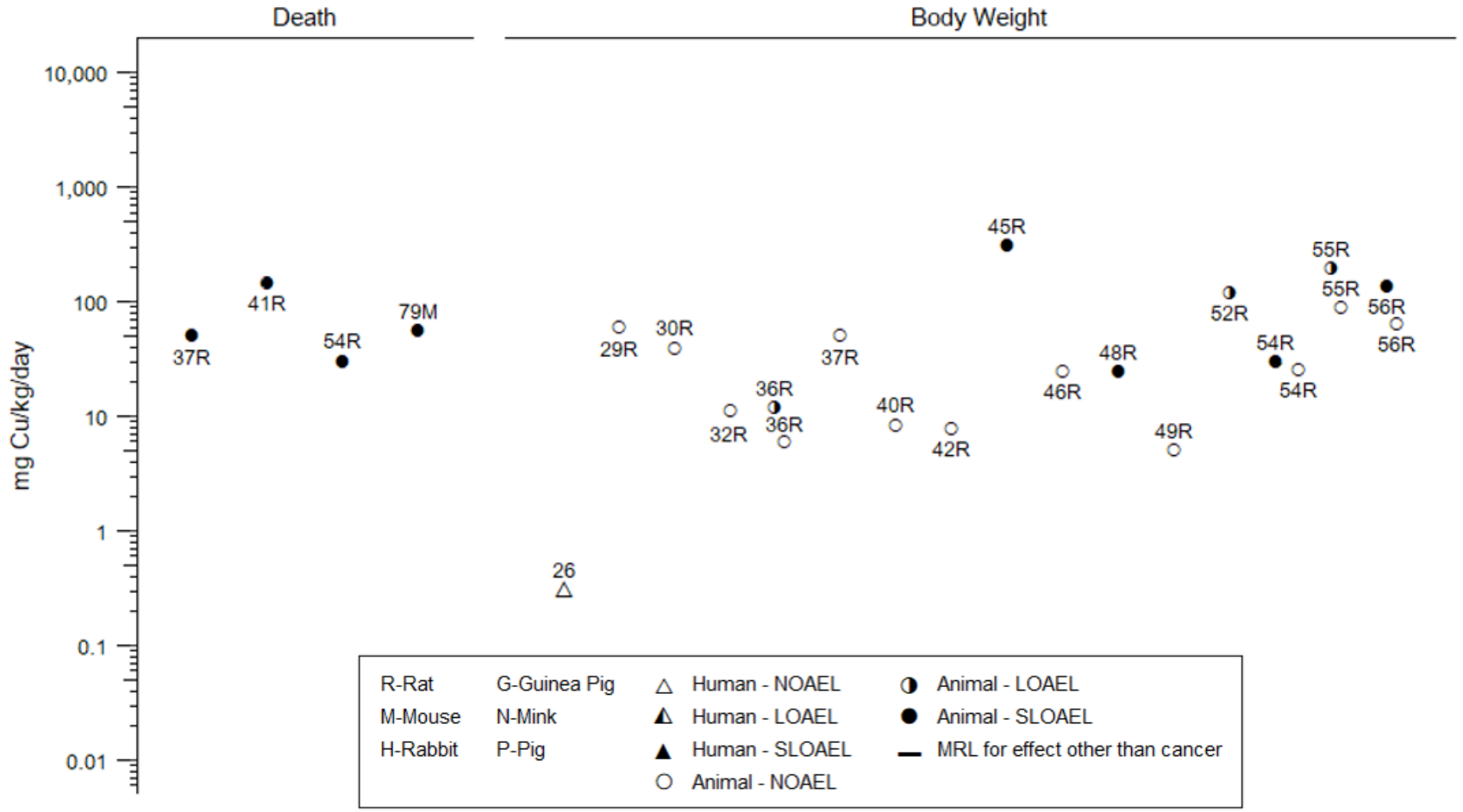


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

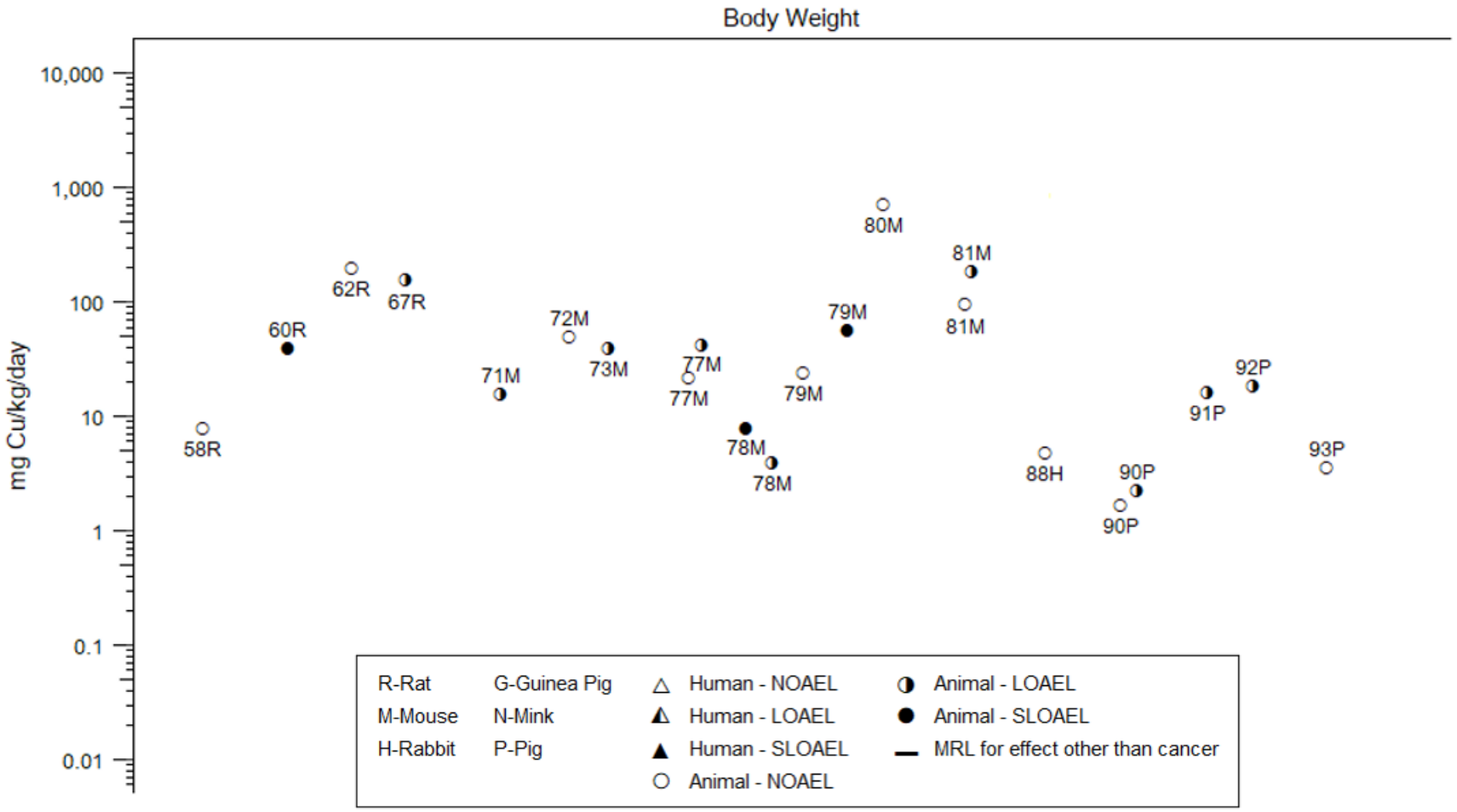


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

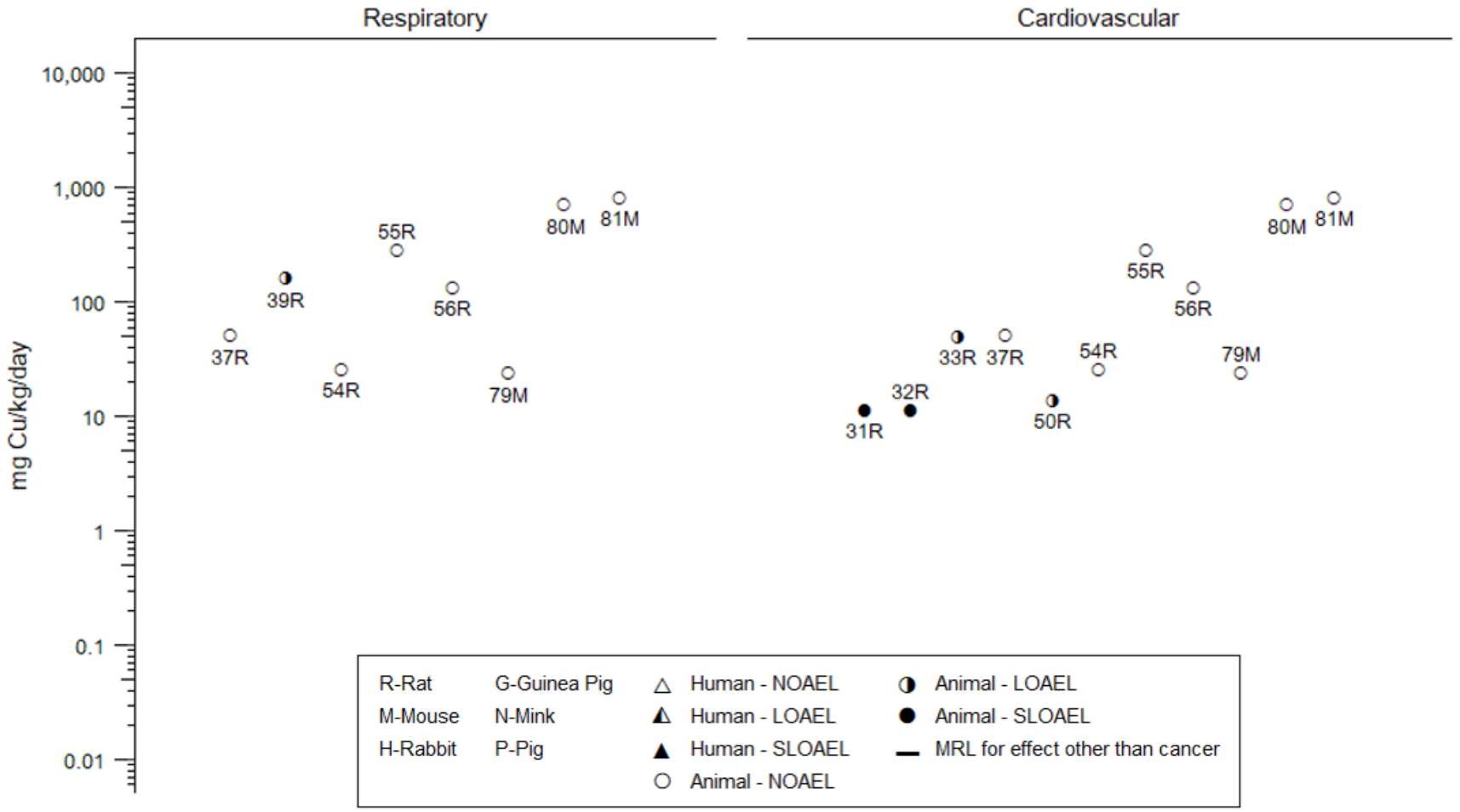


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

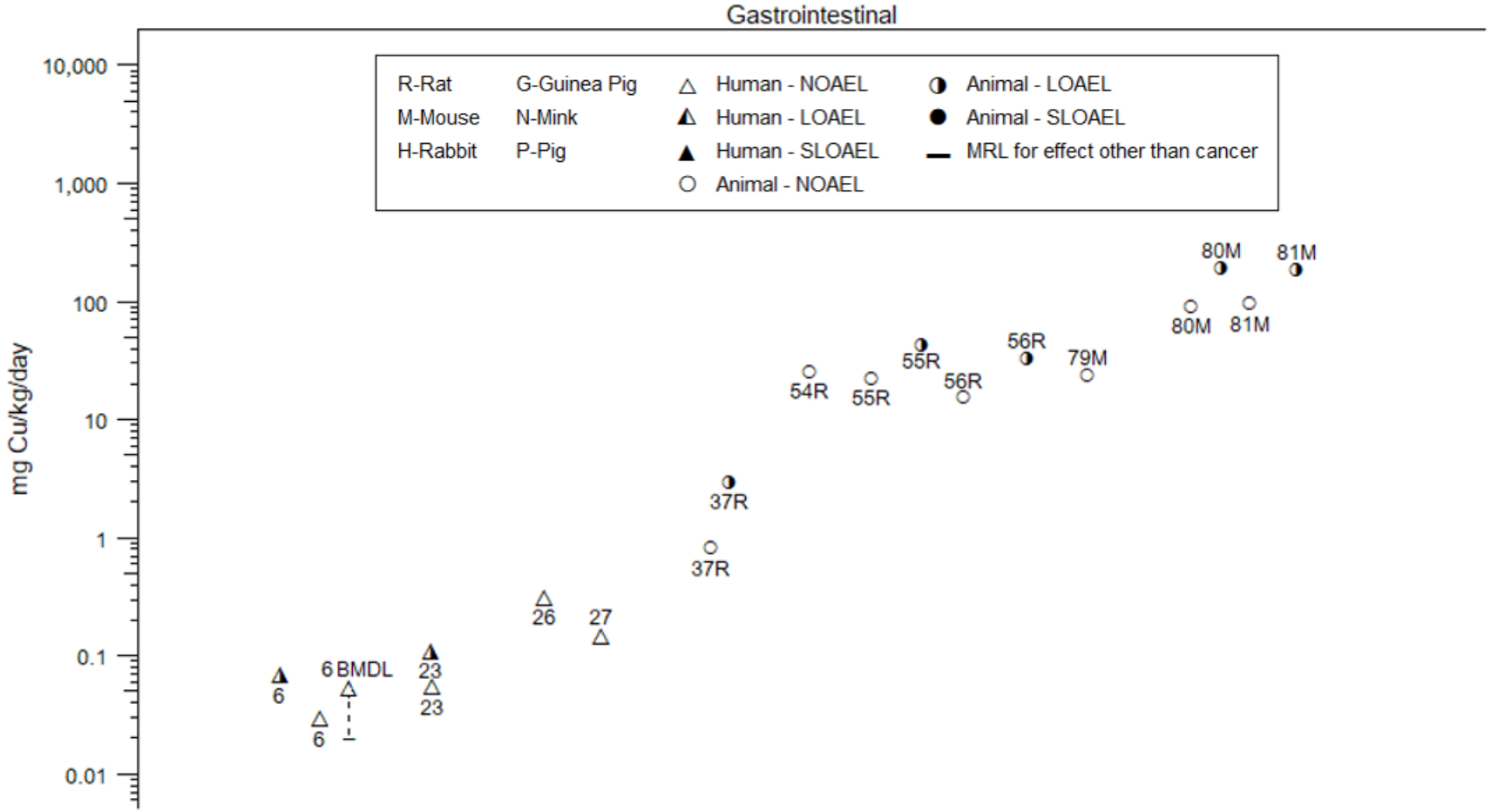


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

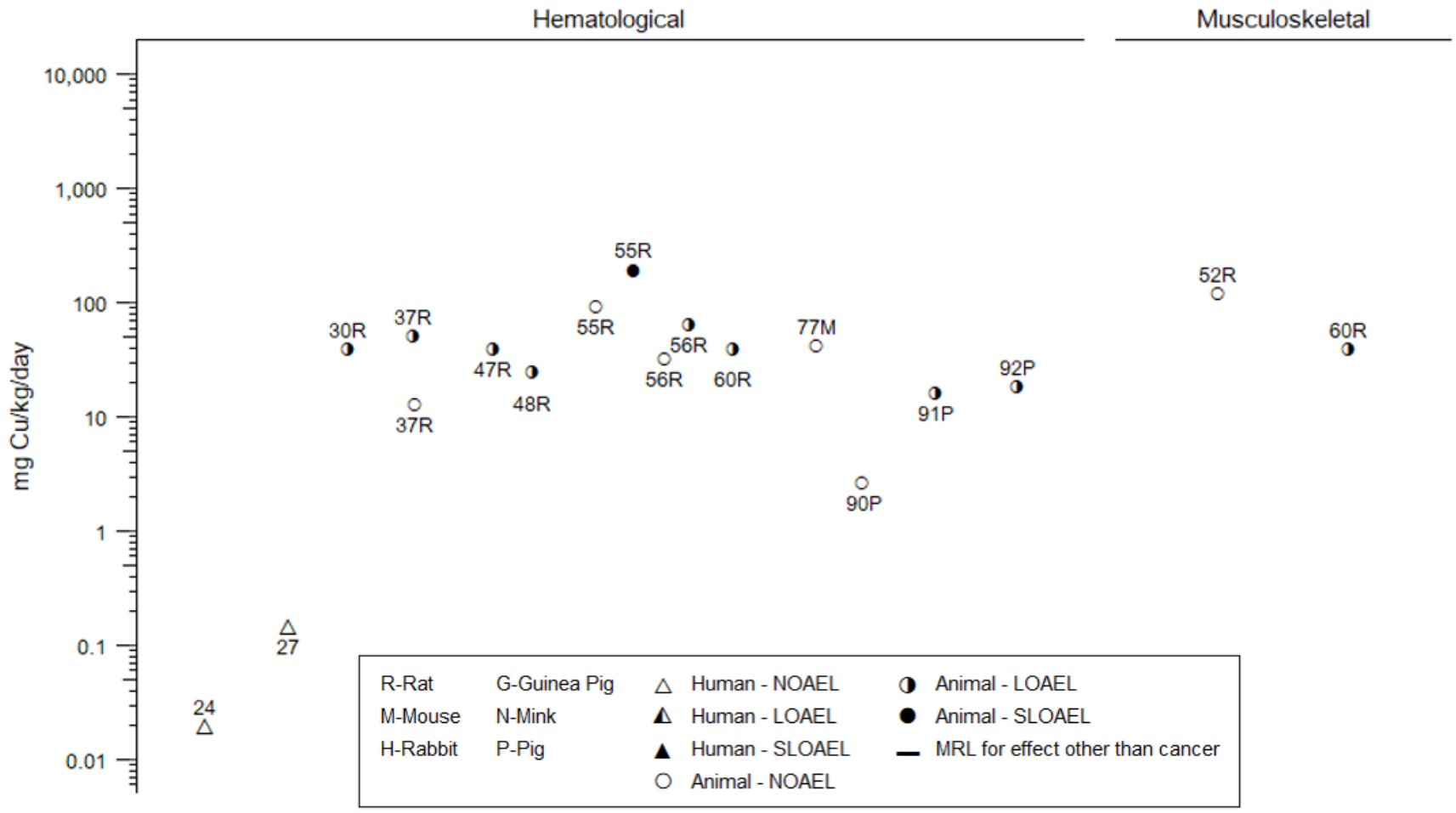


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

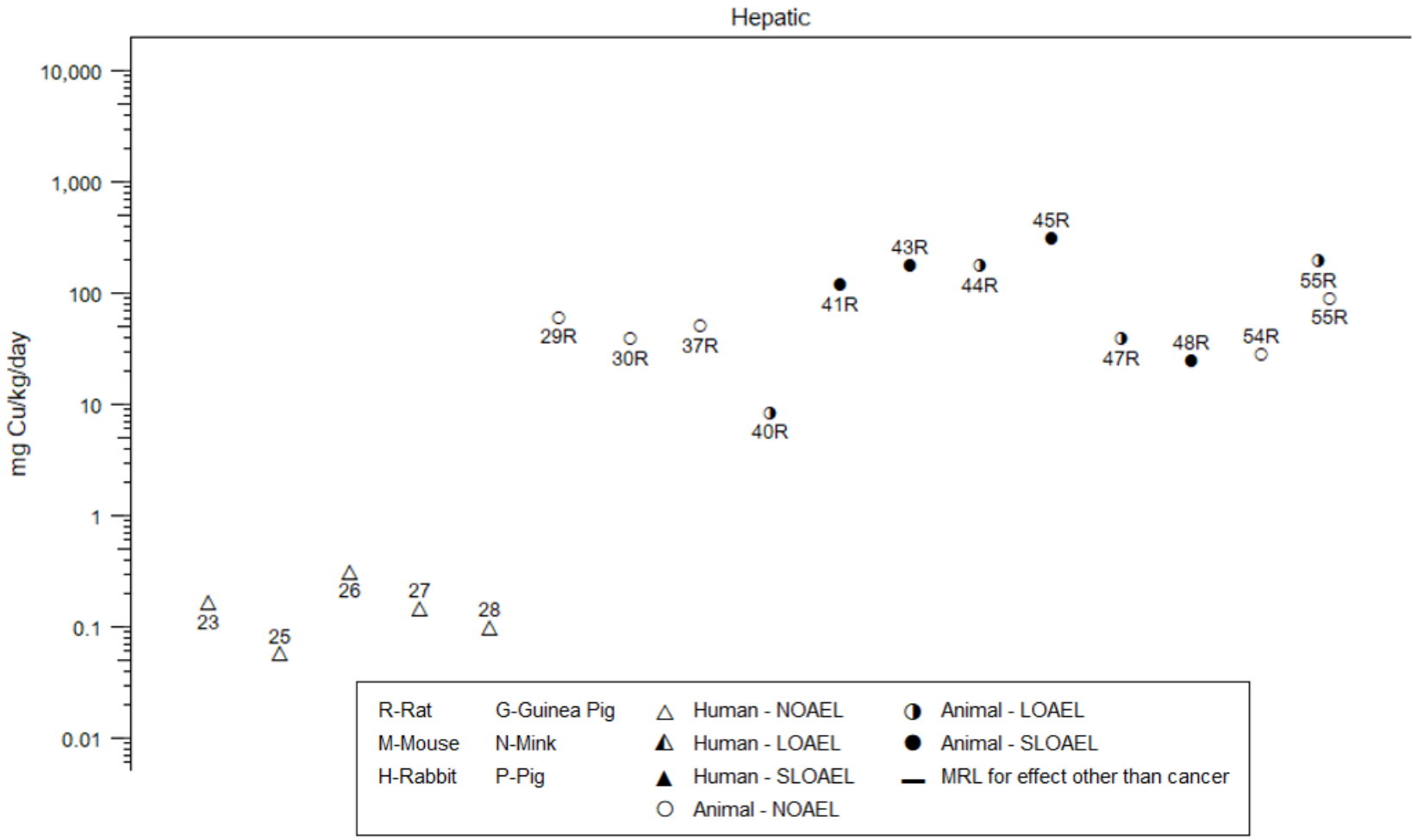


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

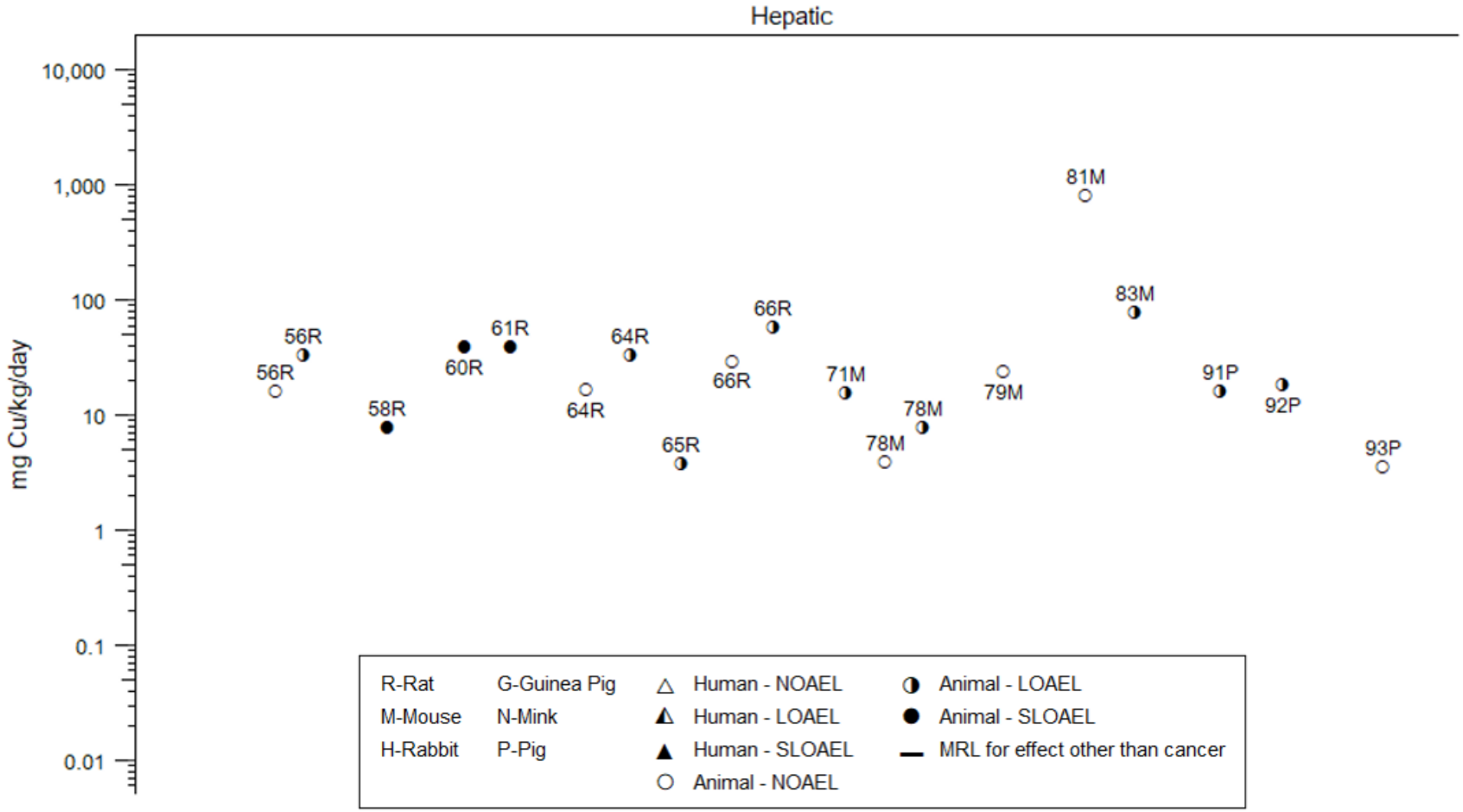


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

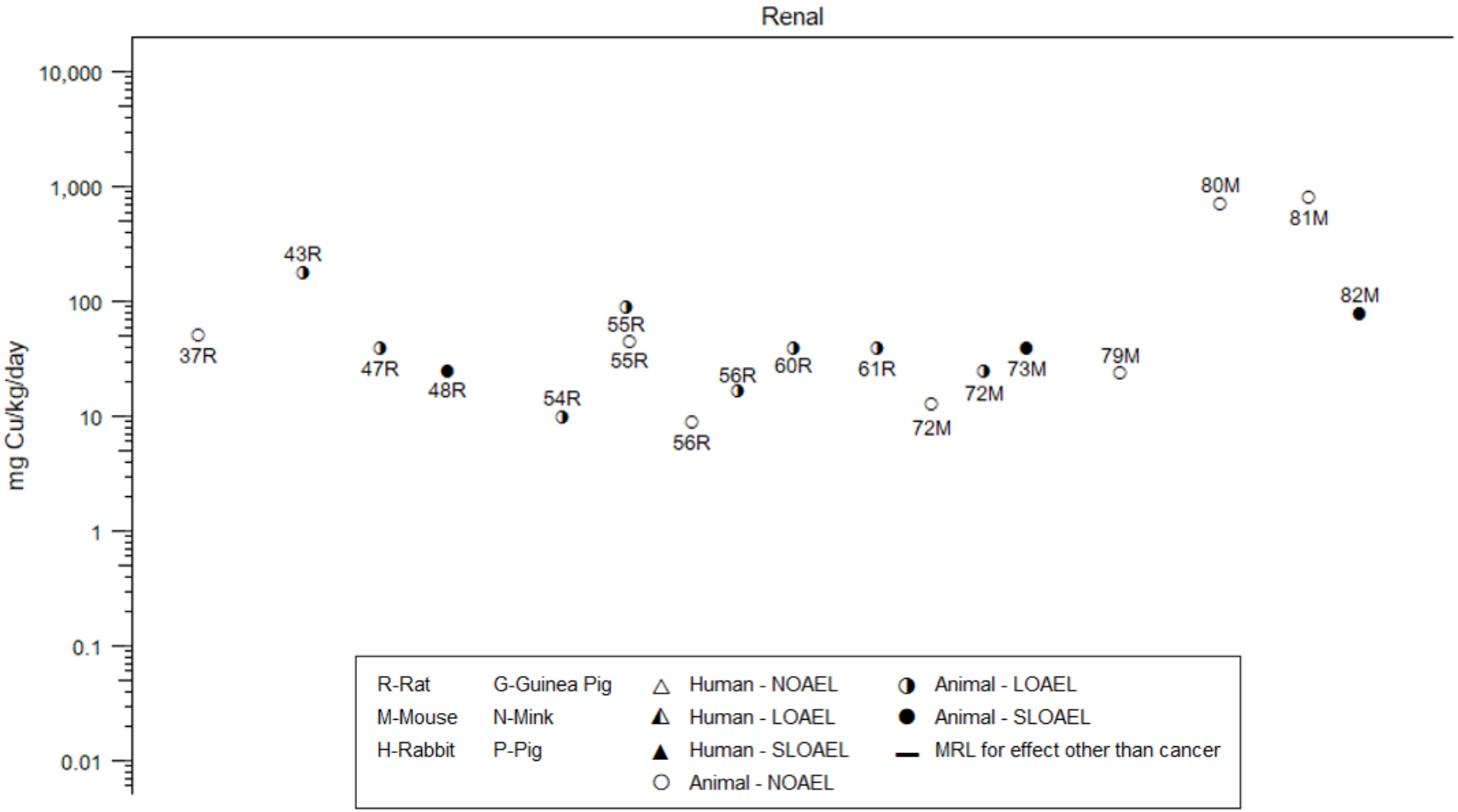


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

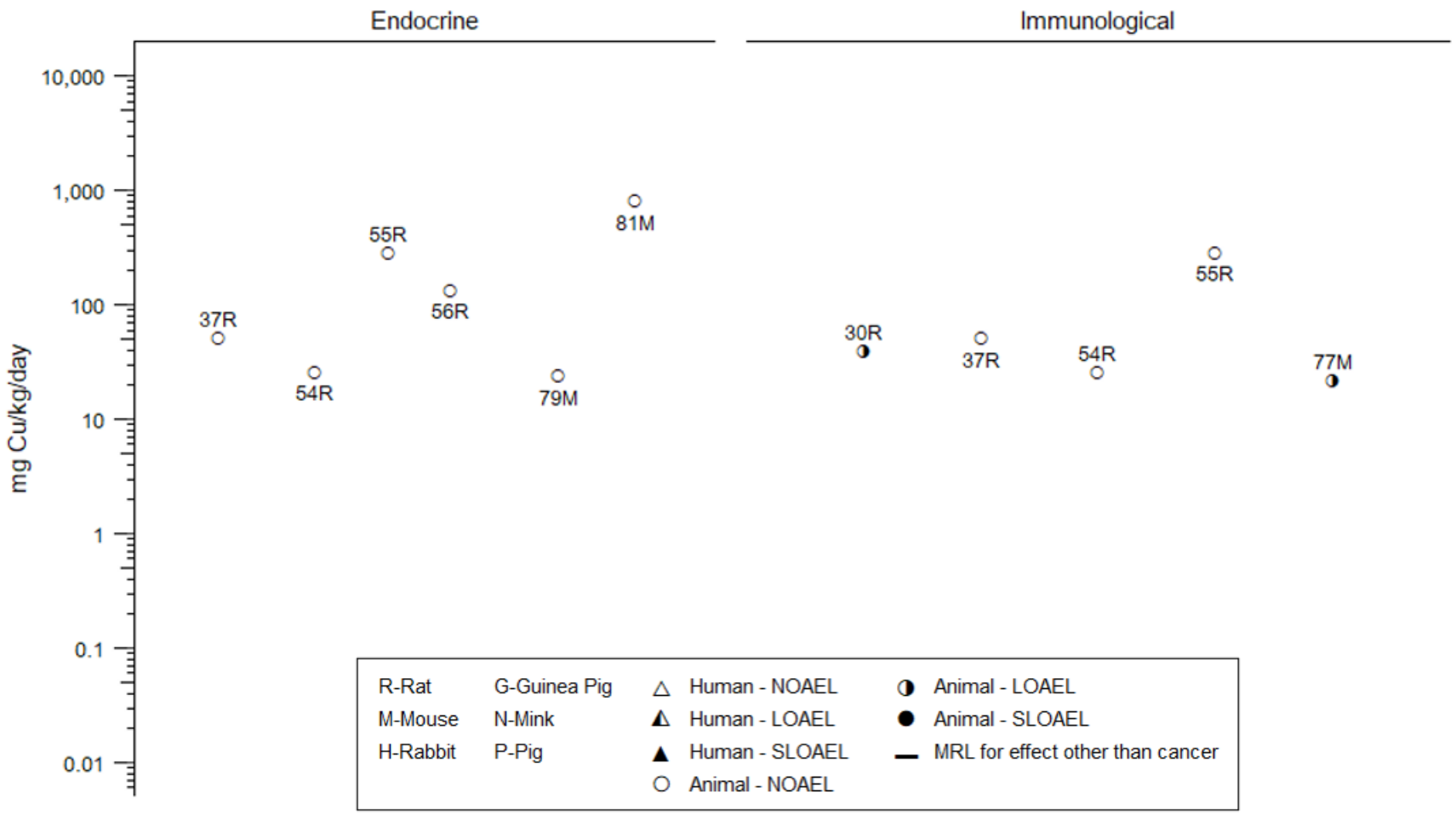


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

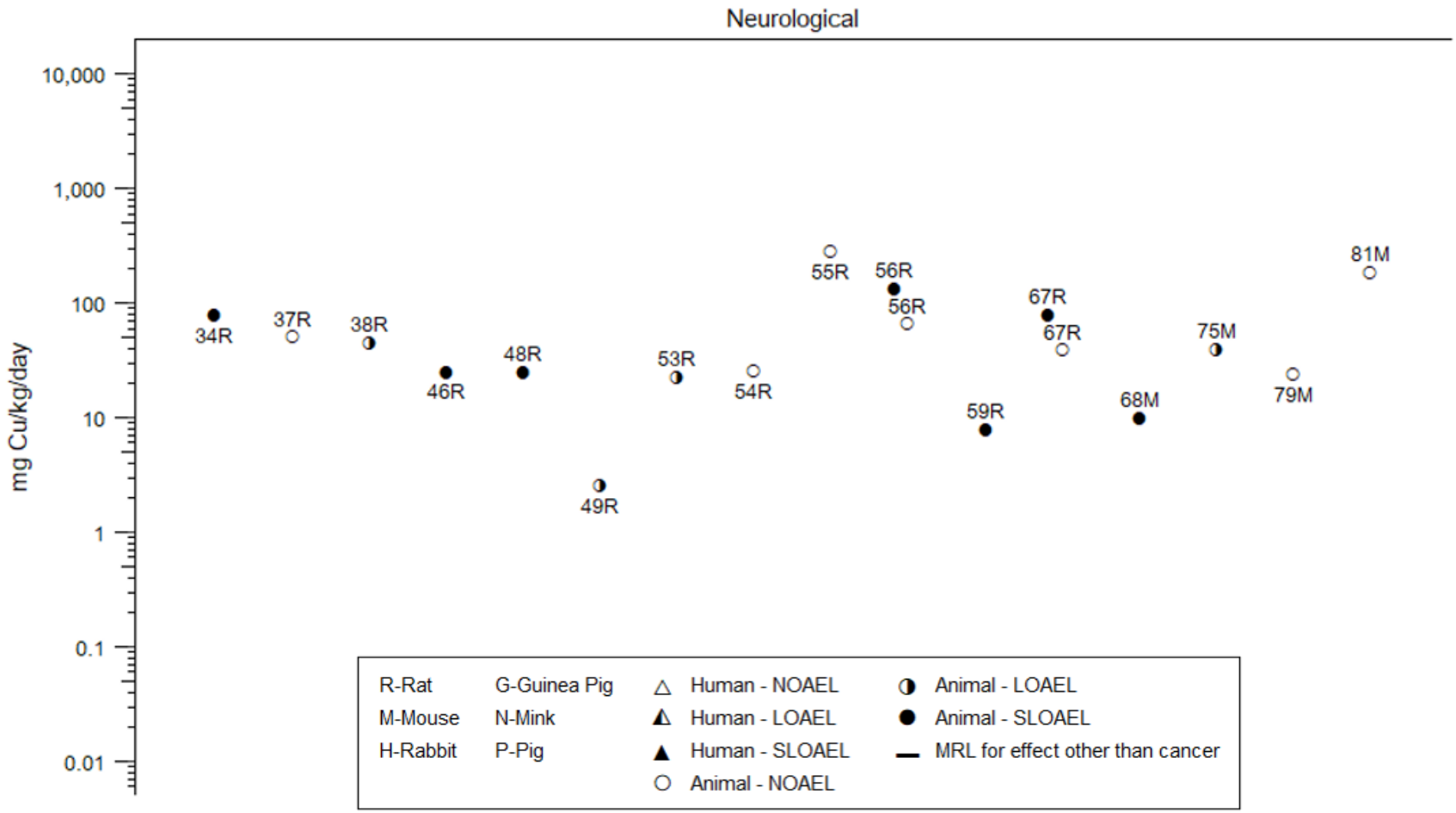


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

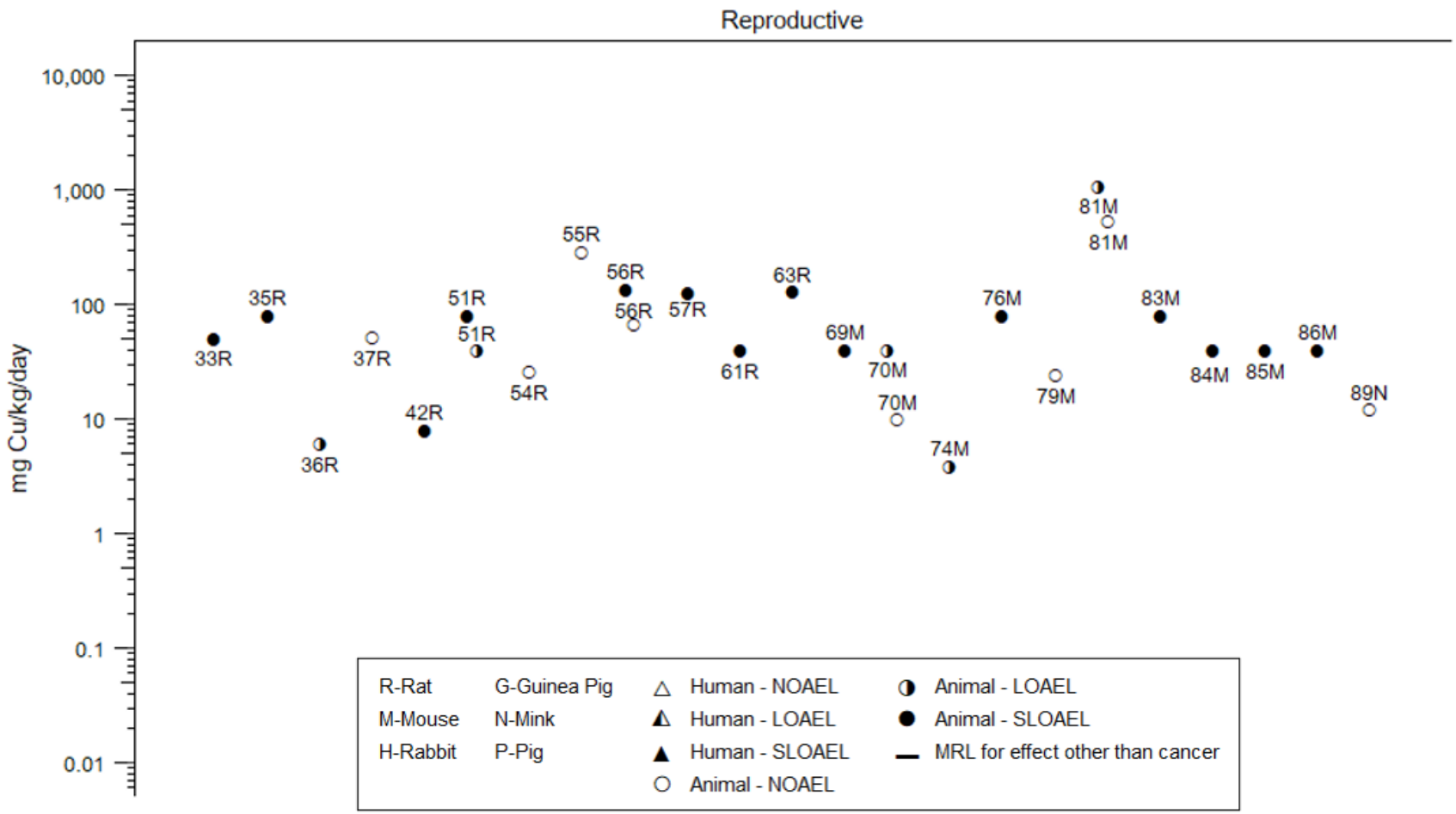


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15–364 days)

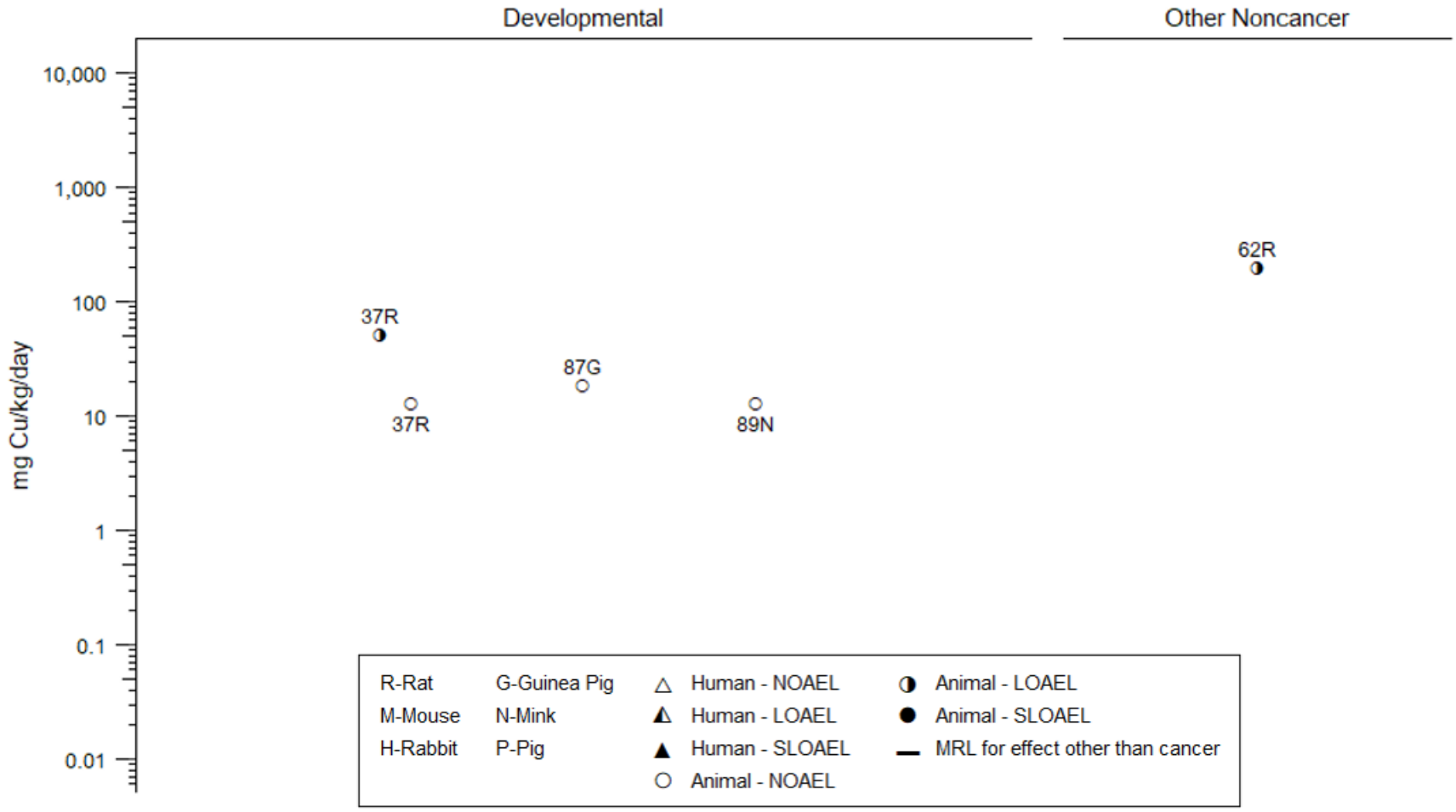
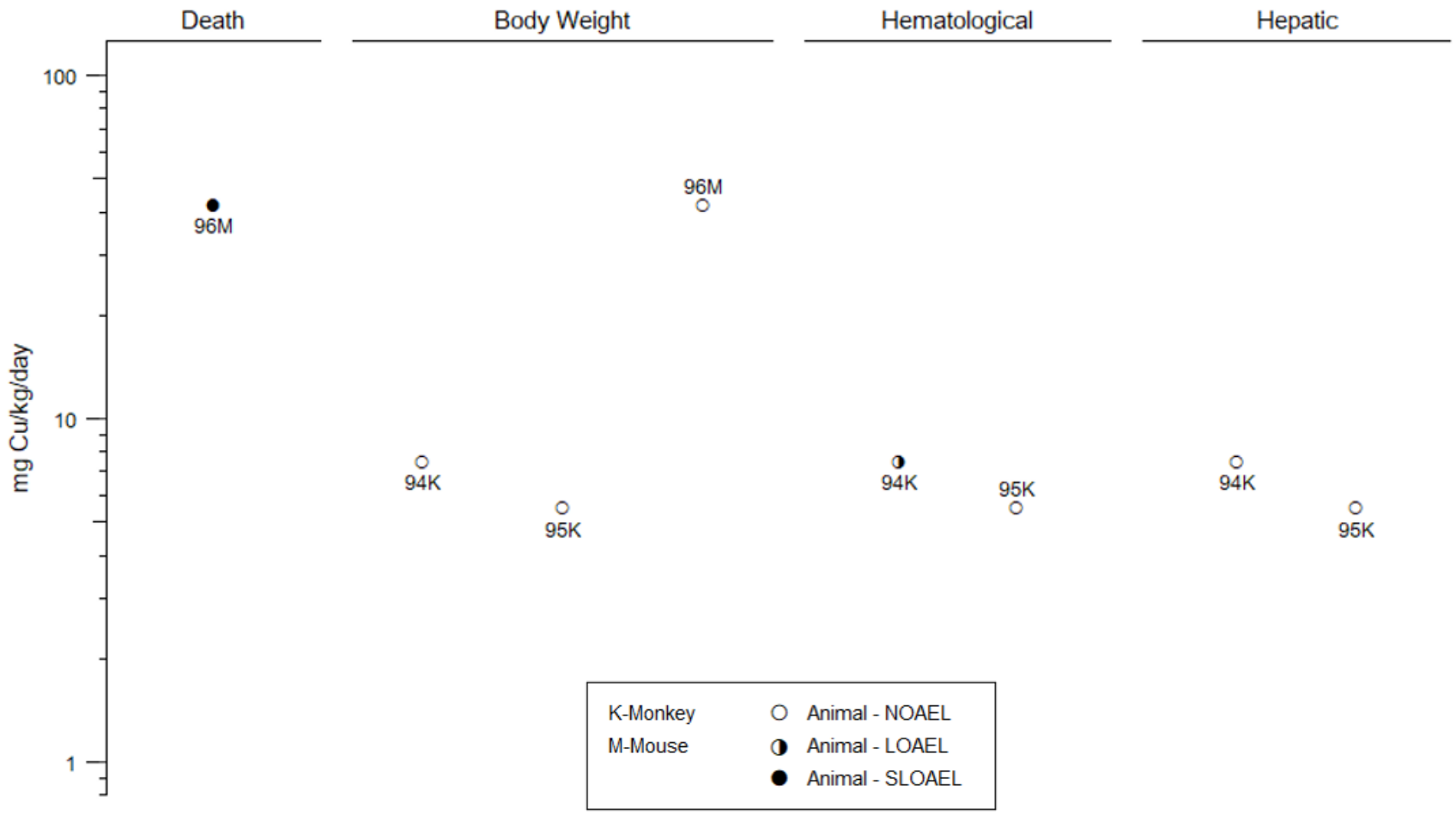


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Chronic (≥365 days)



2.2 DEATH

No studies were located regarding death of humans following inhalation exposure to copper. Several case studies reported death following ingestion of large doses of copper sulfate (Chuttani et al. 1965; Griswold et al. 2017; Gupta et al. 2018; Sharma 2011). For example, death by cardiac arrest following ingestion of copper sulfate crystals was reported in two case studies: one involved a 26-year-old man who intentionally ingested an unknown amount of copper sulfate crystals, and another was a situation where a 60-year-old man accidentally ingested 15–18 mg of copper sulfate as crystals (Griswold et al. 2017; Gupta et al. 2018). In a case series, 7 of 48 individuals admitted with copper sulfate poisoning died (Chuttani et al. 1965). The deaths occurring within 24 hours of ingestion were attributed to shock, and deaths after 24 hours were likely due to hepatic and/or renal complications. Deaths, likely due to central nervous system depression and hepatic or renal failure, were also reported in individuals ingesting "spiritual green water," which contains ≥100 mg Cu sulfate/L (Akintonwa et al. 1989).

No studies were found regarding death in humans following dermal exposure to copper; however, some studies reported deaths from different exposure routes than those reported above. One case study reported death by multi-organ failure in a 22-year-old man who intentionally intravenously injected approximately 1 g copper sulfate dissolved in water into his right arm (Behera et al. 2007). Another case study reported death by hypoxia and multi-organ failure in a 29-year-old pregnant woman who intentionally exposed her vaginal tissues to an unknown amount of copper sulfate dissolved in water (Motlhatlhedi et al. 2014).

Few published data on death after inhalation exposure in animals were located. EPA's 2006 Memorandum, *Coppers: Revised human health chapter of the reregistration Eligibility Decision Document (RED) and response to comments from the Phase 3 public comment period,* reviewed a number of unpublished studies of the acute-duration inhalation lethality of copper compounds. These studies were submitted to EPA and are not in the public domain, so the only information available to ATSDR was from the secondary source; thus, these data are not included in the LSE table or figure. EPA (2006) did not report species or exposure duration for the median lethal concentration (LC_{50}) values, but the inhalation studies submitted to EPA's pesticides program are typically 4-hour rat lethality studies. The LC_{50} values reported by EPA (2006) are shown in the Table 2-3.

Table 2-3. LC_{50} Values for Copper Compounds[a] Reported by EPA (2006)

Compound	Composition information	Sex	LC_{50} (mg/m^3 compound)
Copper hydroxide	77%	Male	1,530
		Female	1,040
Copper oxychloride	94.1%	NR	>1,700
Copper, metallic	23%	NR	>100 and <590
Cupric oxide (CuO)	97.6%	Both	>2,080
Cuprous oxide/dicopper oxide (Cu_2O)	40.9% a.i.	NR	100–590
Copper 8-quinolinolate ($C_{18}H_{12}CuN_2O_2$)	96%	Both	89
KOMEEN and K-Tea (elemental copper, ethylenediamine)	NR	Male	1,360
		Female	560
Copper naphthenate	9.5% Cu	Both	>2960
Copper octanoate, 10% fatty acids	NR	Both	380
Cuprous thiocyanate	99%	NR	>500

[a]EPA (2006) did not report the species tested, but acute-duration inhalation lethality studies are typically conducted in rats and/or mice.

a.i. = active ingredient; NR = not reported

The oral median lethal dose (LD_{50}) for mice administered copper sulfate was reported as 39.8 mg Cu/kg, however, only two mice were tested per dose in an "up and down" method (Kadammattil et al. 2018). Total mortality was observed in rats fed 140 mg Cu/kg/day as copper sulfate for 1 week, compared to controls (Boyden et al. 1938). Reduced food intake, possibly the result of taste aversion, contributed to the deaths.

EPA (2006) reported oral LD_{50} values from unpublished studies of copper compounds; these values are shown in Table 2-4. As with the inhalation values, EPA (2006) did not report the species or mode of administration for these studies; however, these studies are typically conducted using rats or mice exposed by gavage. The lowest LD_{50} values were for KOMEEN and K-Tea (elemental copper, ethylenediamine) and for copper sulfate pentahydrate.

Table 2-4. Oral LD_{50} Values for Copper Compounds Reported by EPA (2006)[a]

Compound	Composition information	Sex	LD_{50} (mg/kg compound)
Copper chloride	57.7%	Male	1,796
		Female	2,006
Copper carbonate	96%	NR	>2,000

Table 2-4. Oral LD_{50} Values for Copper Compounds Reported by EPA (2006)[a]

Compound	Composition information	Sex	LD_{50} (mg/kg compound)
Copper hydroxide	77%	Male	2,253
		Female	2,160
Copper oxychloride	94.1%	Male	1,537
		Female	1,370
Copper sulfate pentahydrate	99%	Male	790
		Female	450
Copper, metallic	50%	Male	1,414
		Female	1,625
Cupric oxide	97.6%	Both	>5,050
Cuprous oxide	57%.	NR	>5,000
Copper 8-quinolinolate	99.5%	Both	>5,000
Copper from triethanolamine complex (K-Tea)	99%	Male	1,170
		Female	1,312
KOMEEN and K-Tea (elemental copper, ethylenediamine)	KOMEEN 96%, K-Tea 99%	Male	527
		Female	462
Copper naphthenate	8% Cu	Both	>5,050
Copper octanoate, 10% fatty acids	NR	Both	>2,000
Copper salts of fatty and rosin acids (Cu and zinc neoisoate 35%)	NR	NR	>7,000
Cuprous thiocyanate	99%	NR	>5,000

[a]EPA (2006) did not report the species tested, but most acute-duration oral lethality studies are typically conducted in rats and/or mice.

NR = not reported

Intermediate-duration animal studies reported deaths from oral exposure to copper in drinking water and via gavage, but not when administered in food. In drinking water studies, all rats died or were sacrificed moribund when groups of five male and five female rats orally exposed to ≥36 and 31 mg Cu/kg/day, respectively, as copper sulfate pentahydrate in water for 15 days (NTP 1993). Similar results were seen in mice. One of five male mice and three of five female mice died following exposure to 57 and 62 mg Cu/kg/day, and all mice died at higher doses when copper sulfate pentahydrate was administered in water for 15 days (NTP 1993). In both rats and mice exposed via the drinking water, there were profound decreases in water consumption at the higher doses, which NTP (1993) attributed to palatability. As a result, the animals were dehydrated, which may have contributed to the mortalities.

Two of 12 rats died following exposure to 51 mg Cu/kg/day as copper chloride via gavage for up to 38 days in a combined repeat-dose and reproductive/developmental toxicity screening study (Chung et al. 2009). No deaths were reported in rats or mice receiving doses up to 324 or 717–781 mg Cu/kg/day, respectively, as copper sulfate pentahydrate in food for 15 days. In 13-week studies, no mortality was reported in male or female rats exposed daily to 140 or 134 mg Cu/kg/day (respectively) or in male or female mice exposed to 815 or 1,058 mg Cu/kg/day (respectively) as copper sulfate pentahydrate in feed (NTP 1993). NTP (1993) did not conduct 13-week studies using drinking water administration due to the premature deaths seen in the 15-day studies.

In an unpublished developmental toxicity study submitted to EPA and reviewed by EPA (2006), doses of 18 mg Cu/kg/day as copper hydroxide administered via gavage over gestation days (GDs) 7–28 resulted in death in 3/22 pregnant New Zealand White rabbits. No deaths were reported at 9 mg Cu/kg/day (reviewed by EPA 2006).

Chronic-duration oral studies in animals were limited. Lifetime exposure of mice to 42 mg Cu/kg/day as copper gluconate in drinking water resulted in an average 12.8% reduction of the maximum lifespan (from 986 to 874 days) and an average 14.4% decrease in their mean survival time (Massie and Aiello 1984).

Dermal LD_{50} values reported by EPA (2006) for copper compounds are shown in Table 2-5; species was not reported in the secondary source, but these studies generally use rats or rabbits. Only copper oxychloride had a dermal LD_{50} value below the upper limit dose of 2,000 mg compound/kg used in these studies.

Table 2-5. Dermal LD_{50} Values for Copper Compounds Reported by EPA (2006)[a]

Compound	Composition information	Sex	LD_{50} (mg/kg as compound)
Copper chloride	57.7%	Both	>2,000
Copper hydroxide	77%	NR	>2,000
Copper oxychloride	94.1%	Both	710
Copper sulfate pentahydrate	99%	NR	>2,000
Copper, metallic	8.5% elemental	NR	>2,000
Cupric oxide	97.6%	Both	>2,020
Cuprous oxide	57%	NR	>2,000
Copper 8-quinolinolate	99.5%	Both	>2,000

Table 2-5. Dermal LD_{50} Values for Copper Compounds Reported by EPA (2006)[a]

Compound	Composition information	Sex	LD_{50} (mg/kg as compound)
Copper from triethanolamine complex (K-Tea)	99%	NR	>2,000
KOMEEN and K-Tea (elemental copper, ethylenediamine)		NR	>2,000
Copper naphthenate	8% Cu	Both	>2,020
Copper octanoate, 10% fatty acids		Both	>2,000
Copper salts of fatty and rosin acids (copper and zinc neoisoate 35%)		NR	>2,000
Cuprous thiocyanate	99%	NR	>2,000

[a]EPA (2006) did not report the species tested, but most acute-duration dermal lethality studies are typically conducted in rats and/or rabbits.

NR = not reported

2.3 BODY WEIGHT

No studies were located regarding body weight effects in humans following inhalation exposure to copper or in humans exposed dermally. No effects on body weight were observed in a controlled exposure study in women exposed to a daily dose of up to 0.1 mg Cu/kg/day as copper sulfate for 2 weeks (Pizarro et al. 1999). In addition, no changes in body weight were reported in infants given daily doses up to 0.319 mg Cu/kg/day as copper sulfate in drinking water for 9 months (Olivares et al. 1998).

Only one study of body weight in animals exposed to copper via inhalation was located. No effects on body weight were observed in rats exposed to 8.9 mg Cu/m^3 as dicopper oxide for 6 hours/day, 5 days/week, for 2 weeks (6 hours/day) or to 1.76 mg Cu/m^3 for 6 hours/day, 5 days/week, for 4 weeks (Poland et al. 2022). In a companion study of copper sulfate pentahydrate in the same publication, male body weights were significantly decreased on day 4 (10.3%) and day 11 (13.8%) in the 8.9 mg Cu/m^3 group (Poland et al. 2022). These body weight decreases were accompanied by decreased food intake, but the study authors did not report food intakes, so it is difficult to establish whether the body weight effects were attributable to the decline in food consumption.

In an acute-duration oral study, a decrease in terminal body weight of 28% was observed in mice exposed to 6.4 mg Cu/kg/day as copper sulfate pentahydrate via gavage for 14 days (Al-musawi et al. 2022).

Intermediate-duration studies had mixed results on body weight changes. Intermediate-duration oral exposure studies to copper sulfate reported 10–28% decreases of body weight and 12–51% decreases in body weight gain in rats following exposure to as little as 12 mg Cu/kg/day for 15–91 days (Chen et al. 2023; Haywood and Loughran 1985; Kumar and Sharma 1987; Kumar et al. 2015, 2016a, 2016b; Rana and Kumar 1980); in mice following exposure to as low as 4 mg Cu/kg/day for 15–133 days (Dai et al. 2023; Kvietkauskaite et al. 2004; Liu et al. 2021c); and in pigs following exposure to 2.3 mg Cu/kg/day for 88 days (Kline et al. 1971). Rats exposed to 160 mg Cu/kg/day as tribasic copper chloride for 12 weeks exhibited decreased terminal body weights by 11% (Yu et al. 2023). Significant decreases in body weight were reported in rats exposed to 39.8 mg Cu/kg/day as copper sulfate for 90 days, but were not further described (Kumar et al. 2016a, 2016b). Decreased body weight gains (22–27%) were observed in pigs following exposure to 16.5–18.7 mg Cu/kg/day as copper carbonate for 46–49 days (Suttle and Mills 1966). A 78% decrease in body weight gain was observed in mice exposed via gavage to 16 mg Cu/kg/day as copper sulfate for 20 days (Dab et al. 2023). A 17% decrease in body weight gain was observed in pigs exposed to 2.3 mg Cu/kg/day as copper sulfate for 88 days; no effects were observed at 1.7 mg Cu/kg/day (Kline et al. 1971). Decreased body weight gains of 23% were observed in rats following exposure to 120 mg Cu/kg/day as copper acetate for 21 weeks (Llewellyn et al. 1985).

NTP (1993) evaluated a comprehensive set of toxicological endpoints including body weight effects in rats and mice exposed to copper sulfate pentahydrate in water or diet for 15 days or 13 weeks. In the 15-day studies, male rats fed 198 mg Cu/kg/day exhibited an 18% decrease in body weights, with no effects observed at 92 mg Cu/kg/day, while female rats fed 285 mg Cu/kg/day exhibited a 13% decrease in body weights, with no effects observed at 196 mg Cu/kg/day. Male and female rats had reduced food intake in these studies, at a range of 37–8%, thus confounding the decrease in body weights and reducing the compound intake. Mice fed up to 780 mg Cu/kg/day had no body weight effects. Rats given 31–36 mg Cu/kg/day in the drinking water had decreased body weights by 48% in males and 46% in females, while doses of up to 29 mg Cu/kg/day in the drinking water for 15 days had no effect on body weights. Mice had 22–34% decreases in body weights when administered 57–62 mg Cu/kg/day in water for 15 days, but no effects were observed at 24–36 mg Cu/kg/day. Due to high toxicity at the highest two doses in drinking water, changes in mice body weight were only observed at the mid dose. The drinking water studies were also confounded by decreased water consumption resulting in dehydration in the animals; therefore, no 13-week drinking water studies were performed. In the 13-week studies by NTP (1993), male rats fed 140 mg Cu/kg/day had a 24% decrease in body weight. No effects on body weights were observed in male rats fed 66 mg Cu/kg/day or in female rats fed 134 mg Cu/kg/day. In mice, males and females, respectively, fed 187 and 536 mg Cu/kg/day had 10 and 12% decreases in body weight. No

effects on body weights were observed in male mice at 97 mg Cu/kg/day or in female mice at 267 mg Cu/kg/day.

Numerous studies reported no effects on body weight in intermediate-duration studies in animals exposed to copper sulfate or copper sulfate pentahydrate at doses up to 50.9 mg Cu/kg/day in rats (Adele et al. 2023; Gupta et al. 2021; Kalita et al. 2020; Khushboo et al. 2018; Kumar et al. 2019; Patwa and Flora 2020; Seven et al. 2018), up to 50.9 mg Cu/kg/day in mice (Dai et al. 2020), up to 18.4 mg Cu/kg/day in guinea pigs (Seffner et al. 1997), and at 3.62 mg Cu/kg/day in pigs (Zhang et al. 2020). No changes in body weights were observed in rats given 62 mg Cu/kg/day as copper gluconate for 6 weeks (Abe et al. 2008) or in rats exposed to 51 mg Cu/kg/day as copper chloride for ~35 days (Chung et al. 2009). No changes in body weights were exhibited in mice exposed to 8.6 mg Cu/kg/day as copper acetate (Epstein et al. 1982).

A chronic-duration study (through the lifespan) found no biologically significant body weight effects in mice exposed to 42 mg Cu/kg/day as copper gluconate in drinking water (Massie and Aiello 1984). A 2-year study in monkeys also found no effects on body weight following exposure to doses of 5.5–7.5 mg Cu/kg/day as copper gluconate delivered to animals in food or milk (Araya et al. 2012).

2.4 RESPIRATORY

In humans, airborne copper particles are respiratory irritants. Workers exposed to copper dust have reported symptoms such as coughing, sneezing, thoracic pain, and runny nose (Askergren and Mellgren 1975; Suciu et al. 1981). In an occupational study of 75–100 workers involved with sieving copper dust, lung radiographs revealed linear pulmonary fibrosis, and in some cases, nodulation (Suciu et al. 1981). The study authors noted that “the workers employed on sieving the copper dust were exposed to a 99.9011% purity of copper.” During the first year of operation, the workers were exposed to an estimated average concentration of 464 mg Cu/m^3; the exposure levels declined each year due and by the third year, the levels were estimated to average 111 mg Cu/m^3 (Suciu et al. 1981). Suciu et al. (1981) did not include a comparison group, so the findings are difficult to interpret. Among sheet metal workers exposed to patina dust (copper-hydroxide-nitrate, copper-hydroxide-sulfate, copper silicate, copper oxide), 6 of the 11 examined workers displayed increased vascularity and superficial epistatic vessels in the nasal mucosa (Askergren and Mellgren 1975); however, copper exposure levels were not reported.

Epidemiological studies of respiratory effects in humans exposed to airborne copper are summarized in Table 2-6. Automotive workers in Iran who were exposed to copper particles from welding reported symptoms of cough, sputum, and wheezing (Saadiani et al. 2023). In this study, exposure to copper in the welding unit (mean concentration 0.107 mg Cu/m^3) was also associated with decreased forced expiratory volume in 1 second (FEV_1). The workers had co-exposure to other heavy metals (lead and iron) and the analyses did not adjust for these co-exposures. Two studies evaluated respiratory effects in workers at secondary copper smelters in Egypt, where coexposures included arsenic, lead, and cadmium (Fouad and Ramadan 2022; Mourad and El-Sherif 2022). These studies did not account for co-exposures. Compared to administrative workers without metal dust exposure, workers exhibited higher prevalence of symptoms of respiratory irritation (cough, expectoration, nasal irritation) and reduced respiratory function as measured by spirometry (Fouad and Ramadan 2022; Mourad and El-Sherif 2022). Chest x-rays showed a significant difference in the prevalence of radiological infiltrates (primarily reticular infiltrations) between the smelter workers (36%) and administrative workers (4%) (Fouad and Ramadan 2022). Copper concentrations in air were not measured or reported in either study of copper smelter workers; however, serum copper concentrations were higher in the exposed groups than the referents, supporting a difference in exposure levels.

Table 2-6. Results of Selected Epidemiological Studies Evaluating Exposure to Copper and Respiratory Effects

Reference, study type, and population	Exposure concentration	Outcome evaluated	Result
Saadiani et al. 2023 Cross-sectional, 1,152 automotive welders and 1,152 administrative staff (mean ages 37.5 and 38.5 years, respectively); welders were exposed to copper, lead, and iron (Iran)	Work in welding unit	Cough, sputum, and wheezing prevalence	↑
	Average air concentration in welding unit: 0.107 mg Cu/m^3	FEV_1	↓
Fouad and Ramadan 2022 Cross-sectional, 75 male copper smelter workers and 75 male administrative workers (mean ages 43.19 and 44.05 years, respectively); workers were exposed to copper and arsenic (Egypt)	Work in smelter operations; mean serum copper was 191.41 µg Cu/dL in exposed and 137.30 µg Cu/dL in controls	Prevalence of nasal irritation, rhinitis, sinusitis, cough, expectoration, wheeze, dyspnea	↑
		Prevalence of radiological infiltrates on chest x-ray	↔
		FVC, FEV_1, FEV_1/FVC, PEF, FEF_{25}, FEF_{50}, FEF_{75}	↓

Table 2-6. Results of Selected Epidemiological Studies Evaluating Exposure to Copper and Respiratory Effects

Reference, study type, and population	Exposure concentration	Outcome evaluated	Result
Mourad and El-Sherif 2022 Cross-sectional, 65 male copper smelter workers and 41 matched male administrative workers (mean ages 43.19 and 44.05 years, respectively); workers were exposed to copper, arsenic, lead, and cadmium (Egypt)	Work in smelter operations; mean serum copper was 175.2 µg Cu/dL in exposed and 93.44 µg Cu/dL in controls	Prevalence of exertional dyspnea, cough, expectoration	↑
Boogaard et al. 2013 Cohort study; 661 individuals (at least 4 years of age) in 12 locations, evaluated before and after implementation of traffic reduction policies (Netherlands)	Decrease in mean concentration in ambient air from 2008 to 2010: 27.2 ng Cu/m^3	FVC change between 2008 and 2010	↑ (improved)
Yu et al. 2021b Prospective cohort study; 706 adolescents in PIAMA birth cohort (47.3% male); respiratory symptoms and spirometry evaluated at 13–16 years of age (Netherlands)	Modeled concentration in ambient air at current residence 2.6 ng Cu/m^3 (mean in $PM_{2.5}$) 11 ng Cu/m^3 (mean in PM_{10})	FEV_1, FVC at age 13–16 years	↔
Gehring et al. 2015 Prospective cohort study; 3,702 participants in PIAMA birth cohort (52% male); respiratory symptoms and spirometry evaluated at 8 and 11–12 years of age (Netherlands)	Modeled concentration in ambient air at birth address: 3.1 ng Cu/m^3 (mean in $PM_{2.5}$) 12.8 ng Cu/m^3 (mean in PM_{10})	FEV_1	↓ for Cu in $PM_{2.5}$ at current address
		$FEF_{25–75}$	↓ for Cu in PM_{10} at current address
		FVC	↔

↑ = association; ↓ = inverse association; ↔ = no association; FEF_{25} = forced expiratory flow at 25% of the pulmonary volume; FEF_{50} = forced expiratory flow at 50% of the pulmonary volume; FEF_{75} = forced expiratory flow at 75% of the pulmonary volume; $FEF_{25–75}$ = forced expiratory flow at 25–75% of the pulmonary volume; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PEF = peak expiratory flow; PIAMA = Prevention and Incidence of Asthma and Mite Allergy; $PM_{2.5}$ = particulate matter ≤2.5 µm; PM_{10} = particulate matter ≤10 µm

Copper was considered the etiologic agent in an occupational disorder referred to as "vineyard sprayer's lung." This condition was found in vineyard workers that used an anti-mildew agent known as the "Bordeaux mixture" that contains 1– 2.5% copper sulfate (with pH neutralized via hydrated lime) (Pimentel and Marques 1969). Published information on this disorder is primarily from case reports

(Pimentel and Marques 1969; Pimentel and Menezes 1975; Stark 1981; Villar 1974; Villar and Nogueira 1980) that lacked quantitative exposure information. Alveolar lavage and biopsy findings consisted of interalveolar desquamation of macrophages, formation of histiocytic and noncaseating granulomas containing inclusions of copper, and healing of lesions in the form of fibrohyaline nodules. These lesions are very similar to those found in silicosis (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, and not in rural workers from the same geographic region who did not work in the vineyards (Plamenac et al. 1985).

A few epidemiological studies evaluated respiratory effects of exposure to copper in particulate matter in ambient air (see Table 2-6). A decline of copper concentration in particulate matter was associated with improved forced vital capacity (FVC) in 661 subjects in a cohort study in the Netherlands (Boogaard et al. 2013). In two studies of the same birth cohort, spirometry measures showed inconsistent relationships to copper concentration in particulate matter. When measured at ages 8 and 11–12 years, FEV_1 was inversely associated with copper concentration in $PM_{2.5}$ (particulate matter ≤2.5 μm) at the child's current home address and $FEF_{25–75}$ (forced expiratory flow at 25–75% of the pulmonary volume) was inversely associated with copper concentrations in PM_{10} (particulate matter ≤2.5 μm) (Gehring et al. 2015). When the children were evaluated during adolescence (ages 13–16 years), spirometry measures were not associated with copper concentration in $PM_{2.5}$ or PM_{10} at the child's current residence.

Several case studies reported respiratory effects in humans following both accidental and intentional ingestion of copper sulfate crystals, powder, or liquid; the most common effects are tachypnea (fast breathing) and dyspnea (labored breathing) (Cho et al. 2018; Franchitto et al. 2008; Gunay et al. 2006; Gupta et al. 2018; Hassan et al. 2010; Higny et al. 2014; Sinkovic et al. 2008; Sood and Verma 2011; Yang et al. 2004). Aspiration pneumonia was reported in two cases of intentional copper sulfate ingestion, one in a 45-year-old man and one in a 29-year-old man (Franchitto et al. 2008; Gamakaranage et al. 2011). Diffuse bilateral infiltration of the lungs was observed in a 44-year-old man who intentionally ingested >100 g copper sulfate (Cho et al. 2018).

Respiratory effects were also documented in case reports of exposure to copper by other routes. A 2-year-old female child developed an acute respiratory distress syndrome with cyanosis, dyspnea, bilateral hyperinflation, and interstitial infiltrates of the lungs following inhalation of copper dust (Donoso et al. 2007). A 24-year-old man developed a deviated septum with persistent sinus pressure and

rhinorrhea after spilling molten copper on his face shield; inhalation of the associated fumes was suggested as a contributing factor (Gibson et al. 2011). A 40-year-old woman developed acute respiratory distress syndrome after intentionally inserting an unknown amount of copper sulfate into her rectum (Moussiegt et al. 2020). A 41-year-old woman developed respiratory failure with bi-basal pneumonia after intentionally injecting 2.5 g copper glycinate subcutaneously (Oon et al. 2006).

The potential for copper to induce respiratory effects has been evaluated in acute-duration studies in rats, mice, and hamsters, as well as in intermediate-duration studies in rats and rabbits.

Drummond et al. (1986) compared respiratory effects in mice and hamsters after single and repeated 3-hour inhalation exposures to several sulfate compounds including copper sulfate. The study authors reported exposure concentrations in terms of sulfate (0.09, 0.1, 0.43, 0.93, and 2.53 mg SO_4/m^3) and in terms of "calculated mg metal/m^3" (reporting values of 0.12, 0.13, 0.56, 1.21, and 3.3 mg metal/m^3, respectively). However, the reported copper concentrations are inconsistent with the concentrations reported in terms of sulfate[1]. Because of the error, the copper exposure concentrations are uncertain and effect levels cannot be determined for the study. Drummond et al. (1986) reported decreased cilia beating frequency and a decreased percentage of normal epithelium in tracheal explants from Syrian-Golden hamsters, but not CD-1 mice, after a 3-hour exposure to copper sulfate. However, after repeated 3-hour exposures to the lowest concentrations of copper sulfate, mice exhibited increased alveolar wall thickening. The severity of the effect increased with the duration of exposure, and was characterized as "extensive" after 10 exposures (Drummond et al. 1986). Pulmonary histology was not assessed in hamsters after single or repeated exposures (Drummond et al. 1986).

Poland et al. (2022) compared the respiratory effects of dicopper oxide and copper sulfate pentahydrate particles in rats exposed by inhalation for 2 weeks at identical copper concentrations of 0, 0.18, 0.71, 1.78, and 8.9 mg Cu/m^3. The results showed that copper sulfate pentahydrate induced respiratory effects at a slightly lower copper exposure level than dicopper oxide, but both compounds induced the same kinds of effects. Both male and female rats exhibited alveolar histiocytosis and males showed bronchioloalveolar hyperplasia after exposure to 0.71 mg Cu/m^3 as copper sulfate pentahydrate. Rats of

[1]For example, Drummond et al. (1986) reported one copper sulfate exposure level as 2.53 mg SO_4/m^3 and 3.3 "mg metal/m^3." However, the copper concentration (from copper sulfate) corresponding to 2.53 mg SO_4/m^3 would be 1.67 mg Cu/m^3 (calculated as mg SO_4 x (molecular weight of copper/molecular weight of sulfate). Copper concentrations based on the reported sulfate concentrations of 0.09, 0.1, 0.43, 0.93, and 2.53 mg SO_4/m^3 would be 0.06, 0.07, 0.28, 0.62, and 1.67 mg Cu/m^3, respectively. This apparent error was limited to the copper concentrations, as the aluminum concentrations reported as "mg metal/m^3" for exposures to aluminum sulfate compounds in the study were consistent with the corresponding sulfate concentrations.

both sexes exhibited markedly increased absolute and relative lung weights (≥69% relative to controls) at ≥1.78 mg Cu/m^3 as copper sulfate pentahydrate. In contrast, neither male nor female rats exhibited respiratory effects at 0.71 mg Cu/m^3 as dicopper oxide. At 1.78 mg Cu/m^3 as dicopper oxide, rats of both sexes showed alveolar histiocytosis and females had increased absolute and relative lung weights (28 and 25%, respectively, compared with controls). Significant increases in absolute and relative lung weights (65 and 62%, respectively) were only seen in males at 8.9 mg Cu/m^3 as dicopper oxide (Poland et al. 2022). Rats exposed to both compounds showed acute neutrophilic inflammation in the lungs and degeneration of the olfactory epithelium in the nose at ≥1.78 mg Cu/m^3 (Poland et al. 2022).

In a 4-week follow up study of dicopper oxide (Poland et al. 2022), pulmonary effects in exposed rats were similar to those seen in the shorter-term experiments described above. Observed effects included exposure-related increases in absolute and relative lung weights and in severity of alveolar histiocytosis and neutrophilic inflammation in the lungs at concentrations ≥0.35 mg Cu/m^3. Minimal to mild lymphocyte infiltration was also seen in the nasal passages of males at the highest tested exposure concentration (1.76 mg Cu/m^3). Respiratory tract inflammation was also evident from BALF analyses that showed increases in neutrophils, total protein, and lactate dehydrogenase (LDH) at the same concentrations (Poland et al. 2022). 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 that was not considered adverse (Johansson et al. 1984). No functional (e.g., phagocytic or bactericidal activity) or morphological (as visualized by transmission and scanning electron microscopy) alterations were observed in the alveolar macrophages of similarly exposed rabbits (Johansson et al. 1983).

Data on the potential of copper to induce respiratory effects after oral exposure in experimental animals are limited to a few studies. NTP (1993) found no histological alterations in the lungs of rats orally exposed to 29–325 mg Cu/kg/day as copper sulfate in the diet for 15 or 90 days, respectively, or in mice exposed to 24 or 1,058 mg Cu/kg/day for 15 or 90 days, respectively.

In an unpublished developmental toxicity study reviewed by EPA (2006), one of three pregnant rabbits that died prematurely during gestational exposure to 18 mg Cu/kg/day (as copper hydroxide via gavage) exhibited irregular respiration. At necropsy, all three rabbits exhibited brown liquid in the chest cavity and dark discoloration and/or mottling of lung tissue (reviewed by EPA 2006).

2.5 CARDIOVASCULAR

Human data on cardiovascular effects from exposure to copper are limited. Suciu et al. (1981) compared the health outcomes of workers involved in the grinding and sieving copper dust in 1970 when concentrations in air were high (up to 464 mg Cu/m^3) to the outcomes of workers later when air concentrations were lower (≤111 mg Cu/m^3 in 1972). Among workers exposed in 1970, 16% showed arterial hypertension. In contrast 6% of workers in 1973 had arterial hypertension and palpitations. However, the findings from this study are limited because other factors that could have impacted the cardiovascular system were not reported (Suciu et al. 1981). In a cross-sectional study comparing copper smelter workers exposed to dusts containing copper, arsenic, lead, and cadmium with unexposed administrative workers, exposure in the smelter operations was associated with higher blood pressure and heart rate (Mourad and El-Sherif 2022).

Two cohort studies examined the association between modeled concentrations of copper in ambient air particulate matter and cardiovascular outcomes (Ostro et al. 2015; Peralta et al. 2021) (see Table 2-7). Ostro et al. (2015) observed an association between increased mortality from ischemic heart disease in a cohort of 101,884 current and former female teachers and administrators and increased copper concentration in particulate matter. In a cohort study of older men in Massachusetts (Peralta et al. 2021), copper concentrations in $PM_{2.5}$ were associated with decreased (improved) heart-rate corrected QT interval (prolongation of the QT interval can lead to life-threatening ventricular tachycardia).

Table 2-7. Results of Epidemiological Studies Evaluating Exposure to Copper and Cardiovascular Effects

Reference, study type, and population	Exposure	Outcome evaluated	Result
Mourad and El-Sherif 2022 Cross-sectional, 65 male copper smelter workers and 41 matched male administrative workers (mean ages 43.19 and 44.05 years, respectively); workers were exposed to copper, arsenic, lead, and cadmium (Egypt)	Work in smelter operations; mean serum copper was 175.2 µg Cu/dL in exposed and 93.44 µg Cu/dL in controls	Blood pressure and heart rate	↑

Table 2-7. Results of Epidemiological Studies Evaluating Exposure to Copper and Cardiovascular Effects

Reference, study type, and population	Exposure	Outcome evaluated	Result
Ostro et al. 2015 Prospective cohort study; 101,884 current and former female teachers and administrators in California (mean age 57.3 years), followed from 2001 to 2007 (United States); exposure modeled for each subject's residence	Modeled concentration in ambient air: 0.5 µg Cu/m^3 (mean in $PM_{2.5}$); 0.03 µg Cu/m^3 (mean in ultrafine particles ≤0.2 µm)	Mortality from ischemic heart disease	↑
Peralta et al. 2021 Cohort study; 563 male participants of the Veterans Administration Normative Aging Study in Massachusetts (mean age 74.1 years); exposure modeled for each subject's residence between 2000 and 2011 (United States).	Modeled concentration in ambient air: 3.7 ng Cu/m^3 (mean in $PM_{2.5}$)	Heart-rate corrected QT interval	↓ (improved) with 4-day moving average copper concentration
Liu and Liang 2023 Cross-sectional, 10,175 adult participants >40 years old (~48% male, mean age 57 years) in NHANES (2013–2014) (United States)	Estimated dietary intake based on 24-hour recall: 1.24 mg Cu/day (mean)	Severity of abdominal aortic calcification	↓
Yin et al. 2021 Cross-sectional, 39,757 adult participants (~49% male, mean age 49.6 years) in NHANES (2005–2018) (United States)	Estimated dietary intake: 1.1 mg Cu/day (median)	Prevalence of cardiovascular diseases	↓
Yang et al. 2022 Cross-sectional, 10,550 adult participants (~48% male, mean age 50 years) in NHANES (2013–2018) (United States)	Estimated dietary intake based on 24-hour recall: Q1: <0.799 mg Cu/day Q2: ≥0.799 to <1.072 Q3: ≥1.072 to <1.42 Q4: ≥1.42	Risk of stroke	↓
Tong et al. 2022 Case-control, 80 hypertensive children and 84 age- and sex-matched controls (6–12 years of age) (China)	Estimated dietary intake based on questionnaire: 1.56 mg Cu/day (cases) 2.09 mg Cu/day (controls)	Hypertension	↓

Table 2-7. Results of Epidemiological Studies Evaluating Exposure to Copper and Cardiovascular Effects

Reference, study type, and population	Exposure	Outcome evaluated	Result
He et al. 2022 Prospective cohort, 12,245 participants in China Health and Nutrition Survey, followed for mean 6.1 years (China)	Estimated dietary intake based on 24-hour recall at baseline: ≥1.57 mg Cu/day	Incident hypertension	↑
	<1.57 mg Cu/day	Incident hypertension	↓

↑ = association; ↓ = inverse association; NHANES = National Health and Nutrition Examination Survey; $PM_{2.5}$ = particulate matter ≤2.5 μm; Q = quartile

Epidemiological studies of dietary copper intake have also evaluated cardiovascular effects (Table 2-7). Inverse associations between estimated dietary intake of copper and cardiovascular effects (including severity of abdominal aortic calcification, prevalence of cardiovascular diseases, or risk of stroke) were observed in three cross-sectional studies of adult participants in NHANES surveys (Liu and Liang 2023; Yang et al. 2022; Yin et al. 2021). A small case-control study in China reported an inverse association between estimated dietary copper intake and childhood hypertension (Tong et al. 2022). In a larger prospective cohort study in China, He et al. (2022) observed a U-shaped dose-response relationship between estimated dietary copper intake and incident hypertension. The incidence of hypertension decreased with intake estimates up to 1.57 mg Cu/day, but at higher doses, the incidence of hypertension increased (He et al. 2022).

A number of case studies reported cardiovascular effects following intentional or accidental ingestion of various copper compounds, including copper sulfate, copper oxychloride, and copper-8-hydroxyquinolate. The most common symptoms were elevated pulse rate, low blood pressure, and tachycardia (Cho et al. 2018; Franchitto et al. 2008; Griswold et al. 2017; Gunay et al. 2006; Gupta et al. 2018; Higny et al. 2014; Sinkovic et al. 2008; Sood and Verma 2011). Two case studies reported elevated blood pressure following accidental ingestion of copper sulfate: one in a 65-year-old man who accidentally ingested approximately 10 g copper sulfate diluted in water and one in a 22-year-old man who accidentally ingested 1 cup of copper sulfate powder (Hassan et al. 2010; Higny et al. 2014). Ingestion of copper sulfate crystals resulted in fatal cardiac arrest in two cases: one in a 26-year-old man who intentionally ingested an unknown amount of crystals and another in a 60-year-old man who accidentally ingested 15–18 mg of crystals (Gupta et al. 2018; Griswold et al. 2017). A 30-year-old female who intentionally ingested dehydrated copper sulfate developed swollen feet in addition to low

blood pressure (Yadla et al. 2015). Thinned arteries, congested veins, and cardiac failure were reported in a 19-year-old woman who intentionally ingested an unknown amount of a liquid fungicide whose sole active ingredient was 50% copper oxychloride (Gunay et al. 2006).

No studies were located regarding cardiovascular effects in humans following dermal exposure to copper, but exposure by routes other than those described previously has led to cardiovascular changes. A 40-year-old woman developed toxic myocarditis followed by a 2-minute-long cardiac arrest after intentionally inserting an unknown amount of copper sulfate into her rectum (Moussiegt et al. 2020). A 29-year-old pregnant woman developed peripheral vasoconstriction after intentionally vaginally inserting an unknown amount of copper sulfate powder diluted in water (Motlhatlhedi et al. 2014). A 22-year-old man who was found dead had developed subpleural and sub-epicardial hemorrhage after intentionally injecting approximately 1 g copper sulfate into his arm (Behera et al. 2007). A 41-year-old woman developed low blood pressure and rapid atrial fibrillation after intentionally injecting 2.5 g copper glycinate subcutaneously into her arm at three sites (Oon et al. 2006).

No toxicity studies were located regarding cardiovascular effects in animals following inhalation exposure to copper. A 7-day gavage exposure to 39.8 mg Cu/kg/day as copper sulfate resulted in a significant increase in serum cardiac troponin I as well as apparent histopathological changes (blood vessel congestion, inflammatory cell infiltration, degenerative changes) in the hearts of rats (Sarawi et al. 2021). However, the study authors did not report the incidence or severity of the histopathological changes, so effect levels could not be determined.

Well-conducted intermediate-duration oral studies have not shown effects on heart histology in rats exposed to copper monochloride by gavage for 4–5 weeks (Chung et al. 2009) or in rats or mice exposed to copper sulfate pentahydrate in drinking water for 15 days or in feed for 15 days or 13 weeks (NTP 1993).

In male Wistar rats exposed to 50.9 mg Cu/kg/day of copper sulfate for 30 days, flabby, enlarged, congested hearts were seen at gross necropsy; histopathology was not examined (Khushboo et al. 2018). Based on marked decreases in reported water and food intake (40 and 30% less than controls, respectively) (Khushboo et al. 2018), it is likely that these animals were dehydrated and malnourished. Increased blood pressure was reported in two intermediate-duration studies of male Wistar rats (Arafa et al. 2019; Liu and Medeiros 1986). Exposure to 14 mg Cu/kg/day as copper carbonate in feed for 15 weeks resulted in ~20% higher systolic blood pressure (Liu and Medeiros 1986), while exposure to

50.9 mg Cu/kg/day as copper sulfate pentahydrate (via gavage) for 90 days resulted in 33% higher systolic blood pressure (Arafa et al. 2019).

2.6 GASTROINTESTINAL

There are few human studies documenting gastrointestinal effects after inhalation exposure to copper, and no human studies of these effects after dermal exposure to copper were located. In workers involved in grinding and sieving copper dust, anorexia, nausea, and occasional diarrhea were reported; more rarely, vomiting was also observed (Suciu et al. 1981). Exposure levels declined over time, from 464 to 111 mg Cu/m^3 over a 3-year period, and gastrointestinal symptom frequency declined over the same time period. While initial exposure was primarily via the inhalation route, it is possible that the gastrointestinal effects were due to oral exposure to copper. Ingestion may have resulted from mucociliary clearance of copper particles deposited in the nasopharyngeal and tracheobronchial regions of the respiratory tract. One case study reported vomiting in a 2-year-old female child following accidental inhalation of a copper powder (Donoso et al. 2007).

Gastrointestinal effects of copper in humans exposed orally have been documented in controlled exposure studies, community health investigations of copper in drinking water, and epidemiological studies. Controlled human exposure studies are included in the LSE table (Table 2-2) and discussed in detail below. Epidemiological studies that met inclusion criteria (see Appendix C, Section C.2.2), community health investigations, and case reports/case series are described in text below.

Controlled Human Oral Exposure Studies. Controlled human exposure studies of gastrointestinal effects primarily used drinking water administration. The doses calculated from these studies represent the exposure from copper in drinking water only; several studies did survey participants on their diets, but copper intake from normal diets was not considered in the dose estimations.

Several experiments designed to identify the threshold for gastrointestinal effects were performed, typically involving adults ingesting a single dose of copper sulfate following an overnight fast (Araya et al. 2001, 2003a, 2003c; Gotteland et al. 2001; Olivares et al. 2001). The lowest exposure level resulting in gastrointestinal effects was identified by Olivares et al. (2001), who observed an increased incidence of nausea at 0.012 mg Cu/kg (4 mg Cu/L). No nausea was reported by subjects exposed to lower doses in this study (Olivares et al. 2001). At 0.018 mg Cu/kg (6 mg Cu/L), 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).

Two multinational studies by Araya et al. (2001, 2003c) reported a threshold of 6 mg Cu/L for increased incidence of nausea. In a study by Araya et al. (2001), no nausea was reported following exposure at doses of ≤0.012 mg Cu/kg (4 mg Cu/L), while nausea occurred in 17/179 adults exposed to 0.018 mg Cu/kg (6 mg Cu/L). In this study, females appeared more sensitive to developing nausea following copper ingestion. In Araya et al. (2003c), a single exposure to 0.09 mg Cu/kg (6 mg Cu/L) resulted in nausea in 50/269 females, while no nausea occurred at 0.06 mg Cu/kg. This study determined that both the copper concentration and the total copper dose are important variables in predicting a gastric response; as the concentration and dose increase, the probability of eliciting nausea increases (Araya et al. 2003c).

Nausea and vomiting effects were confirmed in two studies each testing a single exposure to 10 mg Cu/L as copper sulfate in water: 9/30 adults reported nausea in one study (Araya et al. 2003a) and 6/31 adults reported nausea while 2/31 reported vomiting in the other (Gotteland et al. 2001). These studies also examined physiological alteration in the intestines (Araya et al. 2003a; Gotteland et al. 2001). 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) (Araya et al. 2003a). This change in antral area is suggestive of a delay in gastric emptying. As with gastric permeability, this effect was independent of gastrointestinal symptoms.

Repeated exposure studies conducted in adults exposed to copper in drinking water have confirmed the threshold for gastrointestinal symptoms (Araya et al. 2003b, 2004; Olivares et al. 2001; Pizarro et al. 1999, 2001). Abdominal pain, nausea, and/or vomiting were observed in women drinking water containing 5 mg Cu/L (0.096 mg Cu/kg) copper sulfate or copper oxide for 1 week (Pizarro et al. 2001). The occurrence of gastrointestinal effects was not significantly different between subjects ingesting 10 mg Cu/L as copper sulfate or copper oxide (Pizarro et al. 2001).

A study by Pizarro et al. (1999) demonstrated a dose-response relationship between copper sulfate exposure and gastrointestinal symptoms (nausea, vomiting, and abdominal pain) in healthy adult women.

Each study participant consumed either 0, 1, 3, or 5 mg Cu/L of copper as copper sulfate in their drinking water daily for 2 weeks with a 1-week rest period before starting a new exposure. Based on measured water concentrations and water intake, the study authors reported doses of 0.04 (control), 1.74, 4.68, and 7.94 mg Cu from water[2], corresponding to doses of 0.0006, 0.0272, 0.0731, and 0.124 mg Cu/kg/day, respectively. The incidences of abdominal pain, nausea, diarrhea, and/or vomiting were reported, and no dose-response relationship for copper exposure and diarrhea was found (Pizarro et al. 1999). Abdominal pain, nausea, and vomiting were dose-related, and incidences for these symptoms were significantly higher in groups that consumed ≥0.0731 mg Cu/kg/day (≥3 mg Cu/L) than in groups consuming ≤0.0272 mg Cu/kg/day (≤1 mg Cu/L) (Pizarro et al. 1999).

In a 2-month study by Araya et al. (2003b, 2004), 65/355 male and female adults exposed to 0.106 mg Cu/kg/day as copper sulfate (4 mg Cu/L in water used for drinking and food preparation) reported at least one gastrointestinal symptom, among nausea, vomiting, diarrhea, and abdominal pain, at some point during the exposure period. The incidence of symptoms was significantly higher for this dose group compared to subjects exposed to 0.055 mg Cu/kg/day (2 mg Cu/L) (Araya et al. 2003b, 2004). These investigators showed that the incidence of gastrointestinal symptoms increased with copper exposure (concentration in water and volume of water ingested) and females appeared to be at a higher risk for symptoms than males. As the duration of exposure increased, the concentration in water necessary to achieve a positive gastrointestinal response increased (Araya et al. 2003b, 2004).

Abdominal pain and diarrhea were also reported in several of the controlled exposure studies (Araya et al. 2003b, 2004; Pizarro et al. 1999, 2001), but these symptoms did not show a clear relationship to dose among adults. A study of 56 healthy babies who received 2 mg Cu/L of copper sulfate in water daily for 9 months did not observe any significant difference in the incidence of gastrointestinal effects (Olivares et al. 1998). Two babies who were formula-fed had diarrhea, but this was not likely to be exposure-related, as none of the breastfed babies had symptoms. Controls were exposed to copper doses ranging between 0.123 and 0.174 mg Cu/kg/day and experimental infants were exposed to doses ranging from 0.0522 to 0.319 mg Cu/kg/day (Olivares et al. 1998).

Epidemiological Investigations. Two cohort studies that met inclusion criteria examined the association between occurrence of gastrointestinal symptoms and exposure to copper in drinking water. Buchanan et

[2]Pizarro et al. (1999) estimated that the subjects' copper intake from diet ranged between 1.5 and 1.9 mg Cu/day (corresponding to doses of 0.023–0.29 mg Cu/kg/day) over the study; these amounts were not added to the doses received from water.

al. (1999) observed no increased risk of gastrointestinal symptoms during the prior 2 weeks when comparing individuals in homes with drinking water copper concentrations >3 mg Cu/L and those with drinking water copper concentrations <1.3 mg Cu/L (Buchanan et al. 1999). Similarly, Pettersson et al. (2003) observed no association between risk of diarrhea or vomiting among children and copper concentrations in water. These study authors evaluated children's exposure both by intake (<0.5, 0.05–1.0, or >1.0 mg Cu/day) and by concentration (≤2 or >2 mg Cu/L); neither analysis showed a relationship to diarrhea nor to vomiting (Pettersson et al. 2003).

Case Reports/Case Series and Community Health Investigations. Gastrointestinal effects have been documented in case reports of humans after intentional or accidental ingestion of copper substances, and in health investigations of communities with elevated copper levels in drinking water. The most common effects include abdominal pain, nausea, vomiting, diarrhea, and melena (black stool), which typically occur shortly after ingestion and are not persistent (Gupta et al. 2023; Knobeloch et al. 1994, 1998; Shankar et al. 2023; Tsao et al. 2020).

A 1-year-old infant girl developed vomiting and diarrhea within 20 minutes of consuming cake frosting that had been mixed with a non-edible colored dust containing copper. Analysis of the frosting showed a content of 21 mg Cu/g frosting (Tsao et al. 2020). The child's symptoms resolved within a day and she had no long-term effects (Tsao et al. 2020). Gastrointestinal ulcerations and hemorrhaging were observed following copper sulfate ingestion in several case studies (Banerjee et al. 2023; Du and Mou 2019; Franchitto et al. 2008; Galust et al. 2023; Gamakaranage et al. 2011; Griswold et al. 2017; Lubica et al. 2017; Malik and Mansur 2011; Shankar et al. 2023). There have been several reports of upper gastrointestinal effects, including oral mucositis, pharyngeal or esophageal edema and/or corrosive injury, and odynophagia, following copper sulfate ingestion (Galust et al. 2023; Higny et al. 2014; Hassan et al. 2010; Shankar et al. 2023). Dysphagia was reported in a 66-year-old man whose neighbor frequently treated his orchard with copper sulfate, resulting in a "blue dust cloud" to which the man was exposed (Perestrelo et al. 2021). The nature of the patient's exposure was not clearly defined in the report, but may have included both inhalation and oral routes (Perestrelo et al. 2021). Inflammation of the gallbladder was observed in two cases: one in a 19-year-old woman who intentionally ingested an unknown amount of pesticide containing copper oxychloride and another in a 40-year-old man who intentionally ingested 50 mL of a solution containing 33.5% weight by volume copper-8-hydroxyquinolate (Gunay et al. 2006; Yang et al. 2004).

The Wisconsin Division of Health conducted several community health investigations of copper in drinking water (Knobeloch et al. 1994, 1998). Some were in response to community complaints about gastrointestinal symptoms or bitter-tasting water and others were in response to reports of elevated copper concentrations. During the investigations, residents were asked to complete questionnaires about general health and gastrointestinal symptoms. These community health investigations suggested an association between copper intake from drinking water and gastrointestinal symptoms, but the analyses are not sufficiently rigorous to provide independent evidence; copper concentration data generally reflected convenience samples; participant recruitment (e.g., via public meetings to discuss copper levels) may have led to selection bias; covariates/alternative causes for the symptoms were not considered; and the numbers of participants in the investigations were typically quite small.

Animal Studies. No studies were located regarding gastrointestinal effects in animals following inhalation exposure to copper. Gastrointestinal effects have been reported in multiple animal studies of oral administration. In rats administered a single high dose of 50.9 mg Cu/kg/day as copper sulfate pentahydrate, gross necropsy findings included thickened stomach wall with corrugated mucosa (Khushboo et al. 2018). All four shrews exposed to 31 mg Cu/kg as copper sulfate pentahydrate by gavage experienced emesis (vomiting), while exposure to 2.5 mg Cu/kg did not induce vomiting in shrews (Yamamoto et al. 2004). In the same study, rats, which do not possess an emetic reflex, responded to exposure by consuming more kaolin, a common response of rats to emetic agents (Yamamoto et al. 2004).

A single dose of copper chloride (14 mg Cu/kg) administered to rats resulted in duodenal histopathological changes including loss of enterocyte arrangement and brush border, necrotic debris, and lymphocyte and plasma cell accumulations (Husain et al. 2021). Focal intestinal ulceration was reported in mice exposed to 4 mg Cu/kg/day as copper sulfate for 7 days, with no effects at 2 mg Cu/kg/day (Kadammattil et al. 2018); however, the incidence and severity of the lesions was not reported.

Intermediate-duration animal studies have demonstrated tissue damage in the stomach after oral exposure to copper compounds. Increased incidences of squamous cell hyperplasia in the stomach was observed in male and female rats exposed to 13 and 3 mg Cu/kg/day (respectively) as copper monochloride by daily gavage in a combined repeat-dose and reproductive/developmental toxicity screening study (Chung et al. 2009). Hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach was observed in male and female rats exposed to 44–46 mg Cu/kg/day for 15 days or 33–34 mg Cu/kg/day for 13 weeks in their diet as copper sulfate, and in mice

exposed to 197–216 mg Cu/kg/day for 15 days or 187–267 mg Cu/kg/day for 13 weeks in their diet as copper sulfate (NTP 1993). No effects were seen at lower doses of copper in the diet of rats or mice. Animals exposed to copper sulfate in drinking water for 15 days did not show any gastrointestinal effects, including rats exposed to doses up to 26–29 mg Cu/kg/day and mice exposed to doses up to 24–36 mg Cu/kg/day (higher doses were lethal to many of the animals) (NTP 1993).

EPA (2006) reviewed an unpublished developmental toxicity study in rabbits in which four of 21 rabbits exposed to 18 mg Cu/kg/day (as copper hydroxide administered via gavage on GDs 7–28) exhibited stomach hemorrhage, ulceration, or both; deaths were also observed at this dose (reviewed by EPA 2006). As reported in EPA (2021a), *Registration review draft risk assessment for copper 8-quinolinolate (bis(8-quinolinolato)copper(II))*, an unpublished study submitted to EPA reported that dogs exposed to ≥50 mg/kg/day copper 8-quinolinolate by daily capsule for 90 days exhibited vomiting as well as reddened mucosa and hyperemia in the gastrointestinal tract. EPA (2021a) also reviewed an unpublished study of rats exposed by diet for 90 days in which hypertrophy of the duodenal villi was observed in males at doses ≥100 mg/kg/day copper 8-quinolinolate. Based on another study submitted to the Agency, EPA (2021a) reported that male mice exposed to 207.7 mg/kg/day copper 8-quinolinolate in an 80-week carcinogenicity study exhibited increased incidences of stomach ulcers.

Mechanisms. Studies in monkeys, dogs, shrews, and ferrets provide 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 shrews, abdominal vagotomy prevented emesis at low doses of copper sulfate but not at higher doses (Horn et al. 2014). 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 Andrews 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.

2.7 HEMATOLOGICAL

Decreased hemoglobin and erythrocyte levels were observed in workers exposed to airborne copper dust levels of 0.64–1.05 mg Cu/m^3 (Finelli et al. 1981); however, it is unknown if copper is causally related to the effects, given that results of hair analysis revealed that the workers had also been exposed to iron, lead, and cadmium, and the study authors did not control for co-exposures (Finelli et al. 1981).

In a controlled exposure study in which 60 adult females were exposed to copper in drinking water daily for 2 weeks, no changes in hemoglobin were seen with doses as high as 0.1 mg Cu/kg/day (Pizarro et al. 1999). Likewise, when seven adult subjects were exposed to copper gluconate daily in capsule form for 12 weeks, there were no changes in hematocrit or mean corpuscular volume compared to pre-exposure values (Pratt et al. 1985).

Numerous case studies have reported hematological effects in humans following intentional or accidental ingestion of copper-containing substances. The most common effects are hemolytic anemia, hemoglobinemia, methemoglobinemia, leukocytosis, and reduced reticulocyte count (Banerjee et al. 2023; Cho et al. 2018; Du and Mou 2019; Franchitto et al. 2008; Gamakaranage et al. 2011; Griswold et al. 2017; Gunay et al. 2006; Gupta et al. 2018, 2023; Lubica et al. 2017; Malik and Mansur 2011; Mortazavi and Jafari-Javid 2009; Perestrelo et al. 2021; Shankar et al. 2023; Sinkovic et al. 2008; Sood and Verma 2011; Valsami et al. 2012; Yadla et al. 2015; Yang et al. 2004). Cyanosis, a blueish discoloration of the skin usually associated with methemoglobin accumulation, has also been reported in several case studies (Banerjee et al. 2023; Du and Mou 2019; Hassan et al. 2010; Malik and Mansur 2011; Sinkovic et al. 2008; Yang et al. 2004).

Hypoxemia and hemolytic anemia were observed in a 2-year-old female child who spilled a copper powder on her face and inhaled some of the powder (Donoso et al. 2007). Methemoglobinemia, leukocytosis, and hemolysis were observed in a 53-year-old man following dermal contact with a hot copper sulfate solution (Park et al. 2018). In a child who had been severely burned, copper sulfate crystals were applied to the burn area, which resulted in hemolytic anemia and increased serum and urine copper levels (Holtzman et al. 1966). Intravascular hemolysis was observed in a 22-year-old man who intentionally injected approximately 1 g copper sulfate solution intravenously (Behera et al. 2007). Hemolytic anemia was observed in a 41-year-old female who intentionally subcutaneously injected a total of 2.5 g copper glycinate in solution via syringe among three different sites on the forearm (Oon et al. 2006). Methemoglobinemia, elevated blood glucose, and increased white blood cell counts were

observed in a 29-year-old woman who intentionally vaginally inserted copper sulfate powder diluted in water in order to terminate an unwanted pregnancy (Motlhatlhedi et al. 2014).

Only one study of hematological effects in animals exposed to copper by inhalation was located. Poland et al. (2022) observed a significant increase in circulating neutrophils in rats exposed to dicopper oxide particles by inhalation for 4 weeks. Neutrophil counts in the blood were significantly increased in males (≥93% compared to control) at 0.35 and 0.7 mg Cu/m^3, and were increased (88%), but not statistically significant, at the highest concentration of 1.76 mg Cu/m^3. In females, circulating neutrophils were significantly increased (118 and 120%) at 0.7 and 1.76 mg Cu/m^3, respectively, compared to control. No other hematology changes were observed.

Several studies examined the hematological effects of copper in rats, mice, pigs, and rabbits following intermediate-duration oral exposures. Evidence for effects on hematological parameters comes primarily from studies of rats, as studies in other species are more limited. In rats exposed for intermediate durations (20–90 days) to doses of 25.5–39.8 mg Cu/kg/day as copper sulfate, decreased hemoglobin concentration, red blood cell counts, and/or hematocrit were observed (Adele et al. 2023; Kumar and Sharma 1987; Kumar et al. 2015; Rana and Kumar 1980). At the high end of the dose range (39.8 mg Cu/kg/day), marked decreases in erythrocyte count (48–52% less than controls) and hemoglobin (38–47% less than controls) were observed (Kumar and Sharma 1987; Rana and Kumar 1980); changes of this magnitude could affect oxygenation. Adele et al. (2023) also observed a decrease in the myeloid:erythroid ratio in the bone marrow of female rats given 39.8 mg Cu/kg/day for 5 weeks. NTP (1993) did not evaluate hematology in its 15-day drinking water and feed studies of rats; however, histopathology evaluation revealed depletion of hematopoietic cells in bone marrow of male and female rats exposed to 196–198 mg Cu/kg/day as copper sulfate in feed. Hematology evaluations in the 13-week feed studies of copper sulfate showed decreases in hematocrit, hemoglobin concentration, mean cell hemoglobin, and/or mean cell volume, along with increased reticulocyte counts in both male and female rats exposed to ≥66–68 mg Cu/kg/day (NTP 1993). Platelet levels were increased in both sexes at the same doses; NTP (1993) suggested that these changes were consistent with reactive thrombocytosis (NTP 1993). Rats exposed to copper chloride by gavage in a combination repeat-dose and reproductive/developmental screening study for 30–38 days exhibited hematology changes (Chung et al. 2009). At the highest dose of 51 mg Cu/kg/day, males showed significant decreases in erythrocyte counts, hemoglobin concentration, mean cell hemoglobin, hematocrit, and mean cell volume, as well as increased platelets, white blood counts, and percentage of neutrophils. Females showed nonsignificant decreases in many of

the same parameters, along with a significant decrease in mean cell hemoglobin and a significant increase in platelet count.

NTP (1993) did not evaluate hematology in mice in the 15-day or 13-week studies. In these studies, there were no histopathology findings related to hematopoiesis in mice. Kvietkauskaite et al. (2004) reported that no hematological effects were observed in mice exposed to copper sulfate doses of 42 mg Cu/kg/day for 19 weeks, but the study authors did not specify the hematological parameters that were analyzed, and data were not shown.

In pigs, significantly decreased hemoglobin levels and increased erythrocyte counts were seen with 16.5–18.7 mg Cu/kg/day as copper carbonate for 46–49 days of exposure (Suttle and Mills 1966). Kline et al. (1971) saw no changes in hemoglobin levels in pigs following 88 days of exposure to 2.7 mg Cu/kg/day as copper sulfate.

Chronic-duration exposure studies in young monkeys found no hematological effects after exposure to a daily dose of 5.5 mg Cu/kg/day as copper gluconate for 3 years; however, lower hemoglobin levels were observed in adults receiving a dose of 7.5 mg Cu/kg/day when compared to the controls (Araya et al. 2012).

2.8 MUSCULOSKELETAL

There are very limited data on musculoskeletal effects of copper and copper compounds in humans or animals, and the only data are for oral exposures. Rhabdomyolysis (breakdown of skeletal muscle) was reported in two case reports, one in a 25-year-old man who intentionally ingested an unknown amount of a substance thought to contain copper and another in a 53-year-old man who intentionally ingested 120 g of copper sulfate (Lubica et al. 2017; Valsami et al. 2012).

Depressed skeletal growth, as measured by tail length, was observed in rats administered 39.8 mg Cu/kg/day as copper sulfate via gavage for 20 days (Rana and Kumar 1980). Rabbits fed 4.83 mg Cu/kg/day as copper sulfate pentahydrate for 5 weeks showed 4–9% increases in the weights of the foreleg and hindlegs, but the effects were not dose related and no further evaluations were performed (Li et al. 2021). Based on radiographic findings, 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).

2.9 HEPATIC

Several disorders of copper homeostasis in humans result in hepatic effects. Wilson's disease, Indian childhood cirrhosis (ICC), and idiopathic copper toxicosis (ICT) are diseases largely defined by accumulation of copper in the liver. These disorders are described briefly below, followed by studies of human and animal exposure to exogenous copper.

Hepatic Effects in Human Disorders of Copper Homeostasis. Wilson's disease is a rare, autosomal, recessive genetic disorder with a prevalence of approximately 30–50 cases per million in most parts of the world, with a gene frequency of 0.56% and carrier frequency of 1 in 90 (Rodriguez-Castro et al. 2015). In Western countries, the gene frequency is generally lower at 0.36% (Liu et al. 2017). It is primarily characterized by low levels of serum ceruloplasmin and by elevated urinary copper excretion, elevated copper levels in the liver, elevated serum free copper, or the presence of Kayser-Fleischer rings (excess copper deposits in the cornea) (Rodriguez-Castro et al. 2015). The accumulation of copper in the liver is due to a genetic mutation in the ATP7B region on chromosome 13q14, resulting in impaired biliary excretion of copper (Liu et al. 2017). Clinical manifestation of the disease varies but is predominantly hepatic or neurological. Liver effects can range from asymptomatic to liver failure and cirrhosis (Rodriguez-Castro et al. 2015), and three types of liver damage are seen: cirrhosis, chronic active hepatitis, and fulminant hepatic failure. In infants with Wilson's disease, the disease is first characterized by excess hepatic copper despite no histologic indications. Symptoms appear with age and include degenerative change in hepatocytes, fibrosis, and cirrhosis (Scheinberg and Sternlieb 1996). The manifestations of Wilson's disease are not considered to be related to exposure to high levels of copper, but rather the individual's impaired excretion of copper. Individuals with Wilson's disease have elevated levels of hepatic copper when consuming diets with average copper intakes (Scheinberg and Sternlieb 1996).

ICC is a type of liver cirrhosis that was previously considered endemic to India but has since been documented in children of non-Indian origin in multiple countries. It is typically seen in infants and young children 6 months to 3 years in age but has also been diagnosed in children up to 11 years of age (Nayak and Chitale 2013). Predisposition to ICC is suspected to be inherited due to its random occurrence among siblings (up to 22% of siblings affected) and mortality due to liver disease in second-degree relatives of affected children (Nayak and Chitale 2013; Pandit and Bhave 1996). Two widely recognized distinctive features of ICC are coarse, dark brown orcein hepatic 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.

No specific genetic susceptibilities have been linked to ICC, and evidence is inconclusive on whether ICC is caused by external exposure to copper or endogenously through dysregulation of copper in the body (Nayak and Chitale 2013). Several studies suggest that copper overload and liver injury in ICC-diagnosed children resulted from the use of brass vessels for milk storage (Bhave et al. 1987; Tanner 1998; Tanner et al. 1983). Other studies conversely conclude that excess dietary copper was not a likely cause of copper overload in ICC-diagnosed children, including in a 2006 multi-center study in India that compared 227 cases of confirmed ICC with 426 controls (Nayak and Chitale 2013; Sethi et al. 1993). This conclusion is supported by several epidemiological studies of high copper-exposed populations that failed to reveal liver injury in children (Nayak and Chitale 2013).

ICT is believed to be caused by an autosomal-recessive genetic defect in copper metabolism combined with excess dietary copper (Müller et al. 1998; Nayak and Chitale 2013). In the literature, ICT is also referred to as ICC-like liver disease, primary copper toxicosis, and Tyrolean infantile cirrhosis. In general, a few rare, sporadic cases of ICC-like diseases have been reported in 11 countries other than India (Nayak and Chitale 2013). 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 for one to four children or at least one adult (Harada et al. 2020; Nayak and Chitale 2013). Compiling the data from these studies, Müller et al. (1998) found a number of consistent patterns: (1) the age of onset of clinical symptoms occurring 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 (ALP), bilirubin, albumin, and prothrombin time; and (5) marked panlobular and pericellular fibrosis associated with a usually mild inflammatory infiltrate, ballooning degeneration of hepatocytes, and an abundance of Mallory bodies. Previously, ICT was attributed to excess intake of exogenous forms of copper but is more likely attributable to a genetic defect along with abnormal copper metabolism (Harada et al. 2020; Nayak and Chitale 2013). A genealogic investigation conducted by Müller et al. (1996) provided suggestive evidence that the disease is transmitted in an autosomal recessive mode.

Hepatic Effects of Human Exposure to Exogenous Copper. Hepatomegaly was observed in workers involved in grinding and sieving copper dust (Suciu et al. 1981). The exposure levels declined over time, from 464 to 111 mg Cu/m^3 over a 3-year period; however, the prevalence of hepatomegaly increased, rather than decreased, during this time period. One case study reported elevated AST and bilirubin in a 2-year-old female who accidentally inhaled a copper powder (Donoso et al. 2007).

Hepatic clinical chemistry parameters (serum ALT, AST, γ-glutamyl transferase [GGT], and/or LDH) were evaluated in several controlled human oral exposure studies. No significant changes in serum enzyme activities were seen at doses up to 0.138 mg Cu/kg/day as copper sulfate in drinking water for up to 2 months (Araya et al. 2003b; Pizarro et al. 1999, 2001), in the diet for 6 weeks (O'Connor et al. 2003), or in capsule form for 6 months (Rojas-Sobarzo et al. 2013). Similarly, in a study of seven adults receiving capsules (orally) containing 0.15 mg Cu/kg/day as copper gluconate for 12 weeks, no significant alterations in serum enzyme activities were found (Pratt et al. 1985). No alterations in total bilirubin levels or serum ALT, AST, or GGT activities were found in a study of infants (3 months of age at study initiation) exposed to 0.319 mg Cu/kg/day as copper sulfate in drinking water for 9 months (Olivares et al. 1998).

Numerous case reports documented hepatic effects in humans following accidental or intentional ingestion of copper substances, including copper sulfate, copper oxychloride, and copper-8-hydroxyquinolate. The most common effects were altered liver enzyme activity, including changes in serum AST, ALT, ALP, and LDH (Du and Mou 2019; Griswold et al. 2017; Gunay et al. 2006; Hassan et al. 2010; Malik and Mansur 2011; Mortazavi and Jafari-Javid 2009; Sinkovic et al. 2008; Shankar et al. 2023; Yadla et al. 2015; Yang et al. 2004). Liver impairment was reported in two cases that provided limited details: one in a 26-year-old man who intentionally ingested approximately 30 g copper sulfate and another in a 53-year-old woman who intentionally ingested 120 g copper sulfate (Gamakaranage et al. 2011; Lubica et al. 2017). A 17-year-old boy who ingested 10 g cupric sulfate developed hemolytic jaundice and a 19-year-old woman who ingested an unknown amount of a copper oxychloride-containing pesticide developed jaundice of the conjunctivae (Du and Mou 2019; Gunay et al. 2006). 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 levels and AST activity, and elevated bile salts in the urine were found in five of the individuals with jaundice. In case reports of lethal ingestion of copper sulfate, jaundice (Akintonwa et al. 1989), centrilobular congestion (Lamont and Duflou 1988), and acute hepatotoxicity (Ahasan et al. 1994) have

been reported. O'Donohue et al. (1993) reported a case of an adult with jaundice and hepatomegaly following 3 years of exposure to copper in supplements. For 2 years, the individual had ingested 30 mg Cu/day followed by 1 year of 60 mg Cu/day. Among six patients examined for chronic copper poisoning, five patients suffered from hepatopathy (Eife et al. 1999). Copper concentrations in tap water of the examined patients ranged from 0.1 to 16.9 mg Cu/L (Eife et al. 1999). Two studies of infants up to 12 months of age who were exposed to ≥0.8 mg Cu/L in household water did not find significant alterations in serum parameters of liver function (serum AST, ALT, or GGT) or alterations in liver ultrasound imaging (Zietz et al. 2003a, 2003b).

Data regarding hepatic effects in humans following dermal exposure to copper are limited to one case study. Elevated serum AST and bilirubin and reduced serum albumin and total protein were observed in a 53-year-old man who slipped and landed on a hot copper sulfate solution on the floor of his workplace, resulting in burns primarily to his legs (Park et al. 2018). It is unclear whether the liver effects were attributable to copper exposure or physical burns, which often result in cholestasis.

Hepatic effects were observed in humans following intentional injection of copper substances. A 22-year-old man intravenously injected approximately 1 g copper sulfate mixed with water into his arms and developed substantial hepatic necrosis (Behera et al. 2007). A 41-year-old woman subcutaneously injected 2.5 g copper glycinate and then developed acute hepatic failure with elevated AST and reduced ALT (Oon et al. 2006). Elevated AST and ALT were observed in a 29-year-old pregnant woman who intentionally vaginally inserted an unknown amount of copper sulfate powder dissolved in water (Motlhatlhedi et al. 2014).

Animal Studies. No treatment-related changes in liver weight or liver histopathology were observed in rats exposed to dicopper oxide or copper sulfate pentahydrate by whole-body inhalation at concentrations up to 8.9 mg Cu/m^3 for 2 weeks or to dicopper oxide concentrations up to 1.76 mg Cu/m^3 for 4 weeks (Poland et al. 2022). No other studies of liver effects in animals exposed by inhalation were located.

The hepatotoxicity of copper in animals is described and investigated in numerous acute- and intermediate-duration oral exposure studies. Many of these studies were designed to evaluate the protective effects of various antioxidants (e.g., curcumin) against the hepatic effects of copper. These studies typically reported histopathology findings qualitatively using representative photomicrographs, and these often provided too little information to determine effect levels.

Studies of hepatic effects in acute-duration oral studies were limited by lack of histopathology evaluation or failure to report quantitative histopathological findings. In rats exposed to 119 mg Cu/kg/day as copper sulfate for 7 days, serum ALT[3] was increased nearly 3-fold relative to controls (Alhusaini et al. 2018a). No other hepatic endpoints were evaluated. Another study by the same authors reported a similar change in serum ALT at a dose of 39.8 mg Cu/kg/day; in this study, serum LDH was increased >2-fold, and serum AST was also increased relative to controls (Alhusaini et al. 2018b). Results of histopathology examination were reported qualitatively, and consisted of massive cellular degeneration and necrosis (Alhusaini et al. 2018b). Haywood (1980) reported parenchymal cell hypertrophy in the liver, while Haywood and Comverford (1980) reported increased serum ALT activity, in small groups of male rats given 300 mg Cu/kg/day in feed as copper sulfate for 1–2 weeks. Mice exposed to a single gavage dose of copper sulfate at doses between 0.4 and 4 mg Cu/kg/day reportedly exhibited histopathological changes ("lower cellularity and hemorrhage") in the liver; however, the study authors did not provide incidences or severity of the effect in the exposed or control groups, so effect levels could not be determined. (Kadammattil et al. 2018).

Intermediate-duration oral studies reported hepatic effects in various mammal species. Among these, a few studies were designed to evaluate systemic toxicity and dose-response relationships, including the Chung et al. (2009) combined repeat-dose and reproductive/developmental screening study of copper monochloride in rats and the NTP (1993) 15-day and 13-week studies of copper sulfate pentahydrate in rats and mice. These studies evaluated liver weight and histopathology, and in some cases clinical chemistry as well, and reported results quantitatively. Chung et al. (2009) did not observe any changes in clinical chemistry, liver weights, or liver histology in male or female rats exposed by gavage to doses up to 51 mg Cu/kg/day for 30 and 38 days, respectively. In the NTP (1993) 15-day drinking water studies, no hepatic effects were seen after exposure to doses up to 26–29 mg Cu/kg/day (rats) or 24–36 mg Cu/kg/day (mice); higher doses were associated with animal deaths in both species. In the NTP (1993) feed studies, hepatic effects were seen in rats, but not in mice. Male and female rats exposed for 15 days via feed exhibited minimal to mild mononuclear inflammatory cell infiltrate in the liver at doses of 198 mg Cu/kg/day (males) and 285 mg Cu/kg/day (females). In the 13-week feed study, dose-dependent increases in the incidence of chronic active inflammation in the liver were seen in male rats at 33 mg Cu/kg/day and in female rats at 68 mg Cu/kg/day. Chronic active inflammation with focal necrosis was first seen in 1 of 10 male rats at 33 mg Cu/kg/day and in all male rats at 66 mg Cu/kg/day. Males also showed a doubling of serum ALT at 33 mg Cu/kg/day. No hepatic effects were seen in mice exposed via

[3]In the absence of information on other liver endpoints, increases in serum AST or ALT activities at least 2–3-fold higher than controls were considered to be adverse.

diet to doses up to 717–780 mg Cu/kg/day for 15 days or doses up to 815–1,058 mg Cu/kg/day for 13 weeks NTP (1993).

The majority of other studies in rats exposed to copper sulfate by gavage or via diet provide support for the hepatic effect levels identified by the NTP (1993) studies. In these studies, serum chemistry changes (increases in serum ALT and AST) and histopathological changes consisting of inflammation, necrosis, and hepatocyte degeneration were reported at doses ≥40 mg Cu/kg/day for at least 3 weeks (Fuentealba et al. 2000; Kumar and Sharma 1987; Haywood 1980; Haywood and Comerford 1980; Haywood and Loughran 1985; Rana and Kumar 1980; Sakhaee et al. 2012; Seven et al. 2018). A few intermediate-duration studies reported hepatic effects in rats at lower doses of copper (as the sulfate). Kumar et al. (2015, 2016a, 2016b) reported marked (>2-fold) increases in serum ALT, AST, and bilirubin, as well as significant increases in the severity of liver histopathological changes (hepatocellular degeneration and hemorrhage, necrosis, fatty change) after 90 days of exposure to copper sulfate pentahydrate by daily gavage at doses ≥25.5 mg Cu/kg/day. Incidences of the histopathological changes were not reported, but the liver lesions were graded as severe after 90 days (Kumar et al. (2015, 2016a, 2016b). Patwa and Flora (2020) observed increased severity of necrosis of hepatocytes, distorted sinusoidal space, and central vein distortion (scored as moderate to severe, incidences not reported) in the livers of male rats given 8 mg Cu/kg/day by gavage for 16 weeks. In another study designed to evaluate the mitigating effects of a plant-based antioxidant, Temiz et al. (2021) reported liver lesions (dilatation of sinusoids, hepatocellular degeneration, coagulation necrosis, and inflammatory cell infiltration) without biologically significant changes in serum AST, ALT, or LDH in all rats exposed to 3.9 mg Cu/kg/day by gavage twice per week for 4 weeks.

Hashish and Elgaml (2016) reported similar histopathological changes (acute swelling of hepatocytes, hepatocytes with coagulative necrosis, and mild hyperplasia of the bile duct epithelium) in rats exposed to 1.6 mg Cu/kg/day as copper sulfate for 30 consecutive days, but did not provide quantitative information on the effects (incidence or severity), precluding determination of effect levels. Adele et al. (2023) observed no change in relative liver weight in female rats given 39.8 mg Cu/kg/day as copper sulfate for 5 weeks, but did not evaluate any other hepatic endpoints.

A handful of studies have evaluated limited hepatic effects in rats exposed to other copper compounds. Rats exposed to copper acetate in drinking water at a dose of 8.6 mg Cu/kg/day exhibited increased serum AST (>2-fold) after 90 days; no other hepatic endpoints were evaluated (Epstein et al. 1982). Wistar male rats showed a 30% decrease in relative liver weight and enlarged liver with dark spots and swollen

borders, friable and yellow in color, following 50.9 mg Cu/kg/day as copper sulfate for 30 days (Khushboo et al. 2018). Sugawara et al. (1995) observed >2-fold increases in serum ALT and AST in rats exposed to 34 mg Cu/kg/day as copper chloride in the diet for 60 days. When male rats received 60 mg Cu/kg/day as tribasic copper chloride in food for 24 weeks, decreased hepatocyte count and hepatocyte area; damaged, disordered, or absent hepatic cords; and increased numbers of cells with hyperchromatic nuclei and concentrated cytoplasm were observed (Yu et al. 2021a). No changes in the ratio of liver to brain weight or serum AST, ALT, or ALP activity were seen at this dose, but at the high dose of 120 mg Cu/kg/day, serum enzyme levels were increased nearly 2-fold. Abe et al. (2008) found no significant difference in liver weight in Fischer 344 rats receiving 62 mg Cu/kg/day as copper gluconate for 6 weeks compared to controls.

NTP (1993) conducted a 13-week study in rats fed copper sulfate and noted that copper accumulation in the liver of males appeared dose-related, as did chronic active tissue inflammation in both sexes. In females, there were no effects at doses ≤34 mg Cu/kg/day. However, at 68 mg Cu/kg/day, chronic active liver inflammation was reported in 6/10 females, and it was reported in all females at the highest dose of 134 mg Cu/kg/day (NTP 1993). Chronic active inflammation with focal necrosis was first seen in 1 of 10 male rats at 33 mg Cu/kg/day and in all male rats at 66 mg Cu/kg/day. No effects were noted in males exposed to 8–16 mg Cu/kg/day (NTP 1993). In the 15-day studies, males showed no histological changes at 29–92 mg Cu/kg/day as copper sulfate, but there was liver inflammation manifested as minimal to mild mononuclear inflammatory cell infiltrate at 198 mg Cu/kg/day. No histological changes were observed in any females in the 15-day studies, with no effects at doses of 31–285 mg Cu/kg/day as copper sulfate (NTP 1993). The 15-day NTP animal studies tested lower doses in both sexes but did not evaluate serum chemistry changes. Increased incidences of granular and vacuolar degeneration of hepatocytes, necrotic hepatocytes, and disordered hepatic cord arrangement were reported in mice exposed to ≥4 mg Cu/kg/day as copper sulfate for 42 days (Liu et al. 2021c). The same findings were reported without quantitative information by Liu et al. (2020a, 2020b, 2021b) and Wu et al. (2020); these publications were by the same group of investigators and appear to reflect a single experiment. Biologically significant increases in serum AST and/or ALT were reported in mice exposed to 16 mg Cu/kg/day for 20 days (Dab et al. 2023) or 80 mg Cu/kg/day for 42 days (Sakhaee et al. 2014) (as copper sulfate) in studies that did not evaluate other hepatic endpoints.

An unpublished study submitted to EPA and summarized in EPA (2021a), *Registration review draft risk assessment for copper 8-quinolinolate (bis(8-quinolinolato)copper(II))*, reported significant increases in

serum AST, ALT, and bilirubin (males only), as well as increased incidences of diffuse degeneration of the liver, in male and female rats exposed to doses ≥100 mg/kg/day copper 8-quinolinolate.

Studies of liver toxicity in species other than rats and mice are limited. No hepatic effects were observed in pigs given 3.62 mg Cu/kg/day as copper sulfate in feed for 6 weeks (Zhang et al. 2020). Two out of six pigs fed a diet containing 16.5 mg Cu/kg/day as copper carbonate for 46 days displayed jaundice, while five out of six pigs given 18.7 mg Cu/kg/day for 49 days displayed jaundice and AST levels elevated by >100%, compared to controls (Suttle and Mills 1966). No changes were observed in serum liver enzymes or liver histology changes in rhesus monkeys given supplemental copper in formula (6.6 mg Cu/L) from birth to 5 months of age (Araya et al. 2005). The study authors did not provide information on the intake of formula, so dose estimates could not be made for this study, and it is not included in the LSE table or figure.

Data pertaining to hepatic effects in animals exposed chronically were limited to one monkey study. A study of young tufted capuchin monkeys exposed to copper (as copper gluconate) in milk (5.5 mg Cu/kg/day) and adult monkeys exposed via feed (7.5 mg Cu/kg/day) did not identify any adverse hepatic effects after 3 years of exposure (Araya et al. 2012). The monkeys were evaluated for serum enzyme activities in blood every 2–3 months and liver biopsies were collected for histopathology every 3–6 months during the study (Araya et al. 2012).

2.10 RENAL

Data regarding renal toxicity of copper inhalation in humans is limited to a single case study. A 2-year-old female who inhaled an unknown amount of a copper powder and spilled some on her facial skin developed renal failure accompanied by oliguria (low urine output) (Donoso et al. 2007).

Renal toxicity was observed in a number of case studies following accidental and intentional ingestion of copper sulfate, the most common effects being elevated serum creatinine, oliguria, hemoglobinuria, and hematuria (blood in urine) (Du and Mou 2019; Franchitto et al. 2008; Gamakaranage et al. 2011; Gupta et al. 2018; Hassan et al. 2010; Lubica et al. 2017; Malik and Mansur 2011; Mortazavi and Jafari-Javid 2009; Shankar et al. 2023; Sinkovic et al. 2008; Sood and Verma 2011; Yadla et al. 2015; Yang et al. 2004). In some cases, renal failure was reported in conjunction with other manifestations of copper toxicity without providing further details on the nature of the renal effects (Valsami et al. 2012; Griswold et al. 2017; Gunay et al. 2006). In addition to oliguria and hemoglobinuria, a 40-year-old man also

developed ketonuria and proteinuria following intentional ingestion of copper-8-hydroxyquinolate (Yang et al. 2004). A 19-year-old woman who intentionally ingested an unknown amount of a pesticide containing copper oxychloride developed chronic renal failure (Gunay et al. 2006).

Congestion of the glomeruli and denudation of tubular cells were observed in four individuals who consumed 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, all 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). No studies were located regarding renal effects in humans following dermal exposure to copper.

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

Some experimental rat studies confirm that the kidney is a target of copper toxicity in cases of copper overload. Increased serum levels of creatinine and blood urea nitrogen (BUN) and renal interstitial bleeding were observed in rats given a single gavage does of 2 mg Cu/kg/day as copper chloride (Husain et al. 2023). Doses of 119 mg Cu/kg/day as copper sulfate given to rats for 1 week resulted in increased serum levels of urea, uric acid, and creatinine, and renal histology that includes destroyed glomerular corpuscles and epithelial lining of the proximal and distal convoluted tubules, and glomerular epithelial hyperplasia (Alharbi et al. 2019). Rats gavaged with 25.5 mg Cu/kg/day as copper sulfate pentahydrate for 2 weeks exhibited increased serum levels of urea, uric acid, and creatinine (Abdel-Baky 2019). However, other acute-duration studies found no renal effects. No kidney related serum chemistry changes were observed in rats administered 888 mg Cu/kg/day as copper oxide in gavage for 3 days (Keshavarzi et al. 2019) and no gross or histological lesions were observed in rats administered 300 mg Cu/kg/day as copper sulfate in the diet for up to 2 weeks (Haywood 1980). An acute-duration study by Kadammattil et al. (2018) reported no significant cellular changes in the kidneys of mice dosed with 4 mg Cu/kg/day as copper sulfate.

Several intermediate-duration studies reported kidney dysfunction, indicated by significantly elevated BUN and creatinine, and histological lesions. Rats exposed for 28 days to gavage doses of 25.5–39.8 mg Cu/kg/day as copper sulfate had increased serum levels of BUN and creatinine and renal lesions of tubular degeneration, necrosis, tubular dilation, and glomerular degeneration (Dai et al. 2020, 2023). Mice exposed to 80 mg Cu/kg/day as copper sulfate via gavage for 28 days had increased serum levels of BUN and creatinine and renal histology of tubular degeneration, dilation, and necrosis (Peng et al. 2020).

Significantly increased urea and creatinine were noted in rats following 30 days of exposure to 50.9 mg Cu/kg/day as copper sulfate pentahydrate (Khushboo et al. 2018). Kumar and Sharma (1987) reported significantly elevated urea levels at 39.8 mg Cu/kg/day as copper sulfate for 30 days. Baali et al. (2023) observed increased serum levels of urea, uric acid, and creatinine in rats exposed to 12.1 mg Cu/kg/day as copper quinolate for 8 weeks. While no changes in kidney weights were observed, histology results include glomerular atrophy resulting in glomerular space dilation, and congestion and hypertrophy of the glomerular chamber, although these lesions were not quantified and were therefore not included in the LSE table. Increased serum levels of BUN were observed in rats exposed to 25.5 mg Cu/kg/day as copper sulfate for 90 days (Kumar et al. 2015). Copper-induced histological changes in the kidneys of male rats exposed to 39.8 mg Cu/kg/day as copper sulfate for 20–90 days included necrosis of the tubules, engorged uriniferous tubules, nuclear pyknosis and cell proliferation in the medullary region, hemorrhage, and glomerular capsule degeneration in the cortex (Kumar et al. 2015, 2016a; Rana and Kumar 1980). Kumar et al. (2016a) observed time- and dose-dependent increased severity of histological damage in rats treated for 30, 60, or 90 days with copper sulfate. The severity score criteria used a 1–5 scale to grade vascular, inflammatory, and cellular degenerative changes in the kidney (Kumar et al. 2016a). Kumar et al. (2016a) reported that the histopathological severity score positively correlated with BUN. In a second study, Kumar et al. (2016b), found a time-related, positive correlation between increased BUN and serum creatinine and free copper levels. Sakhaee et al. (2012) reported renal lesions of mild tubular necrosis and hyaline casts following exposure in rats treated with 39.8 mg Cu/kg/day as copper sulfate for 8 weeks. Additional histological observations included necrosis, degeneration, and desquamation to the epithelial lining of the proximal and distal convoluted tubules in rats exposed to doses up to 199 mg Cu/kg/day as copper sulfate (Alharbi et al. 2019; Haywood 1980; Seven et al. 2018). Rats fed up to 12.7 mg Cu/kg/day as copper chloride, exhibited glomerular swelling and proliferation of interstitial cells; however, the incidence and severity of these histological results was not quantified and thus were not included in the LSE table (Wan et al. 2020). Increased relative kidney weights were also noted at 12.7 mg Cu/kg/day but in the absence of body weight or absolute kidney weight data, the relevance is unclear.

NTP (1993) evaluated copper sulfate pentahydrate exposure in drinking water and diet to rats and mice for 2 or 13 weeks. Renal effects observed in rats fed 92–93 mg Cu/kg/day as copper sulfate for 15 days include increased protein droplets in cortical tubules in male and female rats (NTP 1993). Increases in serum levels of BUN and an increase in cortical tubule protein droplets in the proximal tubule were observed in rats fed 17 mg Cu/kg/day as copper sulfate for 13 weeks (NTP 1993). However, NTP (1993) reported no renal effects in mice of both sexes exposed to 24–36 mg Cu/kg/day in water or 717–781 mg Cu/kg/day in the diet for 15 days or in mice fed 815–1,058 mg Cu/kg/day in feed for 13 weeks.

No kidney-related changes in serum chemistry parameters, urinalysis, kidney weights, or histopathology were observed in rats given 51 mg Cu/kg/day as copper chloride via gavage for up to 38 days in a combined repeat-dose and reproduction/developmental toxicity screening study (Chung et al. 2009).

2.11 DERMAL

Information regarding dermal effects in humans following copper inhalation is limited to one occupational study. Impregnation of the squamous nasal epithelium and nails with colored copper deposits was seen in 44 workers involved with grinding and sieving of copper dust (Suciu et al. 1981). These workers made up more than half of the studied workers (Suciu et al. 1981). Forty-three workers had fissured palmo-plantar hyperkeratosis. The workers had been exposed to declining concentrations (from 464 to 111 mg Cu/m^3) over a 3-year period, but the study authors did not evaluate changes in dermal condition prevalence over this time period (Suciu et al. 1981).

Several case studies reported dermal effects in humans following intentional and accidental ingestion of copper substances, including copper sulfate, copper oxychloride, and copper-8-hydroxyquinolate. Reports of skin discoloration in human case studies following copper ingestion used descriptive terms such as mauve lavender (Sood and Verma 2011), yellow (Du and Mou 2019), and green (Yadla et al. 2015). Pallor was reported in conjunction with cyanosis or other skin discoloration in two cases (Malik and Mansur 2011; Yadla et al. 2015) and reported as the only observed dermal effect in two cases (Gunay et al. 2006; Mortazavi and Jafari-Javid 2009).

Second-degree chemical burns were reported in two cases following dermal exposure to copper. One case was a 53-year-old man who developed severe burns and cyanosis after spilling a hot copper sulfate solution on his leg; however, the burns may have been physical in origin (due to temperature) rather than chemical (Park et al. 2018). Copper sulfate solutions are not usually regarded as caustic (causing chemical burns). Another case was an 11-year-old girl who developed burns on her hands with bilateral cellulitis after a blue substance, later identified as copper sulfate, was deliberately applied to her hands in a traditional healing ceremony (Lapid 2008).

One case report documented contact urticaria in a 22-year-old man after dermal exposure to copper (Seki et al. 2021). The man developed a rash on his wrists after using an electric file to shave a copper plate.

Subsequent skin prick testing with copper sulfate solution at a dermatology office yielded positive results, while patch testing was negative (Seki et al. 2021).

Dermal effects have been recorded after injection exposures to copper. A 22-year-old man developed yellow skin discoloration after intentionally injecting approximately 1 g copper sulfate intravenously (Behera et al. 2007). A 41-year-old woman developed necrotic tissue surrounding injection sites after intentionally injecting 2.5 g copper glycinate subcutaneously (Oon et al. 2006).

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

Data on dermal effects in animals following oral exposure to copper are limited to a single study in rats exposed daily to 50.9 mg Cu/kg/day as copper sulfate for 30 days, in which rough, dry skin with alopecia, most notably on the skin of the abdominal region, was reported (Khushboo et al. 2018).

No dermal effects were seen in rats dermally exposed to doses up to 1,000 mg/kg/day copper 8-quinolinolate for 4 weeks in an unpublished study submitted to EPA and reviewed by EPA (2021a).

2.12 OCULAR

Very few reports of ocular effects after copper exposure were located. Kayser-Fleischer rings (excess copper deposits in the cornea) are a common finding in patients with Wilson's disease (Rodriguez-Castro et al. 2015). Eye irritation was reported by factory workers exposed to copper dust (Askergren and Mellgren 1975). A 64-year-old man developed a corneal ulcer with gradual vision loss and pigment discoloration in his left eye 3 years after retiring from a job where he handled copper wire regularly (Cai et al. 2009). It was suspected that a small piece of copper wire was lodged in his eye, causing the ulcer and vision loss.

No animal studies examining ocular effects following inhalation, oral, or dermal copper exposure were located.

2.13 ENDOCRINE

Seven cases of enlargement of the sella turcica and nonsecretive hypophyseal adenoma, accompanied by obesity, arterial hypertension, and "red facies" were observed in a group of 100 workers exposed to 111–

464 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 (Suciu et al. 1981); however, neither the significance of this effect nor its relationship to copper exposure can be determined.

Three case studies reported endocrine effects in humans following intentional ingestion of copper sulfate. A 26-year-old man developed acute pancreatitis after intentionally ingesting approximately 30 g copper sulfate (Gamakaranage et al. 2011). A 53-year-old man also developed acute pancreatitis after intentionally ingesting 120 g copper sulfate (well above reported lethal doses); medical intervention prevented death (Lubica et al. 2017). A 33-year-old woman developed adrenal insufficiency with reduced cortisol after intentionally ingesting an unknown amount of copper sulfate (Sinkovic et al. 2008). In all cases, the endocrine effects were not permanent, and the patients made full recoveries within weeks. No studies were located regarding endocrine effects in humans following dermal exposure to copper.

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

Oral studies in animals consistently showed no evidence of endocrine effects. Rats exposed to up to 20 mg Cu/kg/day as copper monochloride for 30 days had no treatment-related changes in adrenal, thyroid, or pituitary gland weights or histopathology (Chung et al. 2009). No histological differences were observed in the adrenal, parathyroid, or pituitary glands of rats exposed to copper sulfate at doses as high as 31–36 mg Cu/kg/day in water or 285–324 mg Cu/kg/day in feed for 15 days (NTP 1993). Similarly, no differences in these measures were seen in mice exposed to doses as high as 24–62 mg Cu/kg/day for 15 days in water (NTP 1993). Additionally, no effects were observed in the same study in rats exposed to dietary doses of up to 134–140 mg Cu/kg/day or in mice exposed to dietary doses as high as 815–1,058 mg Cu/kg/day for 13 weeks (NTP 1993).

2.14 IMMUNOLOGICAL

A controlled exposure study in humans exposed to copper-containing welding fumes reported a significant increase in blood C-reactive protein (Markert et al. 2016). Men were exposed to 0.41 mg Cu/m^3 in a copper-only (zinc-free) welding fume for 6 hours/period, for 3 periods with 1 week between exposure periods. Welding fumes were generated in a separate room and were connected by a ventilation system to the room where subjects were exposed (Markert et al. 2016). The change in C-reactive protein was <1 mg/L relative to control subjects (control data were published in an earlier study) and absolute

mean values were not reported, so it is uncertain whether the increased C-reactive protein was clearly adverse. Several similar experiments evaluating inflammatory markers and respiratory function were conducted by the same laboratory (Krabbe et al. 2019, 2023; Reisgen et al. 2020), but these used zinc- and copper-containing welding fume which contained ~60% zinc and ~20% copper. As a result, it is not possible to discern effects from copper itself in these experiments.

In a birth cohort in the Netherlands, increased risk of allergic sensitization was positively associated with modeled estimates of copper in airborne PM_{10} in children's current home address (Gehring et al. 2015). Copper in particulate matter was not associated with incident asthma, asthma symptoms, hay fever, or rhinitis (Gehring et al. 2015).

Immunological effects were evaluated in a controlled exposure study in which nine men were exposed to copper in their food (Turnlund et al. 2004). The experiment began with an 18-day period during which the men consumed a controlled diet providing 1.6 mg Cu/day while residing in a metabolic research unit. At the end of that period, the subjects resumed their normal diets at home and took supplements containing 7 mg Cu/day as copper sulfate for 129 days, followed by a second 18-day residential period in the metabolic research unit during which they received a controlled diet providing 7.8 mg Cu/day (Turnlund et al. 2004). The study authors did not report the dietary copper level during the intervening 129 days, so dose levels across the entire exposure period could not be reliably estimated and effect levels could not be determined. Blood samples collected at the end of each 18-day residential period were analyzed for white blood cell, polymorphonuclear (PMN) cell, and lymphocyte counts; immunoglobin G; and interleukins 2R and 6 (IL-2R and IL-6). During the second 18-day period of exposure, the men had significantly lower PMN cells and higher lymphocytes, as well as significantly lower IL-2R levels when compared with the results from the first 18-day period (Turnlund et al. 2004). The study authors also evaluated antibody titer after the men received a trivalent influenza vaccine. The timing of the vaccinations was inconsistently reported in the publication, and it is not clear whether the vaccines were administered during the 129-day "free-living" period or during the second 18-day residential period. Blood was collected for antibody titers before immunization and 14 days after immunization and compared with results for a similarly immunized control group of 10 subjects who did not receive copper supplements (Turnlund et al. 2004). The copper-exposed subjects exhibited smaller increases in antibody titers to all three influenza strains compared than controls (controls showed 32–92-fold increases from pre-immunization titers, while exposed subjects showed 12–14-fold increases), although the difference was statistically significant for only one strain (Turnlund et al. 2004).

Case reports documenting immune system effects in humans are limited. Reduced albumin and globulin were observed in a 17-year-old boy who ingested 10 g copper sulfate (Du and Mou 2019). Eife et al. (1999) reported that, among 29 patients with chronic copper poisoning from plumbing, one had a natural-killer cell deficiency. Copper levels in tap water measured in homes of these patients ranged from 0.1 to 16.9 mg Cu/L (Eife et al. 1999).

In some individuals, exposure to copper metal produced 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; Seki et al. 2021). Axillary lymphadenopathy was reported in an 11-year-old boy who had copper sulfate crystals intentionally applied to his hands (Lapid 2008).

An acute-duration inhalation study in mice reported an impaired immune response in host defense assays following inhalation exposure to copper sulfate (Drummond et al. 1986). The study authors reported exposure concentrations both in terms of sulfate (mg SO_4/m^3) and in terms of "calculated mg metal/m^3." However, the reported copper concentrations were inconsistent with the concentrations reported in terms of sulfate[4]. This apparent error was limited to the copper concentrations, as the aluminum concentrations reported as "mg metal/m^3" for aluminum sulfate compounds in the study were consistent with the corresponding sulfate concentrations. Because of the error, the copper exposure concentrations are uncertain and effect levels cannot be determined for the study. In the study, 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* (Drummond et al. 1986). There were no functional differences in macrophages in rabbits exposed to 0.6 mg Cu/m^3 as copper chloride for 6 hours/day, 5 days for 1 month (Johansson et al. 1983).

Only one study of immune system effects following acute-duration oral exposure to copper in animals met inclusion criteria. In mice, a 7-day exposure to copper sulfate at doses between 1 and 4 mg Cu/kg/day resulted in follicular hyperplasia in the spleen (Kadammattil et al. 2018). Incidences and

[4]For example, Drummond et al. (1986) reported one copper sulfate exposure level as 2.53 mg SO_4/m^3 and 3.3 "mg metal/m^3." However, the copper concentration corresponding to 2.53 mg SO_4/m^3 would be 1.67 mg Cu/m^3.

severities of the lesion were not reported by Kadammattil et al. (2018), precluding identification of effect levels for the spleen.

Intermediate-duration oral studies of spleen weight and histopathology had mixed results. No histopathological changes were seen in the spleens of rats exposed to doses as high as 31–36 mg Cu/kg/day as copper sulfate in drinking water or as high as 285–325 mg Cu/kg/day as copper sulfate in feed for 15 days (NTP 1993). However, rats exposed for 30 days to 50.9 mg Cu/kg/day as copper sulfate showed congested and enlarged spleens (Khushboo et al. 2018).

In rats exposed to 199 mg Cu/kg/day as copper sulfate for 21 days, serum tumor necrosis factor-alpha (TNF-α) levels were increased 1.55 times greater than in controls, but no other evidence of inflammation was examined (Seven et al. 2018). Decreased white blood cell counts of 42% were observed in female rats given 39.8 mg Cu/kg/day as copper sulfate for 5 weeks (Adele et al. 2023). There were no effects on spleen weight or histology in rats exposed to up to 51 mg Cu/kg/day as copper chloride for ~35 days (Chung et al. 2009). A 19-week study in mice exposed to 22 mg Cu/kg/day as copper sulfate showed altered phenotypic properties of immunocompetent cells as evidenced by decreased percentage of suppressor (CD8+CD4), natural killer (NK) and NK precursor (CD4+CD8+) cells, and increased immunoregulatory index (helper to suppressor ratio) (Kvietkauskaite et al. 2004).

Two unpublished studies submitted to EPA and reviewed by EPA (2021a) reported immunological effects of copper 8-quinolinolate. In a 90-day study of rats exposed via diet, females exhibited increased spleen weight at doses ≥100 mg/kg/day copper 8-quinolinolate. In the other study, male rats exposed dermally to 1,000 mg/kg/day copper 8-quinolinolate for 28 days had an increased incidence of necrosis in the thymic lymphocytes. EPA (2021a) did not provide additional information on these findings.

2.15 NEUROLOGICAL

Studies in workers exposed by inhalation and case reports of humans exposed orally to copper have reported neurosensory effects, and some epidemiological studies have suggested effects of excess dietary copper on cognition and/or memory. While robust human data to support a relationship between excess copper exposure and neurodegenerative diseases such as Alzheimer's and Parkinson's diseases are lacking, there are mechanistic data suggesting the possibility that copper may play a role; these data are discussed in Section 2.21, Mechanisms of Toxicity. Neurobehavioral changes have been reported in

animal studies of oral exposure to copper, and at high oral doses (≥25.5 mg Cu/kg/day), copper has been shown to induce neuromuscular effects in laboratory animals.

Neurological effects in humans following copper inhalation were reported in an occupational health study and one case report. Headache, vertigo, and drowsiness were reported in factory workers exposed for 3 years, beginning with a maximum concentration of 464 mg Cu/m^3 and declining over 3 years to 111 mg Cu/m^3 copper dust (Suciu et al. 1981). The prevalence of neurological symptoms declined with declining exposure concentrations (Suciu et al. 1981). A 2-year-old girl who accidentally inhaled copper dust experienced sensory impairment within the first few hours of exposure (Donoso et al. 2007).

Seven adult females exposed to 0.07 mg Cu/kg/day as copper sulfate for 2 weeks in a controlled exposure study experienced headaches (Pizarro et al. 1999). Neurological effects following ingestion of copper substances, including copper sulfate, copper oxychloride, and copper-8-hydroxyquinolate, were also reported in several case reports. The most common effects were headache, dizziness, agitation, and drowsiness (Du and Mou 2019; Gunay et al. 2006; Malik and Mansur 2011; Yang et al. 2004). Dizziness after oral exposure to copper may stem from stimulation of gastrointestinal tract receptors that can alter the brain response to vestibular stimulation (Yates et al. 2014).

Epidemiological investigations of neurological effects in humans exposed to copper in the diet have been conducted; those that met inclusion criteria (see Appendix C, Section C.2.2) are shown in Table 2-8. A large (>10,000 subjects) prospective cohort study of adults in the United States showed an association between an increase in dietary copper intake of 1 mg Cu/day and an increased risk of incident dementia (hazard ratio [HR] 1.49, 95% confidence interval [CI] 1.04, 1.95) among participants whose diets were high in saturated fat, but not among those whose diets were not high in saturated fat (Wei et al. 2022). The study authors also observed an association between dietary copper intake and a decline in scores on word fluency tests (over the 20-year follow-up) in both groups. Dietary intake was estimated at enrollment in the cohort (1987–1989) and again a few years later (1993–1995) based on responses to a validated food frequency questionnaire administered by an interviewer. Cognitive assessments were performed at three time points; the first time point, 1996–1998, served as the baseline assessment. The study authors noted several strengths of their study, including the large sample size, long follow-up, and prospective cohort design (Wei et al. 2022). Limitations highlighted by Wei et al. (2022) included their inability to account for copper intake from water or other local sources and the limited number of cognitive tests administered.

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to Copper and Neurological Effects

Reference, study type, and population	Exposure	Outcome evaluated	Result
Odai et al. 2020 Cross-sectional, 245 women >40 years of age visiting menopause clinic (Japan)	Estimated dietary intake based on questionnaire: 0.15 mg Cu/MJ among women 40–54 years old	Severity of subjective forgetfulness	↔
	0.17 mg Cu/MJ among women ≥55 years old	Severity of subjective forgetfulness	↑
Wang et al. 2021 Cross-sectional, 2,483 adult participants (~50% male, ≥60 years of age) in NHANES (2011–2014) (United States)	Estimated dietary intake based on 24-hour recall: 1.2 mg Cu/day (mean)	Cognitive function scores (word list recall, animal fluency, and digital symbol substitution tests)	↔ for intake >RDI
Wei et al. 2022 Prospective cohort, 10,269 participants (~44% male, mean age 62.9 years old) in Atherosclerosis Risks in Communities Study in four states (United States)	Estimated dietary intake from food and supplements: 1.25 mg Cu/day (mean)	Incident dementia among participants with high intake of saturated fat	↑
		Scores on word fluency test	↓

↑ = association; ↓ = inverse association; ↔ = no association; MJ = millijoule energy; NHANES = National Health and Nutrition Examination Survey; RDI = recommended dietary intake

Other studies that met inclusion criteria were cross-sectional in design, so temporality of the association cannot be established. A small cross-sectional study of women visiting a menopause clinic in Japan reported an association between increased severity of subjective forgetfulness and estimated dietary copper intake among those ≥55 years of age, but not among those between 40 and 54 years of age (Odai et al. 2020). In a cross-sectional study of 2,483 adults at least 60 years old who participated in NHANES (2011–2014) surveys, no significant association was seen between scores on tests of cognitive function and copper intake when estimated dietary intake was greater than the recommended dietary intake (Wang et al. 2021).

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

No treatment-related changes in brain weight or brain histopathology were observed in rats exposed to dicopper oxide by whole-body inhalation at concentrations up to 1.76 mg Cu/m^3 for 4 weeks (Poland et al. 2022). No other studies of neurological effects in animals exposed by inhalation were located.

Neurological effects after oral exposure to copper (as copper sulfate or copper monochloride) were studied in acute and intermediate-duration studies. No histological changes were observed in the brains of mice exposed to doses up to 4 mg Cu/kg/day as copper sulfate for 7 days (Kadammattil et al. 2018). In 15-day exposure studies, no compound-related changes in brain weight or histology were seen in rats after exposure up to 29 mg Cu/kg/day in drinking water or up to 324 mg Cu/kg/day in food or in mice exposed up to 36 mg Cu/kg/day in drinking water or up to 294 mg Cu/kg/day in food in the form of copper sulfate (NTP 1993). In the longer-duration 13-week study, gliosis in the brain was seen in 10/10 female rats exposed to 134 mg Cu/kg/day as copper sulfate in food, but not at 68 mg Cu/kg/day. No neurological effects were observed in male rats exposed up to 140 mg Cu/kg/day or mice exposed to up to 267 mg Cu/kg/day for 13 weeks in food (NTP 1993). Also, rats gavaged with up to 51 mg Cu/kg/day as copper monochloride for 4–5 weeks had no change in brain weight or histology (Chung et al. 2009).

Multiple neurobehavioral effects were observed in rats exposed for intermediate durations. Changes including decreased passive avoidance response (refraining from an act or response that would produce an aversive stimulus), increased immobility time in a forced-swim test, decreased locomotor activity in open field test, and signs of increased anxiety (decreased entries in an open-arm test and decreased exploration time) were observed in rats exposed for 16 weeks to ≥2.6 mg Cu/kg/day as copper sulfate pentahydrate via gavage (Kumar et al. 2019) or 8 mg Cu/kg/day as copper sulfate (Patwa et al. 2022). The rats also exhibited impaired muscle strength and coordination in the rotarod test. The severity of the neurotoxic effects increased with dose (Kumar et al. 2019). Increased depression-like behaviors (assessed in the tail suspension test and forced swim test) and degeneration of neurons in the prefrontal cortex, hippocampus, and striatum were observed in rats following exposure to ≥10 mg Cu/kg/day as copper sulfate for 28 days via gavage (Adeleke et al. 2023). Impaired learning and spatial memory and recognition were also observed in rats following 28 days of exposure to 0.2 mg Cu/kg/day via gavage (Kaur et al. 2021). However, this study was not included in LSE table or figure because the estimated dose is below the recommended dietary intake of copper in rats, and no information on dietary or water copper levels were reported to ensure that the animals' intake was adequate. A feeding study in rats exposed to 23 mg Cu/kg/day as copper sulfate in the diet for 30 days reported no effects on spontaneous motor activity (assessed using an actophotometer), learning ability (assessed using a pole climbing chamber), or relearning capacity and memory (assessed using a Y-maze) (Murthy et al. 1981). The same study

observed 16 and 17% increases in brain levels of dopamine and norepinephrine neurotransmitters, respectively, when the rats were given copper with a high-protein diet (21% casein), but no change when the rats received copper with a low-protein diet (10% casein). De Vries et al. (1986) did not find significant alterations in corpus striatal dopamine levels in rats exposed to 46 mg Cu/kg/day as copper sulfate in drinking water for 11 months. However, a 25% decrease in the levels of a dopamine metabolite, 3,4-dihydroxyphenylacetic acid, in the corpus striatum was observed.

Serious neurotoxic effects observed in rats exposed to a dose of 25.5 mg Cu/kg/day as copper sulfate (by gavage) included impaired motor coordination, cognitive function, and changes in locomotor activity (Kalita et al. 2020; Kumar et al. 2015). Toxicity was demonstrated by reductions in grip strength, fall time latency on a rotarod test, distance traveled, time moving, attention scores, and an increase in resting time (Kalita et al. 2020; Kumar et al. 2015). Changes in grip strength, and rotarod and Y-maze tests results were observed in rats exposed to ≥39.8 mg Cu/kg/day for 30–90 days; neurotoxicity increased with dose (Kumar et al. 2016b). In a similarly designed study by Kumar et al. (2016b), gliosis, pyknotic nuclei, and glial nodule formation in brain sections of rats were observed with doses of ≥39.8 mg Cu/kg/day for 60–90 days. More severe histological findings of neuronal loss and vacuolated spaces marked by depletion of myelin at 79.6 mg Cu/kg/day for 60–90 days were observed in a second study by the same study authors (Kumar et al. 2016a). Severe impairment of spatial learning and memory in the Morris water maze test along with histopathological changes in the cortex and hippocampus (pyknotic hyperstaining, hyperemia, edema, and vacuoles) were seen in rats exposed to 80 mg Cu/kg/day as tribasic copper chloride for 12 weeks via gavage (Yu et al. 2023). Degenerated neurons and focal areas of necrosis in the cerebellum, as well as decreases in acetylcholinesterase (AChE) activity were reported in rats exposed to 79.6 mg Cu/kg/day as copper sulfate for 7 weeks, 3 times/week via gavage (Arowoogun et al. 2021). A study that only tested one dose (50.9 mg Cu/kg/day as copper sulfate) in rats for 30 days reported that copper toxicity slowed brain activity and produced a swollen, congested, and edematous brain (Khushboo et al. 2018). This study was not included in LSE table or figure because the exposed group had a 40% lower water intake and a 30% lower food intake than controls. Effects reported may have stemmed from dehydration and/or malnutrition.

In mice, impaired spatial memory in the Y-maze test and increases in brain AChE activity were seen after exposure to 39.8 mg Cu/kg/day as copper sulfate for 28 days (Isibor et al. 2022). Impaired cognitive function in the Morris water maze test and neuronal degeneration were seen in mice exposed for 90 days to ≥15 mg Cu/kg/day in drinking water (Zhang et al. 2023a). This study was not included in the LSE

table or figure because copper-exposed groups had decreased water intake (20–45% compared to control); dehydration may have been a contributing factor to the effects.

2.16 REPRODUCTIVE

In an occupational health study, sexual impotence was reported in 16% of workers (75–100 workers examined) exposed to copper dust (declining over time from 464 to 111 mg Cu/m^3) during grinding and sieving operations (Suciu et al. 1981). The significance of this finding is difficult to assess because the study did not evaluate whether the prevalence of impotence changed with declining exposure concentrations. No studies were located regarding reproductive effects in humans following oral or dermal exposure to copper.

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

Many animal studies have examined the reproductive toxicity of copper following acute-duration oral exposure. Acute-duration exposure in male rats resulted in decreased serum total testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and/or prolactin following gavage dosing with 25.5 mg Cu/kg/day as copper sulfate pentahydrate for 2 weeks (Abdel-Baky 2019) or 39.8 mg Cu/kg/day as copper sulfate for 7 days (Sarawi et al. 2022). Sarawi et al. (2022) also reported degeneration of seminiferous tubules, loss of spermatogenic series, and an absence of mature spermatozoa in the testes of copper exposed rats. In mice, no changes in testis weight, sperm count, or percentage of abnormal sperm were seen 35 days after a single gavage dose of 4.0 mg Cu/kg/day as copper sulfate (Kadammattil et al. 2018). However, infertility was reported in male mice after 2 weeks of exposure to 6.4 or 8.9 mg Cu/kg/day as copper sulfate pentahydrate via gavage (Al-Musawi et al. 2022). After the 2-week exposure period ended, males were mated with unexposed females until a copulation plug or vaginal sperm was present. No births occurred; histological examination of the testis suggests defective spermatogenesis and death of germ cells occurred in exposed males (Al-Musawi et al. 2022). Female mice exposed to ≥39.8 mg Cu/kg/day as copper sulfate for 14 days had a decrease in the number of antral follicles and ovarian cell damage (Babaei et al. 2012). No differences in number of implantation sites, percentage of viable embryos, or reabsorbed embryos were seen in pregnant mice exposed on GDs 7–12 to 4.0 mg Cu/kg/day as copper sulfate (Kadammattil et al. 2018).

Multiple studies in male rats and mice exposed to copper for intermediate durations suggest that copper plays a role in spermatogenesis and male infertility. Decreases in testicular weight, sperm count, motility, and viability and increases in abnormal sperm morphology were seen in rats exposed to 8 mg Cu/kg/day, as copper sulfate for 24 weeks via gavage; no change in serum testosterone was seen in these rats (Gupta et al. 2021). Similarly, decreases in sperm concentration, count, motility, and viability were observed in rats exposed to ≥39.8 mg Cu/kg/day as copper sulfate for 30–56 days (Liu et al. 2016; Sakhaee et al. 2012); decreases in serum LH and FSH were also seen on one of these studies (Liu et al. 2016). The severity of reproductive toxicity in male animals was found to be dose-dependent in these studies (Liu et al. 2016; Sakhaee et al. 2012). Additionally, at the highest dose tested (79.6 mg Cu/kg/day as copper sulfate), a significant increase in the sperm malformation rate and a decrease in testosterone were noted (Liu et al. 2016). In a separate study in rats by Babaei and Abshenas (2013), significantly decreased sperm count, percentage of live spermatozoa, sperm motility, and testicular weight were seen after 56 days of exposure to 79.6 mg Cu/kg/day as copper sulfate, but not after 28 days of exposure. The signs of reproductive toxicity reported at lower doses were also present in several studies that tested a single higher dose, such as 50.9 mg Cu/kg/day in rats for 30 or 90 days (Arafa et al. 2019; Khushboo et al. 2018), and 127 or 128 mg Cu/kg/day as copper sulfate pentahydrate for 21 days (Parlak Ak et al. 2021; Seven et al. 2020). These effects included significant reductions in testicular weight, testosterone levels, significant increases in sperm head and tail abnormalities, degeneration of epididymides, and testicular degeneration (Arafa et al. 2019; Khushboo et al. 2018). The Khushboo et al. (2018) study was not included in the LSE table or figure because the exposed group had 40% lower water intake and 30% lower food intake, and some effects reported may have stemmed from dehydration and/or malnutrition. No effects on male reproductive organ histology were seen after 15 days of exposure in rats exposed in drinking water (up to 29 mg Cu/kg/day) or diet (up to 324 mg Cu/kg/day) or mice exposed in drinking water (up to 24 mg Cu/kg/day) as copper sulfate pentahydrate (NTP 1993). Thirteen-week feeding studies found no compound-related effects on reproductive organ weights, histopathology, or sperm morphology in rats exposed to doses up to 140 mg Cu/kg/day as copper sulfate or mice exposed up to 815 mg Cu/kg/day as copper sulfate (NTP 1993).

In ICR mice, decreased sperm motility and concentration and increased sperm malformations were seen after 42 days of gavage dosing with ≥3.9 mg Cu/kg/day, and a decrease in testicular weight was seen at ≥7.8 mg Cu/kg/day as copper sulfate (Guo et al. 2021). Chen et al. (2020) reported decreases in epididymal sperm count and motility in CD-1 mice at ≥39.8 mg Cu/kg/day as copper sulfate after 8 weeks of gavage exposure, but not at 10 mg Cu/kg/day. The inconsistent findings may reflect differences in strain of mice; Guo et al. (2021) used ICR mice, whereas Chen et al. (2020) performed their experiment

in CD-1 mice. Significant decreases in sperm concentration, count, motility, and viability were reported in NMRI mice exposed to 39.8 mg Cu/kg/day as copper sulfate once every 2 days for 28–42 days or daily for 42 days via gavage (Sakhaee et al. 2016a, 2016b). In two other studies in male NMRI mice, exposure to 79.6 mg Cu/kg/day as copper sulfate for 42–56 days resulted in changes in sperm parameters similar to those seen in rats, in addition to histological changes including shrinkage and degeneration of seminiferous tubules, moderate to severe degeneration of germinal layers, significantly decreased Sertoli cells nuclei diameter and epithelial height, and significantly less meiotic index (Kheirandish et al. 2014; Sakhaee et al. 2014).

Effects of copper compounds on the female reproductive tract have been reported. In female rats, a 35-day exposure via gavage resulted in changes in ovarian follicular development at ≥6 mg Cu/kg/day as copper sulfate pentahydrate and increases in absolute and relative ovary and uterus weight at ≥12 mg Cu/kg/day (Chen et al. 2023). Changes in ovaries were also seen in mice after a 35-day exposure to copper sulfate at ≥39.8 mg Cu/kg/day (lowest dose tested), including decreases in ovarian follicles and corpora lutea, and structural damage to the ovarian structure (Babaei et al. 2012). Chronic active inflammation of the clitoral gland and ovarian cysts were seen in 10/10 female rats exposed to 134 mg Cu/kg/day as copper sulfate in diet for 13 weeks (NTP 1993). No effects were seen in lower doses of 9–68 mg Cu/kg/day. The NTP (1993) 13-week study in mice reported cysts in the clitoral glands of 8/10 female mice exposed to 1,058 mg Cu/kg/day as copper sulfate in diet and no effects at 52–536 mg Cu/kg/day (NTP 1993). No changes in vaginal cytology were observed in rats or mice (NTP 1993). In 15-day studies, no histological changes in the reproductive organs were reported in female rats exposed up to 26 mg Cu/kg/day as copper sulfate in drinking water or up to 285 mg Cu/kg/day as copper sulfate in food or in mice exposed to 15–36 mg Cu/kg/day in drinking water (NTP 1993).

No reproductive effects were seen in mink exposed up to 13 mg Cu/kg/day as copper sulfate in food for 8 months prior to mating and throughout gestation (Aulerich et al. 1982).

In an unpublished developmental toxicity study of copper hydroxide in rabbits, 2 of 22 pregnant rabbits aborted pregnancies on GD 22 after gavage exposure to 18 mg Cu/kg/day as copper hydroxide; maternal deaths also occurred at this dose (reviewed by EPA 2006). In the EPA (2021a) review of unpublished developmental toxicity studies of copper 8-quinolinolate, reproductive effects were seen in a dose-range-finding study in rabbits exposed orally during gestation. At all doses (≥7 mg/kg/day copper 8-quinolinolate), there were increased pre-implantation losses that led to fewer implantations and live fetuses. However, these results were not confirmed in the definitive rabbit study using doses up to

7 mg/kg/day copper 8-quinolinolate. A two-generation reproductive toxicity study in rats exposed to ≥203 mg/kg/day copper 8-quinolinolate reported decreased numbers of implantation sites in F0 parents, leading to decreased numbers of live F1 pups at birth and on postnatal day (PND 4) (EPA 2021a). No other reproductive effects were seen in this study.

2.17 DEVELOPMENTAL

Only one developmental toxicity study in humans exposed to copper met inclusion criteria (see Appendix C, Section C.2.2): a nested case-control study of 1,172 cases of stillbirth and 7,032 full-term controls in Texas (Rammah et al. 2019). No association was observed between risk of stillbirth and copper concentration in $PM_{2.5}$ modeled for each subject's pregnancy (Rammah et al. 2019). The median modeled copper concentration in $PM_{2.5}$ was 7.06 ng Cu/m^3. No studies regarding developmental effects of humans following oral or dermal exposure to copper met inclusion criteria.

Data on the developmental toxicity of copper in experimental animals are limited. No toxicity studies were identified for developmental effects in animals following inhalation or dermal exposure to copper.

Developmental toxicity following oral exposure to copper has been studied in several species. No significant difference was reported for the number of implantations, nonviable embryos, resorbed embryos, or mean embryo weight when pregnant mice were exposed to 4 mg Cu/kg/day as copper sulfate on days 7–12 of pregnancy as compared to controls (Kadammattil et al. 2018). Rats exposed via gavage 2 weeks prior to mating and throughout gestation to PND 3 with 51 mg Cu/kg/day as copper chloride had litters with increased percentages of runts (defined as weighing at least one-third less than the control means) and pups with icterus, compared to controls (Chung et al. 2009). Decreased litter size and fetal weights were seen when mice were exposed 1 month prior to mating and on GDs 0–19 to ≥208 mg Cu/kg/day as copper sulfate in food (Lecyk 1980). This study was not included in LSE table due to deficiencies in reporting. No developmental effects were observed in the offspring of mink exposed up to 13 mg Cu/kg/day as copper sulfate in food for 8 months prior to mating and throughout gestation (Aulerich et al. 1982).

EPA (2006) reviewed an unpublished developmental toxicity study in rabbits exposed to copper hydroxide. In this study, maternal exposure to 18 mg Cu/kg/day resulted in significantly increased fetal incidences of hemivertebra, delayed ossification (mandible, pelvis, and skull), and supernumerary ribs when compared to the controls; maternal deaths also occurred at this dose. EPA (2021a) summarized the

results of unpublished developmental toxicity studies of copper 8-quinolinolate in rats and rabbits exposed orally during gestation. No developmental effects were seen in rats exposed to doses up to 800 mg/kg/day or in rabbits exposed to doses up to 30 mg/kg/day of copper 8-quinolinolate in guideline (OPPTS 870.3700) studies (EPA 2021a).

Newborn rats exposed on PNDs 7–21 via gavage to ≥0.2 mg Cu/kg/day exhibited changes in serum chemistry and histological changes in the liver; however, this study was not included in the LSE table or figure because the data are inadequately reported to determine an effect level (Dai et al. 2020). No developmental effects occurred in infant guinea pigs exposed to 18.4 mg Cu/kg/day as copper sulfate in water for 6 months (after a month of dosing at 6.6 mg Cu/kg/day in formula milk) (Seffner et al. 1997).

2.18 OTHER NONCANCER

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 Cu/m^3. It has been suggested that other metals present in the workplace could have been the primary causative agents for the metal fume fever, rather than copper (Borak et al. 2000).

One cross-sectional epidemiology study that met inclusion criteria (see Appendix C, Section C.2.2) reported associations between decreased body mass index and waist circumference and estimated dietary intake of copper in 19,952 adult NHANES (2007–2014) participants (Jiang et al. 2020). No other studies of these outcomes met inclusion criteria.

Several experimental oral studies reported reductions in food and/or water intake in animals exposed to copper. In rats fed up to 285–325 mg Cu/kg/day as copper sulfate pentahydrate for 15 days, 37–38% decreased food intake was observed (NTP 1993). Reduced water consumption of 25–67% was observed in mice exposed to doses of 10–62 mg Cu/kg/day as copper sulfate pentahydrate in the drinking water for 15 days (NTP 1993). In rats, decreases in food consumption (by 21–29%) and water intake (41%) were attributed to gavage exposure to 50.9–199 mg Cu/kg/day as copper sulfate for 21–30 days (Khushboo et al. 2018; Seven et al. 2018). Two chronic-duration studies in monkeys reported no differences in food intake following oral intake of 5.5–7.5 mg Cu/kg/day as copper gluconate in diet or milk for 3 years (Araya et al. 2012).

EPA (2006) reviewed an unpublished study of pregnant rabbits exposed to ≥9 mg Cu/kg/day as copper hydroxide on GDs 7–28. The does exhibited significant reductions in mean food consumption accompanied by body weight losses during GDs 7–10.

2.19 CANCER

There are limited data for humans and no data for animals on the carcinogenicity of inhaled copper. Several studies that evaluated the association between lung cancer and exposure to copper in airborne particulate matter or indoor dust measured exposure after the outcome had occurred, and thus were not considered useful for hazard identification. Although a number of studies examined cancer risk among workers at copper smelters, the cancer risk was attributed to arsenic exposure rather than exposure to copper. In a study of >6,700 male workers at a Chinese copper mine, there was a significantly increased risk for cancer (all sites combined) (standardized mortality ratio [SMR] 123, 95% CI 109–139), a significantly increased risk for stomach cancer (SMR 131, 95% CI 105–161), and a significantly increased risk for lung cancer (SMR 147, 95% CI 112–189) (Chen et al. 1993). The cancer risk increased with the duration of employment and time since first exposure (time between first exposure and cancer diagnosis). The risk was also higher in workers employed in the 1950s, when there was a dramatic increase in production, but poor underground ventilation and dry drilling methods were used, which generated high levels of dust. Radon and radon daughters (decay products) were measured in the underground mines; between 1960 and 1990, radioactivity levels of 1.29×10^{-11} Ci/L were recorded. To assess the relative contribution of radon and radon daughters to lung cancer risk, the workers were divided into two groups: underground miners and drilling miners (presumably above ground). Increases in lung cancer risk were observed in both groups, and the study authors suggested that exposure to radiation did not appear to be responsible for the risk of excess death from lung cancer. The copper ore from the Chinese mine also contained silica, iron, manganese, arsenic, titanium, and sulfur (Chen et al. 1993). 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) calculated the risks of cancer for: all sites (SMR 129, 95% CI 117–142), stomach cancer (SMR 141, 95% CI 116–169), and lung cancer (SMR 152, 95% CI 123–187). All risks were still significantly elevated. This study also conducted a worker smoking survey and found that a higher percentage of the miners were smokers (71.7%) than the control population of local residents (64.3%). The increased smoking rate, along with the exposures to radioactivity, silica, iron, and arsenic, could have contributed to the increased cancer risk.

No studies were located regarding cancer effects in humans or animals following dermal exposure to copper.

Two oral studies examined the carcinogenicity of copper compounds in animals; however, these studies used only single dose levels, tested small groups of animals (6–13), exposed animals for far less than lifetime, and examined only selected tissues for tumor formation. These studies did not find increases in the occurrence of liver tumors in rats exposed to 130 mg Cu/kg/day as copper acetate for 24 weeks (Kamamoto et al. 1973) or large intestine tumors in rats exposed weekly to 9 mg Cu/kg/day as an unspecified copper compound for 16 weeks (Greene et al. 1987).

In an intermediate-duration study, rats were orally exposed to 62 mg Cu/kg/day as copper gluconate for 6 weeks, and a significant increase in the number of glutathione S-transferase placental form (GST-P) positive single hepatocytes was seen (Abe et al. 2008). There were no changes in number of GST-P positive lesions or area of such lesions (Abe et al. 2008). GST-P-positive foci are considered preneoplastic changes that may progress to neoplasm.

As reported by EPA (2021a), an unpublished carcinogenicity bioassay of copper 8-quinolinolate in mice exposed via diet did not report increased tumor incidences at doses up to 855.8 mg/kg/day copper 8-quinolinolate in males and 1051.7 mg/kg/day copper 8-quinolinolate in females.

Several studies examined the carcinogenicity of copper compounds following parenteral administration. No clear increases 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); 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 Wistar rats receiving intramuscular injections of 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.

IARC has not evaluated the carcinogenicity of copper. IARC lists copper 8-hydroxyquinoline as not classifiable as to its carcinogenicity in humans due to lack of cancer studies in humans and animals (IARC 1987). Neither NTP nor EPA has evaluated the carcinogenicity of copper (IRIS 1988; NTP 2021).

2.20 GENOTOXICITY

Studies investigating the genotoxicity of copper in humans have given inconsistent results. Significant increases in deoxyribonucleic acid (DNA) damage were observed in the peripheral blood leukocytes of males working at a copper smelting plant (duration varied from 0.2 to 25 years) relative to controls, however, these increases were not associated with copper concentrations measured in the blood (De Olivera et al. 2012). Shubber et al. (1998) analyzed blood lymphocytes of women using copper-containing contraceptive IUDs for various periods (1–4 years). Compared to age- and income-matched control women, those using IUDs had significantly higher plasma copper levels and increased frequencies of both chromosomal aberrations and sister chromatid exchanges. In a human study by O'Connor et al. (2003), healthy adults were provided with copper supplements for 6 weeks at doses up to 0.067 mg Cu/kg/day as copper sulfate. There was no evidence of DNA damage to leukocytes. No studies were located regarding genotoxicity in humans after dermal exposure to copper or its compounds.

Several animal studies assessed the genotoxicity of copper sulfate following oral or parenteral exposures and have consistently shown copper to be genotoxic in these systems. The results of these *in vivo* genotoxicity studies are summarized in Table 2-9. Significant increases in the occurrence of micronuclei and chromosomal aberrations have been observed in chick bone marrow cells and erythrocytes 24 hours after exposure to 1.9–2.5 mg Cu/kg as copper sulfate (Bhunya and Jena 1996) and mouse bone marrow cells following exposure to 0.28–8.25 mg Cu/kg as copper sulfate (Agarwal et al. 1990; Bhunya and Pati 1987; Fahmy 2000; Kadammattil et al. 2018; Prá et al. 2008). Peripheral lymphocytes from rabbits gavaged for 6 days with 7.5 mg Cu/kg as copper sulfate showed significant increases in sister chromatid exchanges and chromosomal aberrations (Georgieva et al. 2013). A study of copper sulfate did not find increases in the number of micronuclei in bone marrow cells 24 hours after mice were injected with up to 5.04 mg Cu/kg (Tinwell and Ashby 1990). The discrepancy in findings from other studies is not clear but could be due to differences in mouse strain and/or administration route. Several studies reported DNA strand breaks in blood cells of mice orally exposed to copper sulfate at doses of 0.498–8.5 mg Cu/kg both 24 hours after a single gavage or after 6 days of exposure (Franke et al. 2006; Prá et al. 2008; Saleha Banu et al. 2004). Husain et al. (2021) reported increased DNA strand breaks in intestinal cells after a single oral dose ≥2 mg Cu/kg as copper chloride in rats. DNA fragmentation was also observed in liver cells of rats after oral exposures to 39.8 or 119 mg Cu/kg/day as copper sulfate for 7 days (Alhusaini et al. 2018a, 2018b).

Table 2-9. Genotoxicity of Copper and Copper Compounds *In Vivo*

Species (test system)	Endpoint	Results	Reference	Compound
Non-mammalian systems				
Drosophila melanogaster (oral exposure)	DNA damage	+	Shukla et al. 2011	Copper sulfate
D. melanogaster (injection into larvae)	Recessive lethals	+	Law 1938	Copper sulfate
Mammalian systems				
Human peripheral blood leukocytes (occupational exposure)	DNA strand breaks	–	De Olivera et al. 2012	Copper
Human leukocytes (oral exposure)	DNA strand breaks	–	O'Connor et al. 2003	Copper
Albino rat liver cells (oral exposure)	DNA strand breaks	+	Alhusaini et al. 2018a	Copper sulfate
Albino rat liver cells (oral exposure)	DNA stand breaks	+	Alhusaini et al. 2018b	Copper sulfate
Wistar rat intestinal cells (oral exposure)	DNA strand breaks	+	Husain et al. 2021	Copper chloride
CF1 mice blood cells (oral exposure)	DNA strand breaks	+	Prá et al. 2008	Copper sulfate
Swiss Albino mice leukocytes (oral exposure)	DNA strand breaks	+	Saleha Banu et al. 2004	Copper sulfate
Swiss Webster mice blood cells (oral exposure)	DNA strand breaks	+	Franke et al. 2006	Copper sulfate
Human blood leukocytes (women with copper IUDs)	Chromosomal aberrations	+	Shubber et al. 1998	Copper
Inbred Swiss mice bone marrow cells (i.p. and/or s.c. injection)	Chromosomal aberrations	+	Bhunya and Pati 1987	Copper sulfate
White Swiss mice bone marrow cells (i.p. injection)	Chromosomal aberrations	+	Agarwal et al. 1990	Copper sulfate
New Zealand rabbit blood cells (oral exposure)	Chromosomal aberrations	+	Georgieva et al. 2013	Copper sulfate
White Leghorn chicken bone marrow cells (i.p. injection and oral exposure)	Chromosomal aberrations	+	Bhunya and Jena 1996	Copper sulfate
White Swiss mice spermatocytes (i.p. injection)	Chromosomal aberrations	+	Fahmy 2000	Copper sulfate
Human blood leukocytes (women with copper IUDs)	Sister chromatid exchanges	+	Shubber et al. 1998	Copper
New Zealand rabbit blood cells (oral exposure)	Sister chromatid exchanges	+	Georgieva et al. 2013	Copper sulfate
CBA mice bone marrow cells (i.p. injection)	Micronuclei	–	Tinwell and Ashby 1990	Copper sulfate
CF1 mice bone marrow cells (gavage exposure)	Micronuclei	+	Prá et al. 2008	Copper sulfate
Inbred Swiss mice bone marrow cells (i.p. and/or s.c. injection)	Micronuclei	+	Bhunya and Pati 1987	Copper sulfate

Table 2-9. Genotoxicity of Copper and Copper Compounds *In Vivo*

Species (test system)	Endpoint	Results	Reference	Compound
Swiss Albino mice bone marrow cells (oral exposure)	Micronuclei	+	Kadammattil et al. 2018	Copper sulfate
White Leghorn chicken bone marrow cells (i.p. injection and oral exposure)	Micronuclei	+	Bhunya and Jena 1996	Copper sulfate
White Leghorn chicken erythrocytes (i.p. injection and oral exposure)	Micronuclei	+	Bhunya and Jena 1996	Copper sulfate
White Swiss mice bone marrow cells (intraperitoneal injection)	Micronuclei	+	Fahmy 2000	Copper sulfate
Inbred Swiss mice (i.p. injection)	Sperm abnormalities	+	Bhunya and Pati 1987	Copper sulfate
Swiss Albino mice (oral exposure)	Sperm abnormalities	+	Kadammattil et al. 2018	Copper sulfate
White Swiss mice (i.p. injection)	Sperm abnormalities	+	Fahmy 2000	Copper sulfate
ICR mice testis and spleen (gavage)	γ-H2AX levels	+	Guo et al. 2021, 2022a	Copper sulfate

+ = positive results; – = negative results; DNA = deoxyribonucleic acid; i.p. = intraperitoneal; IUD = intrauterine device; s.c. = subcutaneous

Sperm abnormalities, including spermatocyte chromosome aberrations, double-headed, and double-tailed sperm, were observed in mice after intraperitoneal exposure to 0.524 mg Cu/kg as copper sulfate for 3 days or 1 mg Cu/kg for 5 days (Bhunya and Pati 1987; Fahmy 2000) and oral exposure to 4 mg Cu/kg as copper sulfate once (Kadammattil et al. 2018). Levels of the DNA damage marker, γ-H2AX, were significantly increased in the testis in mice gavaged with 8.0 mg Cu/kg as copper sulfate for 21 days (Guo et al. 2021). In *Drosophila*, exposure to copper sulfate resulted in significant increases in the occurrence of recessive lethal mutations after 10 minutes (at 0.1% copper concentration) (Law 1938) and DNA damage after 24 hours (at 20 μM Cu) (Shukla et al. 2011).

The results of *in vitro* genotoxicity studies are summarized in Table 2-10. There were no 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 were found in studies testing for DNA damage including errors in DNA synthesis using viral DNA polymerase (Sirover and Loeb 1976), a reduction in DNA synthesis in Chinese hamster ovary cells (Garrett and Lewtas 1983), and increased oxidative DNA damage in HeLa cells (Schwerdtle et al. 2007). Occurrence of DNA strand breaks in primary human blood cells following copper exposure has not been consistent. Two studies by Husain and Mahmood (2019, 2020) found that DNA damage occurred in human

lymphocytes at copper concentrations of 0.2–1.2 mM (15–76 mg Cu/L) as copper chloride after 1 hour of exposure, whereas no DNA damage was observed in human CD4+ T lymphocytes exposed to copper at concentrations of 5 mM (318 mg/L) for 48 hours (Caicedo et al. 2008) or human blood cells exposed to up to 40 mM (2.5 g/L) for 30 minutes (Prasad et al. 2006). Several studies conducted in nonprimary human and animal cells have consistently shown increased DNA strand breaks following copper exposure in the absence of activation (Anchordoquy et al. 2017; Dai et al. 2020; Grillo et al. 2010; Jing et al. 2016; Mandil et al. 2020; Schwerdtle et al. 2007; Sideris et al. 1988; Sina et al. 1983; Urbina-Cano et al. 2006). One study reported no change in the number of strand breaks in pulmonary alveolar epithelial cells following a 4-hour exposure up to 24 µg Cu/mL as copper chloride (Boyadzhiev et al. 2022). An increase in sister chromatid exchange in Chinese hamster cells occurred after a 24-hour exposure to 10^{-5}M copper nitrate (Sideris et al. 1988) and is consistent with the clastogenic effects observed in *in vivo* assays. Increased micronuclei formation was observed in rat splenocytes following exposure to 40 µM of copper for 12 hours (Mandil et al. 2020). Unscheduled DNA repair synthesis occurred in rat hepatocytes at copper concentrations of 7.9–78.5 µM, both in the presence or absence of hydroxyurea (Denizeau and Marion 1989).

Table 2-10. Genotoxicity of Copper and Copper Compounds *In Vitro*

Species (test system)	Endpoint	Results		Reference	Compound
		With activation	Without activation		
Prokaryotic organisms					
Avian myeloblasts virus, DNA polymerase	Errors in DNA synthesis	No data	+	Sirover and Loeb 1976	Copper chloride
Salmonella typhimurium TA 102	Reverse mutation	No data	–	Marzin and Phi 1985	Copper sulfate
S. typhimurium TA98, TA102, TA1535, TA1537	Reverse mutation	–	–	Wong 1988	Copper chloride
S. typhimurium TA100	Reverse mutation	No data	–	Tso and Fung 1981	Copper chloride
Escherichia coli	Reverse mutation	No data	+	Demerec et al. 1951	Copper sulfate
Bacillus subtilis	DNA damage (rec-assay)	No data	–	Nishioka 1975	Copper chloride
Eukaryotic organisms					
Fungi:					
S. cerevisiae	Recombination	No data	–	Sora et al. 1986	Copper sulfate
Saccharomyces cerevisiae	Reverse mutation	No data	–	Singh 1983	Copper sulfate

Table 2-10. Genotoxicity of Copper and Copper Compounds *In Vitro*

Species (test system)	Endpoint	Results		Reference	Compound
		With activation	Without activation		
Mammalian cells:					
Human blood cells	DNA fragmentation	No data	–	Prasad et al. 2006	Copper chloride
Human lymphocytes	DNA strand breaks	No data	+	Husain and Mahmood 2019	Copper chloride
Human CD4+ T lymphocytes	DNA strand breaks	No data	–	Caicedo et al. 2008	Copper
Human lymphocytes	DNA strand breaks	No data	+	Husain and Mahmood 2020	Copper
Human HeLa S3 cells	DNA strand breaks	No data	+	Schwerdtle et al. 2007	Copper sulfate
HEK293 (human embryonic kidney)	DNA strand breaks	No data	+	Dai et al. 2020	Copper sulfate
Rat hepatocytes	DNA strand breaks	No data	+	Sina et al. 1983	Copper sulfate
Rat splenocytes	DNA strand breaks	No data	+	Mandil et al. 2020	Copper
Mouse Balb-C lymphocytes (comet assay)	DNA strand breaks	+	+	Urbina-Cano et al. 2006	Copper
Mouse primary lymphocytes	DNA strand breaks	No data	+	Jing et al. 2016	Copper
FE1 Mouse pulmonary alveolar epithelial cells	DNA strand breaks	No data	–	Boyadzhiev et al. 2022	Copper chloride
Bovine ovary cells	DNA strand breaks	No data	+	Anchordoquy et al. 2017	Copper
CHO cells	DNA strand breaks	No data	+	Grillo et al. 2010	Copper
Chinese hamster V79 cells	DNA strand breaks	No data	+	Sideris et al. 1988	Copper nitrate
CHO cells	DNA synthesis	No data	+	Garrett and Lewtas 1983	Copper chloride
Chinese hamster V79 cells	Sister chromatid exchange	No data	+	Sideris et al. 1988	Copper nitrate
Rat splenocytes	Micronuclei formation	No data	+	Mandil et al. 2020	Copper
Rat hepatocytes	Unscheduled DNA synthesis	+	+	Denizeau and Marion 1989	Copper sulfate
Human HeLa S3 cells	Oxidative DNA damage	No data	+	Schwerdtle et al. 2007	Copper sulfate
Porcine oocytes	Oxidative DNA damage	No data	+	Chen et al. 2021	Copper sulfate

+ = positive results; – = negative results; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid

Changes in DNA methylation and acetylation caused by exposure to copper can lead to modifications on the epigenome, which could potentially have transgenerational effects. Recent evidence indicates that exposure to copper can influence gene expression by binding to metal response elements and also via epigenetic mechanisms (Cheng et al. 2012). Increased copper levels in the placenta or serum of pregnant mothers have been associated with changes in DNA methylation of placental (Kennedy et al. 2020) and cord blood cells (Weyde et al. 2021). In addition, lower methylation levels of four CpGs sites in blood leukocytes were associated with higher plasma copper concentrations in a Chinese population study (Long et al. 2021). On the other hand, no association was seen between urinary copper levels or pregnant women and DNA methylation in cord blood (Zhang et al. 2022) or between serum copper levels in pregnant women and DNA methylation in peripheral blood cells (Xu et al. 2022). Human cell line and animal studies have been used to demonstrate alterations to the epigenome. Melino et al. (2009) suggested that copper might also modulate histone deacetylase (HDAC) activity in *E. coli* cells, a crucial enzyme in the epigenetic machinery. In another study, rats were exposed to 6.5 mg/kg copper in their feed, which increased DNA methylation (Ognik et al. 2019). No significant trends in global DNA methylation related to inhalation copper exposure in ICR mice were observed (Rossner et al. 2020). Human hepatocyte Hep3B cells treated with Cu^{2+} at 100–200 mM showed significant decreases in global histone acetylation (Kang et al. 2004). Hypoacetylation detected in histones demonstrates that copper is capable of altering the epigenome (Cheng et al. 2012).

2.21 MECHANISMS OF TOXICITY

The molecular mechanisms of copper toxicity were reviewed by Gaetke et al. (2014). Many of the systemic effects of excess copper intake stem from copper's ability to undergo redox cycling, leading to increases in reactive oxygen species and oxidative damage (Gaetke et al. 2014). In cells and tissues, copper exists primarily in the cupric form (Cu^{++}), which can be reduced to Cu^{+} in the presence of reducing agents (e.g., glutathione) or superoxide (Gaetke et al. 2014). The reduction reaction can form hydroxyl radicals, which then catalyze formation of protein and lipid radicals and induce oxidative DNA damage. Evidence from animal studies supports a role for oxidative stress in copper-induced liver, kidney, and neurotoxic effects. Increases in oxidative stress parameters (malondialdehyde, nitric oxide, etc.) and depletion of antioxidants (glutathione [GSH], superoxide dismutase [SOD], catalase) have been demonstrated in the liver (Alhusaini et al. 2018a, 2018b; Hashish and Elgaml 2016; Kumar et al. 2016b; Kvietkauskaite et al. 2004; Liu et al. 2020b; Seven et al. 2018), kidneys (Alharbi et al. 2019; Hashish and Elgaml 2016; Kumar et al. 2016b; Seven et al. 2018), and brain (Behzadfar et al. 2017; Kumar et al. 2016b, 2019) of rats and/or mice exposed orally to excess copper. Kumar et al. (2016a) reported that the

severity of renal histopathological changes in rats exposed to copper correlated positively with malondialdehyde (MDA) levels and inversely with GSH and tacrolimus (TAC) levels in the kidney. Similarly, in rats exposed to 39.8 mg Cu/kg/day for 30–90 days, changes in TAC, GSH, and MDA correlated with functional neurological impairment (Kumar et al. 2016b). Studies of copper-exposed animals concurrently treated with antioxidant preparations (e.g., quercetin, curcumin, *Salvia officinalis* extract) showed mitigation of copper's renal and hepatic effects (Alhusaini et al. 2018b; Dab et al. 2023; Peng et al. 2020), providing further support for the role of oxidative stress.

Several reviews have examined potential mechanisms by which altered copper homeostasis may be involved in the development of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Lewy body dementia (Aaseth et al. 2021; Acevedo et al. 2019; Coelho et al. 2022; Mezzaroba et al. 2019; Zhang et al. 2022; Zubčić et al. 2020). The redox properties of copper appear to be important because reactive oxygen species lead to both oxidative protein damage and derangements of protein structure (misfolding and aggregation) (Acevedo et al. 2019). Copper interacts with both amyloid and tau proteins that accumulate in the brain in Alzheimer's disease and with alpha-synuclein, which accumulates in patients with Parkinson's disease. For example, in the brain, copper accumulates in amyloid plaques, often associated with extracellular amyloid-β (Aβ) (Acevedo et al. 2019; Zhang et al. 2022). When bound to Aβ, copper redox cycling results in oxidative damage to Aβ, and oxidized Aβ has a higher tendency to aggregate (Acevedo et al. 2019; Wärmländer et al. 2019; Zhang et al. 2022). In addition, high-affinity binding of Cu^{2+} to Aβ peptides induces structural changes that promote aggregation (Acevedo et al. 2019). Copper has also been shown to bind to tau protein, inducing its aggregation, and to accumulate in neurofibrillary tangles characteristic of Alzheimer's disease (Acevedo et al. 2019; Mezzaroba et al. 2019). Similarly, there is also evidence that copper enhances the aggregation of alpha-synuclein (Acevedo et al. 2019; Gaetke et al. 2014; Mezzaroba et al. 2019).

Copper intake has been implicated in neurodegenerative prion diseases such as Creutzfeldt-Jakob disease, as discussed in a review by Oliveri (2023). Prions, misfolded versions of the normal cellular Prion Protein (PrP^C), are able to self-replicate and aggregate in the nervous system and brain. The normal form of the PrP^C is believed to play a role in metal homeostasis, and it has several copper binding sites. Some studies have suggested that copper is involved in the conversion of normal PrP^C to the abnormal form that occurs in prion disease, but further research is needed (Oliveri 2023).

Other potential mechanisms may also be involved in the observed systemic effects of excess copper. In their review, Gaetke et al. (2014) noted that perturbations of copper homeostasis may impair the function

of key catalytic enzymes including cytochrome P450 isozymes through nonspecific binding. Impairment of lipid metabolism, a common finding in Wilson's disease, may be a downstream effect of redox cycling or may occur through altered gene expression. In fish exposed to copper, concentration-related changes in gene expression, including downregulation of cholesterol biosynthesis genes, were observed (Gaetke et al. 2014).

2.22 COPPER NANOPARTICLES

The following section provides a brief overview on toxicity of copper nanoparticles, including copper oxide nanoparticles when indicated, and is focused on highlighting findings from experimental animal studies. Occupational populations are more likely to be exposed to copper nanoparticles than the general population, and emissions may come from industrial facilities such as for asphalt and rubber production (Ameh and Sayes 2019). Copper nanoparticles are also found in pesticides, fertilizers, and personal care products, which may result in its presence in wastewater and sewage (Ameh and Sayes 2019). Crops such as cucumbers or alfalfa can uptake copper nanoparticles from applied agricultural products, and these plants can present another potential source of human exposure (Ameh and Sayes 2019). No epidemiology studies using copper nanoparticles were identified. *In vitro* models using human cell lines have demonstrated that copper nanoparticles induce dose- and time-dependent increases in cytotoxicity, reactive oxygen species, and DNA damage (Alarifi et al. 2013; Karlsson et al. 2008). Research on the effects of copper nanoparticles in animals is limited but suggest that copper nanoparticles may induce a wide range of effects in laboratory animals, as discussed below. Several *in vivo* and *in vitro* studies have demonstrated that copper nanoparticles increase the production of reactive oxygen species and reactive nitrogen species both associated in other studies with serious adverse effects such as genotoxicity, inflammation, apoptosis, and fibrosis (Ameh and Sayes 2019).

The primary target organs for copper nanoparticle toxicity include the liver, kidneys, and spleen. Oral administration of copper oxide nanoparticles can cause significant alterations in the activity of antioxidant enzymes including decreased activity for GSH, catalase, and SOD, plus increases in the lipid peroxidation product, malondialdehyde, at doses as low as 5 mg/kg/day in rats (Anreddy 2018). Hepatic effects in rats and mice resulting from acute- or intermediate-duration oral exposure to copper, copper oxide, or copper carbonate nanoparticles include an enlarged liver; histopathological changes in liver tissues including congestion, hepatocellular degeneration, and steatosis around the central veins of the hepatic tissue; inflammatory responses; increased mitosis; and significantly diminished cytochrome P450 enzyme activities (Chen et al. 2006; De Jong et al. 2019; El Bialy et al. 2020; Lee et al. 2016; Tang et al. 2018).

Oral exposure to copper oxide nanoparticles in mice resulted in increased levels of serum ALT, AST, BUN, ALP, and creatinine. Histopathological effects on the kidneys of rats and mice resulting from exposure to copper nanoparticles include degenerated tubular cells, inflammatory cell infiltration, glomerular hypercellularity, severe coagulative necrosis, detached tubular epithelia, loss of brush border, and narrowing of tubular lumen (Chen et al. 2016; De Jong et al. 2019; El Bialy et al. 2020; Lee et al. 2016). In the spleen, copper nanoparticle exposure resulted in splenic, lymphatic, and thymus atrophy and lymphoid depletion in rats and mice after acute- or intermediate-duration oral exposure (Chen et al. 2016; De Jong et al. 2019; El Bialy et al. 2020; Lee et al. 2016).

Other adverse effects that were observed in animals exposed to copper nanoparticles include evidence for neurological, gastrointestinal, and pulmonary toxicity. Neurotoxic findings following oral or intravenous copper nanoparticle injection in rodents include changes in motor activity and oxidative stress in various brain regions (thalamus, hypothalamus, and medulla), in addition to increasing levels of AChE in the hippocampus and striatum along with decreased exploratory behavior (Fahmy et al. 2020; Luo et al. 2020). In rats and mice, copper nanoparticle exposure altered the cecum microbiome; induced ulcerations in the cecum, colon, and rectum; and caused apoptosis in the duodenum, ileum, and cecum (Cholewińska et al. 2018; De Jong et al. 2019; Luo et al. 2020). A murine pulmonary infection model presents some evidence that copper nanoparticles cause pulmonary inflammation and may reduce lung clearance, thus increasing the risks of pulmonary infections (Kim et al. 2011). No studies to date have directly linked copper nanoparticle exposure to carcinogenicity.

Hematological effects in rats and mice from copper nanoparticle exposure include decreased red blood cell counts, white blood cell counts, hematocrit, and hemoglobin levels (De Jong et al. 2019; El Bialy et al. 2020). Copper nanoparticles appear to affect reproduction in rats and mice as evidenced by decreased sperm count and testes weight in males and decreased FSH, LH, and progesterone in females. Exposure to copper nanoparticles also resulted in ovarian atrophy, disturbance in follicular development, follicular atresia, and reduction in mature follicles (Kadammattil et al. 2018; Yang et al. 2010). Kadammattil et al. (2018) reported that exposure to copper nanoparticles was more toxic to the reproductive functioning of male mice than copper sulfate exposure. Copper nanoparticle exposure resulted in fetal toxicity in rats, including a dose-dependent change in fetal weight, induction of oxidative stress in fetal liver, and increased expression of pro-inflammatory cytokines (Luo et al. 2020).

The toxicokinetics of copper nanoparticles can vary widely depending on particle size, other physicochemical properties, and the preparation. Identified studies were limited to inhalation and

ingestion of copper nanoparticles. A higher rate of aggregation in the brain (direct translocation via the olfactory bulb) was observed than in the gastrointestinal system (as seen with copper) (Naz et al. 2020). Copper homeostasis in the brain is maintained by a coordinated system of copper transporters and chaperones that transport copper across the membranes as required (Haywood 2019). Copper nanoparticles can be distributed throughout the body. The primary target organs in animals tend to be the brain, liver, kidney, and spleen where the copper nanoparticles induce pathological changes and organ injuries. It is hypothesized that the smaller particle size of copper nanoparticles increases surface area, which in turn increases its reactivity with hydrogen ions in gastric fluids. This then enables conversion to ionic copper resulting in increased systemic uptake of copper (Ameh and Sayes 2019). The ionic copper is distributed to the liver with some excreted in bile like other copper compounds. The unabsorbed copper nanoparticles are primarily excreted in the feces of mammals with minimal excretion in urine.

Evidence to date suggests that copper nanoparticles and soluble copper compounds share several target organs including the liver, kidney, and stomach. Specifically, since copper nanoparticles are smaller, they can cross the cellular membrane and induce oxidative injury. In addition, the small particle size also assists them in evading phagocytosis and other immune response mechanisms allowing for translocation to other organs (Chen et al. 2006). The overall database for copper nanoparticles in mammals is limited to a few studies in rats and mice. Most of the copper nanoparticle toxicity studies use *in vivo* and *in vitro* approaches, and most of the toxicity studies thus far focus on aquatic organisms and/or microorganisms (Chang et al. 2012).